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College of Medicine
Baghdad, Iraq
Tel. + 964 7717516090
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E-mail: : iraqijms@colmed.nahrainuniv.edu.iq, iraqijms2000@gmail.com
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Therapeutic Role of Green Tea on Human Body Function, Some Diseases and Weight loss: A Review

Sawsan J. Al-Harbi[1] *PhD*, **Fouad K. Gatea**[2] *PhD*

[1]Dept. of Human Anatomy, College of Medicine, University of Babylon, Iraq, [2]Dept. of Pharmacology and Therapeutics, College of Medicine, Al-Nahrain University, Baghdad, Iraq

Abstract

After water, tea is the most consumed nutrient on the planet. However, black tea accounts for 78% of global tea consumption, while green tea accounts for only 20%. Except for flavored tea, all types of tea are made from the dried leaves of the tea bush. The type of tea is determined by the degree of oxidation of the leaves. Unoxidized tea leaves are used to make tea leaf, which is one of the less processed varieties of tea. It therefore, contains the most powerful antioxidants and beneficial polyphenols. Green tea polyphenols include epigallocatechin gallate (EGCG), epicatechin gallate, epicatechins, and flavanols, all of which are being studied in the lab for their potential in vivo effects. Kaempferol, quercetin, and myricetin are three types of flavonoids found in various parts. Although the caffeine in green tea can improve mental alertness, there is only weak, inconclusive evidence that it reduces the risk of most cancers or cardiovascular diseases, and there is no evidence that it aids weight loss. Using green tea as a health supplement has been linked to a slight improvement in general well-being. In a 2020 review, the Cochrane Collaboration identified a few potential negative effects, including gastrointestinal issues, higher levels of liver enzymes, and, more rarely, insomnia, elevated blood pressure, and skin reactions. Its anticancer and anti-inflammatory properties are well-known. Catechins are the main antioxidant dealers among the biologically active compounds found in Camellia sinesis. According to recent medical studies, the presence of function structural agencies and the range of hydroxyl agencies have a major impact on catechins' antioxidant activity. Unfermented inexperienced tea is the best source of those compounds.

The review on green tea and its catechins focused on language literature in English. The literature search was conducted in the following databases: Pubmed (1997-2020), EMBASE (1997-2020), Allied and complementary Medicine Database (AMED, 1997-2020) and China Journals Full Text Database (1997-2020). The keywords used were selected from the following terms: green tea, catechins, anticancer, diabetes, polyphenols, in vivo studies, general pharmacology and toxicology. The health benefits and adverse effects of green tea and its catechins were reviewed.

Keywords Green Tea, Human, diseases, weight loss

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List of abbreviations: ALT = Alanine aminotransferase, EGCG = (-)-epigallocatechin-3-gallate, GTE = Green tea extract, IQ = 2-amino-3-methylimidazol (4,5-f)quinoline, LDL = Low-density lipoprotein

Green tea

Tea is a type of tea made from *Camellia sinensis* leaves and buds that haven't been subjected to the constant withering and oxidation process used to make tea leaf teas and black teas [(1)]. Tea originated in China, but it is now grown and manufactured in a number of East Asian countries. Green tea comes in a variety of styles, each of which is well supported by the variety of *Camellia sinensis* used, growing conditions, farming methods, production processing, and harvest time [(2)]. Though there has been extensive

research into the possible health benefits of drinking strong tea on a regular basis, there is very little evidence that drinking inexperienced tea has any health benefits [3].

Kinds of green tea

There are many different types of green tea, each with its own style and inhibitor properties. Sencha is the most popular type of green tea, and it's most commonly made in Japan [4]. Sencha tea is used to make Bancha, Matcha, and Gyokuro species after proper treatment [5]. When compared to Sencha infusion, Bancha infusion contains significantly less caffeine, as well as L-theanine, the organic compound responsible for the formation of proteins responsible for neurotransmitter assembly, internal secretion, and vasoconstrictor function [6]. In comparison to other types of infusions, Matcha tea leaves infusion has the highest amount of alkaloid and L-theanine [7]. Tea comes in a variety of forms, including bottled and sweetened with sugar or artificial sweetener, single tea bags, loose-leaf, instant-powder, and green tea supplements, which are available in capsule form or liquid extracts [8].

Structure of inexperienced tea

Though many catechins are found in small amounts, (-)-epigallocatechin-3-gallate or EGCG is by far the most abundant of the inexperienced tea polyphenols by weight [9]. EGCG consists of a benzenediol ring (category A) joined to a tetrahydropyran moiety (C), a pyrogallol ring (B), and a galloyl organization (with the B' ring) and is thought to contain approximately 80–100 mg of polyphenols in a single bag of inexperienced tea [10]. The basic torsional angles that determine the orientation of the earrings and the conformers of the molecule are also divided into three categories φ, θ, and γ. EGCG has been defined as the most effective of the inexperienced tea catechins and is the most important component of inexperienced tea extract, which is commonly found in health food stores as a supplement [11]. As a result, the majority of research into the effects of inexperienced tea has focused on this molecule [12]. Despite the fact that catechins are much less abundant, three different representatives for comparison were chosen [13]. These are distinguished by one-of-a-kind businesses that result in one-of-a-kind capacity interactions with the solvent or organic environment [14]. They are (-)-epicatechin-3-gallate (ECG), which lacks a hydroxyl organization at the pyrogallol B ring; (-)-epigallocatechin-3-O-(3-O-methyl)-gallate (EGCMG), which has a methoxyl organization at the galloyl B' ring rather than a hydroxyl organization; and (-)-epigallocatechin (EGC), which has a [15]. The number and positions of the hydroxyl businesses (or their substituents) at the earrings, which determine their capacity to interact with organic count number via hydrogen bonding, or electron and hydrogen switch strategies within their antioxidant activities, are structural features of green tea catechins that significantly contribute to their organic motion [16]. As a result, the catechins chosen are a significant group to study and compare [17]. Antioxidants, such as polyphenols found in green tea, will neutralize free radicals, reducing or even preventing some of the damage they cause [18]. Polyphenols, chemicals with potent inhibitor properties, are largely responsible for green tea's health benefits [19]. Polyphenols appear to have greater antioxidant properties than vitamin C. Catechins are a type of polyphenol found in teas [20]. Catechin, gallaocatechin, epicatechin, epigallocatechin, ECG, and EGCG are the six primary catechin compounds found in tea leaves [21]. EGCG is the most researched polyphenol in tea leaves [22]. The ability of catechin and epicatechin molecules to scavenge is determined by their atomic number 1 donating ability. Polyphenols have an undeniable inhibitory effect on the production of reactive oxygen species (ROS) as well as the discharge of lysosomal enzymes [23]. Catechins' anti-oxidant effects include scavenging reactive element species, inhibiting the formation of free radicals, and preventing

supermolecule peroxidation [24]. According to market literature, catechins in green tea have inhibitory activity and have a significant impact on the interference of civilization diseases due to the presence of structural teams within the molecules, as well as the variety of hydroxyl radical groups [25]. Lung, esophageal, stomach, intestinal, pancreatic, breast, prostate, and bladder cancers are among the cancers that tea leaf may help to prevent [26]. However, it is worth considering catechins' aerobic potential, for example, when using green tea in the form of dietary supplements, because there's a chance for the formation of incredibly highly reactive metabolites with compound structure [27]. Quinones have the potential to produce large amounts of reactive element species as a result of oxidation-reduction reactions [28].

Role of tea on body operate and forestall some disease

Tea has been shown to increase blood flow while also lowering cholesterol levels. Tea has been shown to help with a variety of heart-related issues, ranging from high vital signs to symptoms of heart failure. What's good for the heart isn't always good for the brain; your brain craves healthy blood vessels [29].

Tea with cancer

Green tea does not appear to help people prevent or treat cancer, according to research [30]. Because of inconsistencies or insufficient evidence, the link between green tea consumption and the risk of certain cancers such as stomach cancer and non-melanoma skin cancers is unclear [31]. According to the National Cancer Institute, the polyphenols in tea have been shown to reduce tumor growth in laboratory and animal studies and will protect against ultraviolet B radiation injury [32]. Cancer rates are lower in countries where tea consumption is high, but it is impossible to know whether it's the green tea or other lifestyle factors that prevent cancer in these specific populations [33]. Tea has also been shown to have beneficial effects on the following types of cancer: breast, bladder, ovarian, body part (bowel), passage (throat), lung, prostate, skin, and abdomen in some studies [34-37]. Tea's high polyphenol content, according to researchers, aids in the killing and stopping of cancerous cells. The precise mechanisms by which tea interacts with cancerous cells, however, are unknown [38]. Tea, on the other hand, has not been shown to reduce the risk of cancer in various studies. In addition, the amount of tea required for cancer prevention varies widely in studies, ranging from 2 to 10 cups per day [39]. “There isn't any credible evidence to support qualified health claims for tea consumption and a reduced risk of gastric, lung, colon/rectal, esophageal, pancreatic, ovarian, and combined cancers,” the Food and Drug Administration (FDA) stated [40].

Green tea with decrease low-density lipoprotein (LDL) cholesterol

In 2012, a review of published research found that drinking green tea, either as a beverage or as a tablet, was linked to significant but modest reductions in total and LDL (or “bad”) cholesterol [41]. Green tea is made from both unfermented and fully fermented leaves of the same plant and can aid in the reduction of LDL cholesterol levels [42]. Researchers agree that catechins, a type of antioxidant found in tea, are responsible for the lowering of LDL cholesterol [43,44]. According to the researcher, the effects of inexperienced tea on LDL cholesterol are due to chemical compounds known as catechins, which reduce the absorption of LDL cholesterol within the gut [45]. Another study of 14 randomized, placebo-controlled studies found that inexperienced tea significantly reduced LDL cholesterol and triglyceride levels [46,47].

Green tea with cardiovascular disorder

An observational study discovered a minor link between daily inexperienced tea consumption and a 5% lower risk of dying from cardiovascular disease [48,49]. A rise in a single

cup of inexperienced tea per day was linked to a marginally lower risk of dying from cardiovascular reasons, according to some research [50]. Green tea consumption has been linked to a reduced risk of stroke [51,52]. Meta-analyses of randomized controlled trials discovered that drinking green tea for three to six months can result in small reductions in systolic and diastolic blood pressures (roughly 2-3 mmHg each) [53,54]. A separate systematic review and meta-analysis of randomized controlled trials discovered that drinking 5-6 cups of green tea per day was associated with a small reduction in systolic blood pressure (2 mmHg), but not with a significant difference in diastolic blood pressure [55]. A study of 40,530 Japanese adults discovered that those who drank more than 5 cups of green tea per afternoon had a 26% lower risk of dying from a coronary heart attack or stroke, and a 16% lower risk of dying from any cause than those who drank less than one cup of green tea per afternoon [56]. A meta-analysis of observational studies found that those who drank the most inexperienced tea had a 28% lower risk of coronary artery disease than those who drank the least inexperienced tea. Thirteen studies were conducted in inexperienced tea drinkers and five in black tea drinkers. Black tea had no effect on the risk of coronary heart disease [57].

Green tea with diabetes mellitus

Most studies on the effects of green tea on people with diabetes have focused on type 2 diabetes, which is far more common, accounting for 90-95% of diabetes seen in the United States [58]. The evidence for a link between inexperienced tea and diabetes has been mixed. Some studies have found a lower risk of developing type 2 diabetes in inexperienced tea drinkers than in non-tea drinkers, while others have found no link between tea consumption and diabetes at all [59]. There are signs that inexperienced tea may reduce the risk of developing diabetes. According to a study published in Japan's Trusted Source, people who drank six or more cups of green tea per day were 33% less likely to develop type 2 diabetes than those who drank only one cup per week [60]. However, tea's benefits do not stop at prevention; in people who have already been diagnosed with diabetes, green tea can help them manage their blood sugar levels [61,62]. According to a comprehensive review by trusted source, inexperienced tea consumption is linked to lower fasting glucose and glycated hemoglobin (HbA1c) levels, as well as lower fasting insulin levels, which is a dimension of diabetes health. While not all studies have confirmed those positive outcomes, inexperienced tea has been shown to be beneficial in a variety of ways [63].

Green tea with inflammatory skin diseases

Tea leaf has the potential to be a replacement treatment for skin disorders such as eczema and dandruff [64]. Researchers looked at an animal model for inflammatory skin diseases, which are marked by patches of dry, red, flaky skin caused by inflammation and skin cell production [65,66]. Green tea treatment resulted in slower skin cell growth as well as the presence of a factor that controls cell life cycles [67,68]. It also shows promise in treating inflammatory skin conditions like dandruff, lupus-induced lesions, and psoriasis, according to a new study. Skin cells multiply out of control in diseases like psoriasis, causing the skin to thicken and flake off. Immune cells in the body are also activated, resulting in inflammation [69].

Tea leaf with liver toxicity

Green tea consumption has not been linked to liver injury or increased levels of liquid body substance transferase; in fact, cross-sectional studies show that regular green tea consumption is linked to lower serum alanine aminotransferase (ALT) and asparate amniotransferase (AST) values. Nonetheless, case studies and a scientific review by the United States Pharmacopeia have raised concerns about the possibility of green tea extract (GTE) causing hepatotoxicity [70]. GTE

was linked to elevations in 6.7% of patients in a large prospective study of biological time girls in danger of breast cancer, compared to 0.7% of controls. Although no clinically apparent liver injury was observed in these studies, the extract was quickly stopped in patients with elevated ALT levels [71]. In some patients, restarting GTE was followed by a rapid recurrence of ALT elevations, which resolved when the GTE was stopped. The incidence of acute liver injury with symptoms or jaundice caused by tea leaf extract is unknown, but it is low when compared to the widespread use of these products [72,73]. Liver injury usually occurs one to six months after starting the product, but longer and shorter latencies (especially with re-exposure) have been reported. Acute hepatitis-like syndrome and a markedly hepatocellular pattern of liquid body substance accelerator elevations are seen in the majority of cases [74]. Although fatal cases of acute liver failure have been reported, most patients recover quickly after stopping the extract or herbal and dietary supplements (HDS). The results of the diagnostic assay reveal necrosis, inflammation, and eosinophils in a pattern that resembles acute hepatitis. Immunoallergic and response options are not always available or are limited [75]. A small number of similar cases have also been reported when tea leaf "infusions" were consumed instead of oral preparations of green tea extracts [76].

Green tea with weight loss

Tea leaves contain a number of beneficial compounds. Caffein is one of the compounds found in tea. Though a cup of green tea contains less caffeine (24-40 mg) than a cup of black tea (100-200 mg), it still has enough to have a light effect [77]. Caffeine is a well-known stimulant that has been shown in studies to aid fat burning and improve exercise performance. Green tea, on the other hand, shines in terms of its inhibitor content. Drinking a cup of green tea increases the amount of antioxidants in your blood, according to studies [78]. Catechins, which are potent inhibitors, are abundant in this healthy beverage. The most important of these is EGCG, a metabolism-boosting substance. Tea has gained widespread attention as a weight-loss product around the world, making it the second most popular beverage after water [79]. Green tea has been linked to a variety of health benefits, including weight loss, due to its organic process and antioxidant content. It's been used in traditional Chinese medicine for centuries to treat a variety of ailments [80]. The process by which your body converts your food and drink into usable energy is known as metabolism. Tea is thought to be beneficial for weight loss because it aids in the body's metabolism becoming more efficient. It's the flavonoid catechin, which acts as an inhibitor and boosts metabolism [81]. According to some research, green tea supplements containing caffeine or catechins have a minor but positive impact on weight loss programs. The most effective weight loss strategy is to use elbow grease frequently and eat a healthy diet rich in fruits and vegetables. Green tea, on the other hand, when used in these ways, will boost the positive outcome [82]. According to a study conducted by the University of Maryland Medical Centre, drinking two to three cups of tea in the future is sufficient for supplementing weight loss. The exact amount varies from person to person depending on their natural metabolism [83]. Except for weight loss, inexperienced teas are available in a variety of types, with little distinction between them. The richest organic process content is found in plain, minimally processed green teas, which are thought to be the best for weight loss and other health benefits. Another advantageous feature of green tea is that it contains almost no calories in comparison to the amount of nutrients it contains [84].

Effects on drug-metabolizing enzymes

long-term consumption of green tea enhances uridine di-phospatase (UDP)-glucuronosyl transferase activity in rats, and catechins are processed by drug-metabolizing enzymes in

several organs following absorption [85-89]. Accordingly, it is hypothesized that the enhanced glucuronidation caused by UDP-glucuronosyl transferase activation helps green tea's anticarcinogenic impact by promoting the conversion of chemical carcinogens into inert byproducts that are easily eliminated. The relationship between green tea catechin metabolism and 2-amino-3-methylimidazol (4,5-f) quinoline (IQ) was investigated [90]. IQ is a precarcinogen that was first discovered in a fried beef extract. Cytochrome P450 is the primary pathway for rat IQ biotransformation, which is then coupled to a sulfate and a glucuronide conjugate. Rats with altered IQ metabolism produce more IQ glucuronides, which are then eliminated in the urine. Green tea catechins may also protect against malignancies brought on by polycyclic aromatic hydrocarbons by inhibiting their cytochrome P450 metabolism, however this depends on the specific form of green tea consumed. In normal rats, long-term green tea drinking enhances cytochrome P450 1A1 and 1A2 activities but not 2B1 and 2E1 activities. Conclusions regarding a protective effect of green tea against carcinogens involving solely this metabolic pathway's modification, however, are challenging to establish [91].

Conclusion

Tea leaf is thought to improve blood flow and lower cholesterol levels. Several studies found that inexperienced tea prevented a variety of heart-related issues, ranging from sickness to symptom. The effects of green tea on disease have been mixed in studies. More and more emphasis is being placed to define events at the cellular level. Much interest has been centered on the role of oxidant/antioxidant activity in regards to the aging process and degenerative diseases like cancer, cardiovascular disease and diabetes. There are some signs that green tea may aid in the elimination of cancer cells, but this research is still in its early stages. On the other hand, the National Cancer Institute's website states that it "doesn't recommend" the use of tea to reduce the risk of cancer. Future study is required to determine the precise quantity of health benefits, establish the safe range of tea consumption associated with these advantages, and clarify the mechanisms of action because the human clinical data is currently limited. A deeper knowledge of how green tea interacts with endogenous systems and other external elements will be possible with the development of more precise, sensitive, and representative approaches with more representative models. Only carefully planned observational epidemiological research and intervention trials will be able to draw definitive findings about the preventive impact of green tea. Future research in this field will be made easier by the creation of biomarkers for green tea consumption and molecular markers for its biological effects.

References

1. Xu J, Wang M, Zhao J, et al. Yellow tea (*Camellia sinensis L.*), a promising Chinese tea: Processing, chemical constituents and health benefits. Food Res Int. 2018; 107: 567-77. doi: 10.1016/j.foodres.2018.01.063.
2. Kim YK, Jombart L, Valentin D, et al. A cross-cultural study using Napping®: Do Korean and French consumers perceive various green tea products differently? Food Res Int. 2013; 53(1): 534-42. doi: https://doi.org/10.1016/j.foodres.2013.05.015.
3. Cabrera C, Artacho R, Giménez R. Beneficial effects of green tea - A review. J Am Coll Nutr. 2006; 25(2): 79-99. doi: 10.1080/07315724.2006.10719518.
4. Senanayake Namal SPJ. Green Tea Extract: Chemistry, Antioxidant Properties and Food Applications - A Review. J Funct Foods. 2013; 5(4): 1529-41. doi: https://doi.org/10.1016/j.jff.2013.08.011.
5. Komes D, Horžić D, Belščak A, et al. Green tea preparation and its influence on the content of bioactive compounds. Food Res Int. 2010: 43(1): 167-76. doi: https://doi.org/10.1016/j.foodres.2009.09.022.
6. Malinowska E, Inkielewicz I, Czarnowski W, et al. Assessment of fluoride concentration and daily intake by human from tea and herbal infusions. Food Chem Toxicol. 2008; 46(3): 1055-61. doi: 10.1016/j.fct.2007.10.039.
7. Jakubczyk K, Kochman J, Kwiatkowska A, et al. Antioxidant properties and nutritional composition of matcha green tea. Foods. 2020; 9(4): 483. doi: 10.3390/foods9040483.

8. Chou CC, Lin LL, Chung KT. Antimicrobial activity of tea as affected by the degree of fermentation and manufacturing season. Int J Food Microbiol. 1999; 48(2): 125-30. doi: 10.1016/s0168-1605(99)00034-3.

9. Ishii T, Mori T, Tanaka T, et al. Covalent modification of proteins by green tea polyphenol (-)-epigallocatechin-3-gallate through autoxidation. Free Radic Biol Med. 2008; 45(10): 1384-94. doi: 10.1016/j.freeradbiomed.2008.07.023.

10. Almatroodi SA, Almatroudi A, Khan AA, et al. Potential therapeutic targets of Epigallocatechin Gallate (EGCG), the most abundant catechin in green tea, and its role in the therapy of various types of cancer. Molecules. 2020; 25(14): 3146. doi: 10.3390/molecules25143146. doi: 10.17795/jjnpp-18406.

11. Esmaeelpanah E, Rahmatkhah A, Poormahmood N, et al. Protective effect of green tea aqueous extract on acrylamide induced neurotoxicity. Jundishapur J Nat Pharm Prod. 2015; 10(2): e18406. doi: doi: 10.17795/jjnpp-18406.

12. Nagle DG, Ferreira D, Zhou YD. Epigallocatechin-3-gallate (EGCG): chemical and biomedical perspectives. Phytochemistry. 2006; 67(17): 1849-55. doi: 10.1016/j.phytochem.2006.06.020.

13. Steinmann J, Buer J, Pietschmann T, et al. Anti-infective properties of epigallocatechin-3-gallate (EGCG), a component of green tea. Br J Pharmacol. 2013; 168(5): 1059-73. doi: 10.1111/bph.12009.

14. Sae-tan S, Grove KA, Lambert JD. Weight control and prevention of metabolic syndrome by green tea. Pharmacol Res. 2011; 64(2): 146-54. doi: 10.1016/j.phrs.2010.12.013.

15. Chu C, Deng J, Man Y, et al. Green Tea extracts epigallocatechin-3-gallate for different treatments. Biomed Res Int. 2017; 2017: 5615647. doi: 10.1155/2017/5615647.

16. Song JM, Lee KH, Seong BL. Antiviral effect of catechins in green tea on influenza virus. Antiviral Res. 2005; 68(2): 66-74. doi: 10.1016/j.antiviral.2005.06.010.

17. Botten D, Fugallo G, Fraternali F, et al. Structural Properties of Green Tea Catechins. J Phys Chem B. 2015; 119(40): 12860-7. doi: 10.1021/acs.jpcb.5b08737.

18. Suzuki-Sugihara N, Kishimoto Y, Saita E, et al. Green tea catechins prevent low-density lipoprotein oxidation via their accumulation in low-density lipoprotein particles in humans. Nutr Res. 2016; 36(1): 16-23. doi: 10.1016/j.nutres.2015.10.012.

19. Thasleema SA. Green tea as an antioxidant - A short review. J Pharm Sci Res. 2013; 5(9): 171-3.

20. van Driem GL. The tale of tea. A Comprehensive history of tea from prehistoric times to the present day. Brill; 2019.

21. Pham-Huy LA, He H, Pham-Huy C. Free radicals, antioxidants in disease and health. Int J Biomed Sci. 2008; 4(2): 89-96.

22. Devasagayam TP, Tilak JC, Boloor KK, et al. Free radicals and antioxidants in human health: current status and future prospects. J Assoc Physicians India. 2004; 52: 794-804.

23. Nugala B, Namasi A, Emmadi P, et al. Role of green tea as an antioxidant in periodontal disease: The Asian paradox. J Indian Soc Periodontol. 2012; 16(3): 313-6. doi: 10.4103/0972-124X.100902.

24. Li XX, Liu C, Dong SL, et al. Anticarcinogenic potentials of tea catechins. Front Nutr. 2022; 9: 1060783. doi: 10.3389/fnut.2022.1060783.

25. Naponelli V, Ramazzina I, Lenzi C, et al. Green tea catechins for prostate cancer prevention: Present achievements and future challenges. Antioxidants (Basel). 2017; 6(2): 26. doi: 10.3390/antiox6020026.

26. Grzesik M, Naparło K, Bartosz G, et al. Antioxidant properties of catechins: Comparison with other antioxidants. Food Chem. 2018; 241: 480-92. doi: 10.1016/j.foodchem.2017.08.117.

27. Vuong QV, Stathopoulos CE, Golding JB, et al. Optimum conditions for the water extraction of L-theanine from green tea. J Sep Sci. 2011; 34(18): 2468-74. doi: 10.1002/jssc.201100401.

28. Chung MY, Lazaro RA, Lim D, et al. Aerosol-borne quinones and reactive oxygen species generation by particulate matter extracts. Environ Sci Technol. 2006; 40(16): 4880-6. doi: 10.1021/es0515957.

29. Maron DJ, Lu GP, Cai NS, et al. Cholesterol-lowering effect of a theaflavin-enriched green tea extract: A randomized controlled trial. Arch Intern Med. 2003; 163(12): 1448-53. doi: 10.1001/archinte.163.12.1448.

30. Bushman JL. Green tea and cancer in humans: a review of the literature. Nutr Cancer. 1998; 31(3): 151-9. doi: 10.1080/01635589809514697.

31. Filippini T, Malavolti M, Borrelli F, et a. Green tea (*Camellia sinensis*) for the prevention of cancer. Cochrane Database Syst Rev. 2020; 3(3): CD005004. doi: 10.1002/14651858.CD005004.pub3.

32. Elmets CA, Singh D, Tubesing K, et al. Cutaneous photoprotection from ultraviolet injury by green tea polyphenols. J Am Acad Dermatol. 2001; 44(3): 425-32. doi: 10.1067/mjd.2001.112919.

33. Wu D, Guo Z, Ren Z, et al. Green tea EGCG suppresses T cell proliferation through impairment of IL-2/IL-2 receptor signaling. Free Radic Biol Med. 2009; 47(5): 636-43. doi: 10.1016/j.freeradbiomed.2009.06.001.

34. Amin ARMR, Wang D, Nannapaneni S, et al. Combination of resveratrol and green tea epigallocatechin gallate induces synergistic apoptosis and inhibits tumor growth in vivo in head and neck cancer models. Oncol Rep. 2021; 45(5): 87. doi: 10.3892/or.2021.8038.

35. Wang Y, Wang M, Wu HX, et al. Advancing to the era of cancer immunotherapy. Cancer Commun (Lond). 2021; 41(9): 803-29. doi: 10.1002/cac2.12178.

36. Singh N, Bhalla M, de Jager P, et al. An overview on ashwagandha: A Rasayana (rejuvenator) of Ayurveda. Afr J Tradit Complement Altern Med. 2011; 8(5 Suppl): 208-13. doi: 10.4314/ajtcam.v8i5S.9.

37. Hayakawa S, Ohishi T, Miyoshi N, et al. Anti-cancer effects of green tea epigallocatchin-3-gallate and

coffee chlorogenic acid. Molecules. 2020; 25(19): 4553. doi: 10.3390/molecules25194553.

38. Shirode AB, Kovvuru P, Chittur SV, et al. Antiproliferative effects of pomegranate extract in MCF-7 breast cancer cells are associated with reduced DNA repair gene expression and induction of double strand breaks. Mol Carcinog. 2014; 53(6): 458-70. doi: 10.1002/mc.21995.

39. Lambert JD, Elias RJ. The antioxidant and pro-oxidant activities of green tea polyphenols: a role in cancer prevention. Arch Biochem Biophys. 2010; 501(1): 65-72. doi: 10.1016/j.abb.2010.06.013.

40. Aguilera Y, Martin-Cabrejas MA, de Mejia EG. Phenolic compounds in fruits and beverages consumed as part of the mediterranean diet: their role in prevention of chronic diseases. Phytochem Rev. 2016; 15(3): 405-23.

41. Cooper R. Green tea and theanine: health benefits. Int J Food Sci Nutr. 2012; 63 Suppl 1: 90-7. doi: 10.3109/09637486.2011.629180.

42. Sharangi AB. Medicinal and therapeutic potentialities of tea (Camellia sinensis L.) - A review. Food Res Int. 2009; 42: 529-35. doi: https://doi.org/10.1016/j.foodres.2009.01.007.

43. Gramza A, Korczak J, Amarowicz R. Tea polyphenols - their antioxidant properties and biological activity. Polish J food Nutrition Sci. 2005; 14(3): 219-35.

44. Moore RJ, Jackson KG, Minihane AM. Green tea (Camellia sinensis) catechins and vascular function. Br J Nutr. 2009; 102(12): 1790-802. doi: 10.1017/S0007114509991218.

45. Ikeda I, Kobayashi M, Hamada T, et al. Heat-epimerized tea catechins rich in gallocatechin gallate and catechin gallate are more effective to inhibit cholesterol absorption than tea catechins rich in epigallocatechin gallate and epicatechin gallate. J Agric Food Chem. 2003; 51(25): 7303-7. doi: 10.1021/jf034728l.

46. Huang LH, Liu CY, Wang LY, et al. Effects of green tea extract on overweight and obese women with high levels of low density-lipoprotein-cholesterol (LDL-C): a randomised, double-blind, and cross-over placebo-controlled clinical trial. BMC Complement Altern Med. 2018; 18(1): 294. doi: 10.1186/s12906-018-2355-x.

47. Xu R, Yang K, Li S, et al. Effect of green tea consumption on blood lipids: a systematic review and meta-analysis of randomized controlled trials. Nutr J. 2020; 19(1): 48. doi: 10.1186/s12937-020-00557-5.

48. Eilat-Adar S, Sinai T, Yosefy C, et al. Nutritional recommendations for cardiovascular disease prevention. Nutrients. 2013; 5(9): 3646-83. doi: 10.3390/nu5093646.

49. Yi M, Wu X, Zhuang W, et al. Tea consumption and health outcomes: Umbrella review of meta-analyses of observational studies in humans. Mol Nutr Food Res. 2019; 63(16): e1900389. doi: 10.1002/mnfr.201900389.

50. Wolfram S. Effects of green tea and EGCG on cardiovascular and metabolic health. J Am Coll Nutr. 2007; 26(4): 373S-88S. doi: 10.1080/07315724.2007.10719626.

51. Pang J, Zhang Z, Zheng T, et al. Association of green tea consumption with risk of coronary heart disease in Chinese population. Int J Cardiol. 2015; 179: 275-8. doi: 10.1016/j.ijcard.2014.11.093.

52. Kafeshani M, Entezari MH, Karimian J, et al. A comparative study of the effect of green tea and sour tea on blood pressure and lipid profile in healthy adult men. ARYA Atheroscler. 2017; 13(3): 109-116.

53. Liu G, Mi XN, Zheng XX, et al. Effects of tea intake on blood pressure: a meta-analysis of randomized controlled trials. Br J Nutr. 2014; 112(7): 1043-54. doi: 10.1017/S0007114514001731.

54. Peng X, Zhou R, Wang B, et al. Effect of green tea consumption on blood pressure: a meta-analysis of 13 randomized controlled trials. Sci Rep. 2014; 4: 6251. doi: 10.1038/srep06251.

55. Mesas AE, Leon-Muñoz LM, Rodriguez-Artalejo F, et al. The effect of coffee on blood pressure and cardiovascular disease in hypertensive individuals: a systematic review and meta-analysis. Am J Clin Nutr. 2011; 94(4): 1113-26. doi: 10.3945/ajcn.111.016667.

56. Mirtavoos-Mahyari H, Salehipour P, Parohan M, et al. Effects of coffee, black tea and green tea consumption on the risk of non-hodgkin's lymphoma: A Systematic Review and dose-response meta-analysis of observational studies. Nutr Cancer. 2019; 71(6): 887-97. doi: 10.1080/01635581.2019.1595055.

57. Zheng XX, Xu YL, Li SH, et al. Green tea intake lowers fasting serum total and LDL cholesterol in adults: a meta-analysis of 14 randomized controlled trials. Am J Clin Nutr. 2011; 94(2): 601-10. doi: 10.3945/ajcn.110.010926.

58. Neuenschwander M, Ballon A, Weber KS, et al. Role of diet in type 2 diabetes incidence: umbrella review of meta-analyses of prospective observational studies. BMJ. 2019; 366: l2368. doi: 10.1136/bmj.l2368.

59. Iso H, Date C, Wakai K, et al. The relationship between green tea and total caffeine intake and risk for self-reported type 2 diabetes among Japanese adults. Ann Intern Med. 2006; 144(8): 554-62. doi: 10.7326/0003-4819-144-8-200604180-00005.

60. Tsubono Y, Nishino Y, Komatsu S, et al. Green tea and the risk of gastric cancer in Japan. N Engl J Med. 2001; 344(9): 632-6. doi: 10.1056/NEJM200103013440903.

61. Imai K, Suga K, Nakachi K. Cancer-preventive effects of drinking green tea among a Japanese population. Prev Med. 1997; 26(6): 769-75. doi: 10.1006/pmed.1997.0242.

62. Yu J, Song P, Perry R, et al. The Effectiveness of green tea or green tea extract on insulin resistance and glycemic control in type 2 diabetes mellitus: A meta-analysis. Diabetes Metab J. 2017; 41(4): 251-62. doi: 10.4093/dmj.2017.41.4.251.

63. van Dam RM, Pasman WJ, Verhoef P. Effects of coffee consumption on fasting blood glucose and insulin concentrations: randomized controlled trials

in healthy volunteers. Diabetes Care. 2004; 27(12): 2990-2. doi: 10.2337/diacare.27.12.2990.

64. Sandeep K, Nisha S. Green tea polyphenols: versatile cosmetic ingredient. Int J Adv Res Pharmaceut Bio Sci. 2012; 1(4): 348-63.

65. Duke JA, Castleman M, Feinstein A. The green pharmacy: New discoveries in herbal remedies for common diseases and conditions from the world's foremost authority on healing herbs. A Prevention, Magazine Health Book; 1997.

66. Shenefelt PD, Norman RA. Dermatologic conditions. In: Spar MD, Munoz GE (eds). Integrative men's health. New York: Oxford Academic; 2014. p. 333-61.

67. Gaeddert A. Healing skin disorders: Natural treatments for dermatological conditions. North Atlantic Books; 2003.

68. Kim H, Park SY, Lee G. Potential therapeutic applications of bee venom on skin disease and its mechanisms: A literature review. Toxins (Basel). 2019; 11(7): 374. doi: 10.3390/toxins11070374.

69. Turkington C, Dover JS. The encyclopedia of skin and skin disorders. 3rd ed. Infobase Publishing; 2009.

70. Hodges JK, Sasaki GY, Bruno RS. Anti-inflammatory activities of green tea catechins along the gut-liver axis in nonalcoholic fatty liver disease: lessons learned from preclinical and human studies. J Nutr Biochem. 2020; 85: 108478. doi: 10.1016/j.jnutbio.2020.108478.

71. Cadden IS, Partovi N, Yoshida EM. Review article: possible beneficial effects of coffee on liver disease and function. Aliment Pharmacol Ther. 2007; 26(1): 1-8. doi: 10.1111/j.1365-2036.2007.03319.x.

72. Kowdley KV, Belt P, Wilson LA, et al. Serum ferritin is an independent predictor of histologic severity and advanced fibrosis in patients with nonalcoholic fatty liver disease. Hepatology. 2012; 55(1): 77-85. doi: 10.1002/hep.24706.

73. Mahmoodi M, Hosseini R, Kazemi A, et al. Effects of green tea or green tea catechin on liver enzymes in healthy individuals and people with nonalcoholic fatty liver disease: A systematic review and meta-analysis of randomized clinical trials. Phytother Res. 2020; 34(7): 1587-98. doi: 10.1002/ptr.6637.

74. Arundel C, Lewis JH. Drug-induced liver disease in 2006. Curr Opin Gastroenterol. 2007; 23(3): 244-54. doi: 10.1097/MOG.0b013e3280b17dfb.

75. Xu Y, Zhang M, Wu T, et al. The anti-obesity effect of green tea polysaccharides, polyphenols and caffeine in rats fed with a high-fat diet. Food Funct. 2015; 6(1): 297-304. doi: 10.1039/c4fo00970c.

76. Hsu YW, Tsai CF, Chang WH, et al. Protective effects of Dunaliella salina--a carotenoids-rich alga, against carbon tetrachloride-induced hepatotoxicity in mice. Food Chem Toxicol. 2008; 46(10): 3311-7. doi: 10.1016/j.fct.2008.07.027.

77. Heckman MA, Weil J, Gonzalez de Mejia E. Caffeine (1, 3, 7-trimethylxanthine) in foods: a comprehensive review on consumption, functionality, safety, and regulatory matters. J Food Sci. 2010; 75(3): R77-87. doi: 10.1111/j.1750-3841.2010.01561.x.

78. Musial C, Kuban-Jankowska A, Gorska-Ponikowska M. Beneficial properties of green tea catechins. Int J Mol Sci. 2020; 21(5): 1744. doi: 10.3390/ijms21051744.

79. Siddiqui IA, Adhami VM, Afaq F, et al. Modulation of phosphatidylinositol-3-kinase/protein kinase B- and mitogen-activated protein kinase-pathways by tea polyphenols in human prostate cancer cells. J Cell Biochem. 2004; 91(2): 232-42. doi: 10.1002/jcb.

80. Monteiro JP, Alves MG, Oliveira PF, et al. Structure-Bioactivity relationships of methylxanthines: Trying to make sense of all the promises and the drawbacks. Molecules. 2016; 21(8): 974. doi: 10.3390/molecules21080974.

81. Heckman MA, Sherry K, De Mejia EG. Energy drinks: An assessment of their market size, consumer demographics, ingredient profile, functionality, and regulations in the United States. Compr Rev Food Sci Food Saf. 2010; 9(3): 303-17. doi: 10.1111/j.1541-4337.2010.00111.x.

82. Rains TM, Agarwal S, Maki KC. Antiobesity effects of green tea catechins: a mechanistic review. J Nutr Biochem. 2011; 22(1): 1-7. doi: 10.1016/j.jnutbio.2010.06.006. Epub 2010 Nov 5. PMID: 21115335.

83. Diepvens K, Westerterp KR, Westerterp-Plantenga MS. Obesity and thermogenesis related to the consumption of caffeine, ephedrine, capsaicin, and green tea. Am J Physiol Regul Integr Comp Physiol. 2007; 292(1): R77-85. doi: 10.1152/ajpregu.00832.2005.

84. Wang H, Wen Y, Du Y, et al. Effects of catechin enriched green tea on body composition. Obesity (Silver Spring). 2010; 18(4): 773-9. doi: 10.1038/oby.2009.256.

85. Nelson M, Poulter J. Impact of tea drinking on iron status in the UK: a review. J Hum Nutr Diet. 2004; 17(1): 43-54. doi: 10.1046/j.1365-277x.2003.00497.x.

86. Maliakal PP, Coville PF, Wanwimolruk S. Tea consumption modulates hepatic drug metabolizing enzymes in Wistar rats. J Pharm Pharmacol. 2001; 53(4): 569-77. doi: 10.1211/0022357011775695.

87. Sohn OS, Surace A, Fiala ES, et al. Effects of green and black tea on hepatic xenobiotic metabolizing systems in the male F344 rat. Xenobiotica. 1994; 24(2): 119-27. doi: 10.3109/00498259409043226.

88. Donovan JL, Crespy V, Manach C, et al. Catechin is metabolized by both the small intestine and liver of rats. J Nutr. 2001; 131(6): 1753-7. doi: 10.1093/jn/131.6.1753.

89. Okushio K, Suzuki M, Matsumoto N, et al. Methylation of tea catechins by rat liver homogenates. Biosci Biotechnol Biochem. 1999; 63(2): 430-2. doi: 10.1271/bbb.63.430.

90. Embola CW, Weisburger JH, Weisburger MC. Urinary excretion of N-OH-2-amino-3-methylimidazo[4,5-f]quinoline-N-glucuronide in F344 rats is enhanced by green tea. Carcinogenesis. 2001; 22(7): 1095-8. doi: 10.1093/carcin/22.7.1095.

91. Chacko SM, Thambi PT, Kuttan R, et al. Beneficial effects of green tea: a literature review. Chin Med. 2010; 5: 13. doi: 10.1186/1749-8546-5-13.

Correspondence to Dr. Sawsan J. Al-Harbi
E-mail: drsawsamjasiem@gmail.com

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Barriers to Blood Glucose Control in Type 2 Diabetic Patients with Poor Glycemic Control in Kirkuk City/Iraq

Zina A. Rashid[1] *FIBMS CABMS/FM*, **Aseel A. Naji**[2] *FICMS/CM*, **Zahraa H. Ismaeel**[2] *FICMS/FM*

[1]Fellowship of Clinical Nutrition, Kirkuk Health Dept, Kirkuk, Iraq, [2]Fellowship of Clinical Nutrition, Ghazi Al-Harriri Specialized Hospital, Medical City Directorate, Baghdad, Iraq

Abstract

Background Despite the presence of glycemic control guidelines, and the global trends in the delivery of patient care, especially on the primary health care level, many diabetics do not achieve the needed glycemic, blood pressure and cholesterol levels. Better glycemic control is critical in allowing patients to perform at their best in terms of diabetes management and preventing long-term complications.

Objective To identify barriers that may contribute to poor glycemic control in a sample of Iraqi diabetic patients in Kirkuk city, Iraq.

Methods A cross-sectional study conducted during 2019 in outpatient's diabetic clinic in Azadi Teaching Hospital in Kirkuk, Iraq on diabetic patients with poor glycemic control.

Results The study included 195 diabetic patients with mean age of 55.54±9.260 years; while their mean HbA1c was 9.74±1.696 mg/dl. Duration of diabetes was 6 to 10 years among 86 (44.1%) patients, 154 (74.4%) patients used oral antidiabetic drug, 5 (2.6%) used insulin treatment. Regarding barriers, 126 (65.3%) lack of confidence in using insulin regimens, lack of necessary knowledge about insulin therapy 117 (60.6%), difficulty to perform exercise 125 (64.4%) and influence of complications or other chronic disease 100 (51.8%).

Conclusion Main barriers against good glycemic control reported by type 2 patients in this study were mainly poor knowledge regarding treatment, side effects of the disease itself or the medication and some social factors like unemployment and poor access to health care.

Keywords Glycemic control, barriers, diabetes complication

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List of abbreviations: BMI = Body mass index, DM = Diabetes mellitus, HbA1c = Hemoglobin A1c, NCDs = Non communicable diseases

Introduction

Diabetes mellitus (DM) type 2 is an increasing health problem worldwide and specially in Middle East region. DM is becoming more common in Middle Eastern countries, as Iraq [(1)]. Prevalence in Iraq ranges from 8.5% to 13.9%, according to reports. Iraq has set targets for preventing and controlling non-communicable diseases (NCDs) such as DM, hypertension, and breast cancer [(2)]. However, safety confrontation and governmental insecurity have made these objectives difficult to achieve. Physical inactivity, bad food, body over weight, lack of health awareness, health beliefs, stance and life style are central auxiliary factors for type 2 DM, which is prevalent in Middle Eastern countries. Diabetes care need a high focus in

order to reduce morbidity and costs, as diabetic patients use greater resources in the ambulatory and in-patient settings than non-diabetic patients [3].

Improved glycemic control is crucial for individuals to reach their full potential in managing their diabetes and avoiding complications. Glycemic management, hemoglobin A1c (HbA1c), concentration of less than 7.0%) is challenging to accomplish but essential for delaying or preventing diabetes-related consequences [4]. It is critical to identify the barriers to better glycemic control so that patients can do their best to improve diabetes control and reduce long-term complications. Health, wealth, availability of care, prior care experiences, and individual circumstances are just few of the variables that might affect how well an individual is able to self-manage their condition. A comprehensive understanding of patient, provider, and system-level barriers are needed to inform the development of interventions to support self-management and improve outcomes for diabetes patients [5].

In Iraq, primary care facilities and knowledge are weak, and healthcare supply is mainly reliant on secondary and tertiary care. Primary care schedule for soon diagnosis of hypertension, DM, and breast cancer were designed, but they were not successful. At the same time, patients with diabetes are burdened by complex treatment regimens including administration of medications, clinical monitoring, and dietary and lifestyle changes. Patients must also make decisions about when and how to seek medical care [6].

Many diabetics still do not achieve their target glycemic, blood pressure, and cholesterol levels, despite the existence of glycemic control guidelines and global trends in the delivery of patient care, particularly at the primary health care level [7]. It had been proved that a positive attitude, self-management, and motivation are as essential as diet and exercise therapy, which are the basis of DM type 2 therapy. Patient education was reported as useful in achieving good glycemic control and in reducing the incidence and risk of complications. When there is a barrier to optimum glycemic control, even proper medical therapy delivered by a specialist physician will not be enough and probably will not be carried out by the patient [8,9]. Therefore, this study aimed to identify barriers that may contribute to poor glycemic control in a sample of Iraqi diabetic patients.

Methods

Study design, duration and setting

A cross-sectional study was conducted from January to July 2019 in Outpatients Diabetic Clinic in Azadi Teaching Hospital in Kirkuk, Iraq. Kirkuk city is located 238 kilometres north of Baghdad, with current population in 2022 is 1,052,000, a 2.04% increase from 2021. The city involves a wide sociodemographic diversity as it is a home to a diverse population of Turkmens, Arabs, Kurds, and Assyrians [3].

Sampling

A convenient sample of 195 type 2 DM patients who had been diagnosed by a specialist clinician/ endocrinologist in the same hospital after full investigation. Comorbidities was relying on self-reporting by patients.

Inclusion criteria

Patients who were more than 35 years old, whose period of diagnosis was at least 6 months, also, patients who were taking medication but whose compliance with lifestyle adjustments was uncertain were also considered.

Exclusion criteria

Patients on steroid therapy, pregnant women, gestational DM, pediatrics, and in patients were excluded.

Data collection tool

A detailed questionnaire obtained from qualified published article [10] was distributed to the patients and filled by the researcher, it contained two parts:

First part: sociodemographic data of the patients including age, gender, smoking status

and comorbidities in addition to current HbA1c level and body mass index (BMI).

Second part: contained detailed questions regarding barriers faced by the patients regarding good glycemic control. The answers of the barrier's questions were arranged in 5 points Likert scale (extremely agree, agree, uncertain, disagree, and extremely disagree).

Definition of variables

Body mass index

BMI was calculated by dividing the dry weight over the squared height in meters, and was classified according to the World Health Organization categorization for patients as follows: underweight (<18.5 kg/m^2), underweight to normal (≥18.5-<22.5 kg/m^2), normal (>22.5-<25 kg/m^2), overweight (≥25-<30 kg/m^2), and obese (≥30 kg/m^2) [11].

Access to care

The utilization of personal health services at the appropriate time to get the greatest possible outcomes for one's health, as well as the patient's capacity to see a qualified health practitioner within a reasonable amount of time [12].

Barriers to glycemic control

Any factor that makes progress difficult in the process of blood glucose control [9].

Current smokers

Adults who reported they have smoked at least 100 cigarettes in their entire life and that they now smoke some days or every day [11].

Ex-smokers

Individual who has quit cigarette and/or tobacco smoking for at least 6 months [11].

Ethical considerations

The hospital administration was provided with the information, and approval from the Ethics Review Committee of the Kirkuk Health Directorate was acquired. Participants were informed orally and verbal agreement was sought for the research. Each participant's anonymity and confidentiality were ensured, and they gave their informed consent to take part in the study.

Statistics

Analysis of data with SPSS (Statistical Package for the Social Sciences) (SPSS 20). Statistics (frequency, percentage, mean, and standard deviation) used to describe the subjects and their demographics. To determine if there is a statistically significant correlation or dissimilarity between the variables, analytic statistics, Chi square test (X2), was used. A p-value of less than 0.05 indicates statistical significance.

Barrier scoring

Two points was given for the strong agree and agree answer, 1 point for uncertain answers, and zero for strongly disagree and disagree answers. Total score of 50% or less was considered as poor. Total score of 51-74% was considered fair. Total score of equal to or more than 75% was considered as good.

Results

The participants in this study consisted of 195 people with diabetes who had inadequate glycemic control. Participants' ages ranged from 30-76, with a mean of 55.54±9.26 years; their mean HbA1c was 9.74±1.7 mg/dl; and 52 (or 26.7%) were male (Table 1). Out of the total; there were 130 (66.7%) patients were within age group (50-59) years, 143 (73.3%) females, 146 (74.9%) patients' lives in urban area, and 145 (74.4%) married patients. Unemployed patients represented 63.6% (124) of the total, 60 (30.8%) patients were illiterate, 49 (25.1%) with primary education. Current smoker represented 31.3% (61) of the total. Patients who smoking >20-40 Cigarette/day were 44 (62.9%). Irregular access to the care units was found among 147 (75.4%) patients.

According to the disease onset; 92 (47.2%) patients have DM at 40-49 years old, duration of DM was 6 to 10 years among 86 (44.1%) patients. Family history of DM was positive among 154 (79.0%) patients, 145 (74.4%) patients used oral antidiabetic drug, 5 (2.6%) used insulin treatment, while 41 (21.0%) used combined treatment. Diet and life style

modification was used by only 4 (2.1%) patients. Only 51 (26.3%) patients have self-monitoring of blood glucose as shown in figure 1.

Table 1. Baseline features of participants

Features		N	%
Age groups/ years	30-49	51	26.2
	50-69	130	66.7
	≥70	14	7.2
Sex	Male	52	26.7
	Female	143	73.3
Residency	Urban	146	74.9
	Rural	49	25.1
Marital status	Single	7	3.6
	Married	145	74.4
	Divorced	10	5.1
	Widow	33	16.9
Occupation	Employed	71	36.4
	Unemployed	124	63.6
Education	Illiterate	60	30.8
	Primary	49	25.1
	Intermediate	39	20.0
	Secondary	28	14.4
	Higher education	19	9.7
Cigarette smoking	Ex-smoker	9	4.6
	Current smoker	61	31.3
	None	125	64.1
Duration of smoking/ years n=70	≤10 years	32	45.7
	>10 years	38	54.3
No. of Cigarette smoking/day n=70	≤20	18	25.7
	>20-40	44	62.9
	>40-60	8	11.4
Access to care	Regular	48	24.6
	Irregular	147	75.4
BMI	Normal	69	35.4
	Overweight	65	33.3
	Obese	61	31.3
Total		195	100.0

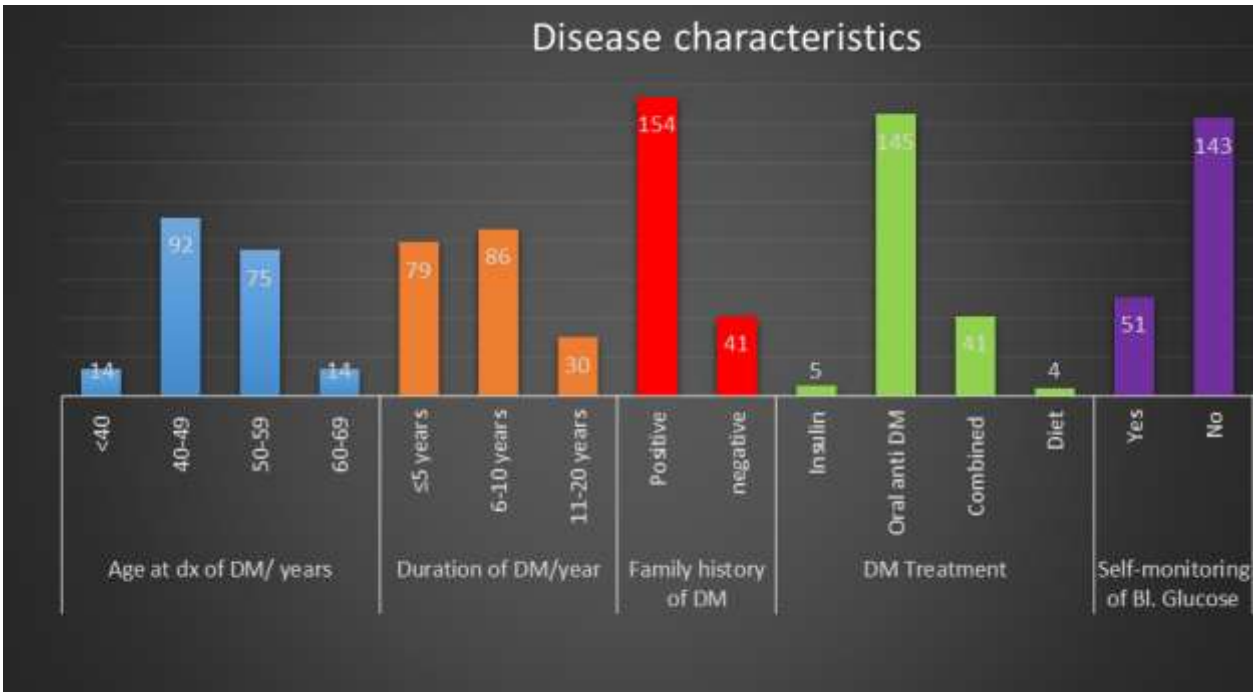

Figure 1. Disease characteristics and treatment

Past medical history (PMH) was represented by Figure 2. There were 32 (16.4%) patients without comorbidity, while there were 163 (83.6%) patients have other comorbidities (Figure 2).

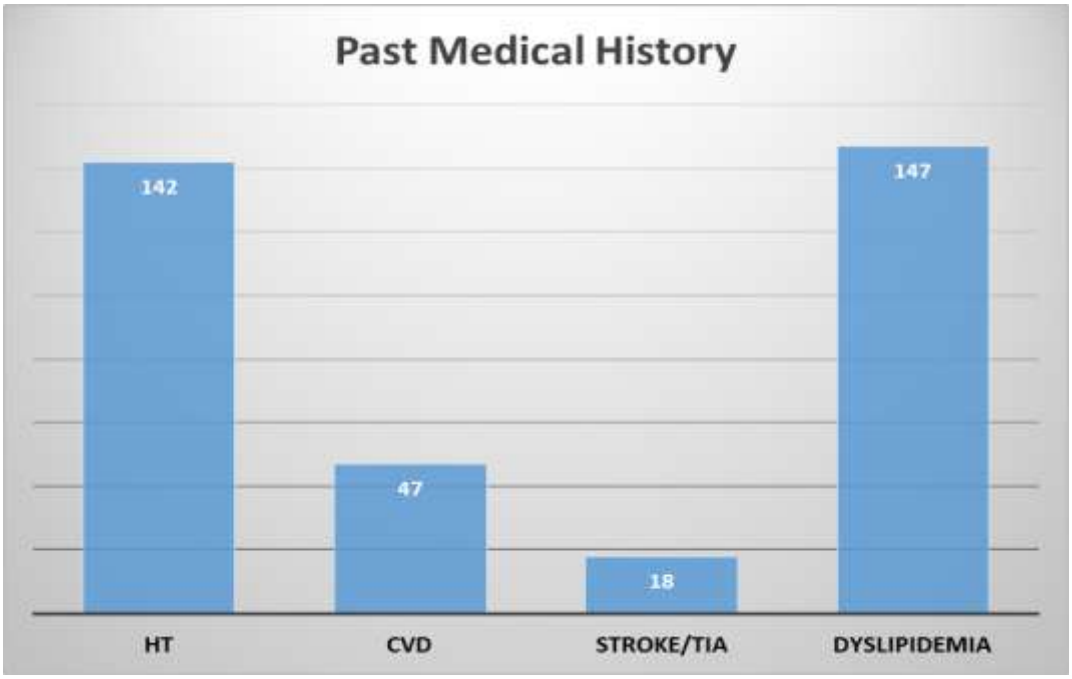

Figure 2. Disease characteristics and treatment

Table (2) demonstrated the responses of participants regarding the obstacles to glycemic management. About two third of participants were agreed that the [Difficulty to control because of lack of confidence in using insulin regimens and how to intensify therapy for patients not reaching glycemic control, 126 (65.3%); and difficulty to control because of lack of necessary knowledge about insulin therapy (storage, injection sites and titration) 117 (60.6%)]; and strongly agreed that (difficulty to perform exercise therapy, 125 (64.4%)). About half of participants stated that the cause behind difficult glycemic control were [difficulty to control due to the influence of complications or other chronic disease 100 (51.8%); difficulty to undergo examination regularly 109 (55.9%), difficulty to control due to polypharmic (≥4 drugs) 96 (49.2%), difficulty to control because of side effects of medication 102 (52.3%), difficulty to control due to psychosocial factors related to the patient 102 (52.3%).

Table 2. Participants' respondents about barriers against glycemic control

Barriers		Strongly disagree	Disagree	Uncertain	Agree	Strongly agree
Difficulty to perform diet therapy	N	2	21	5	72	95
	%	1.0	10.8	2.6	36.9	48.7
Difficulty to perform exercise therapy	N	2	7	6	54	125
	%	1.0	3.6	3.1	27.8	64.4
Difficulty to perform drug therapy	N	7	47	21	84	36
	%	3.6	24.1	10.8	43.1	18.5
Lack of concern about diabetes mellitus	N	5	27	31	66	66
	%	2.6	13.8	15.9	33.8	33.8
Difficulty to control due to the influence of complications or other chronic dis.	N	5	16	30	100	42
	%	2.6	8.3	15.5	51.8	21.8
Difficulty to undergo examination regularly	N	1	17	22	109	46
	%	0.5	8.7	11.3	55.9	23.6
Difficulty to control due to polypharmic (≥4 drugs)	N	0	17	46	96	36
	%	0.0	8.7	23.6	49.2	18.5
Patient lack necessary knowledge about DM, being not familiar with diabetes	N	2	26	50	89	28
	%	1.0	13.3	25.6	45.6	14.4
Difficulty to control because of side effects of medication	N	0	27	41	102	25
	%	0.0	13.8	21.0	52.3	12.8
Difficulty to control due to financial burden, cost and availability of medication	N	4	36	24	87	44
	%	2.1	18.5	12.3	44.6	22.6
Difficulty to control due to psychosocial factors related to the patient	N	0	4	12	77	102
	%	0.0	2.1	6.2	39.5	52.3
Difficulty to control because of health believes of the patient (herbal using instead of medication and using of oral antidiabetic or insulin therapy will cause adaptation or causing cancer	N	6	64	63	56	5
	%	3.1	33.0	32.5	28.9	2.6
Difficulty to control due to resistance to taking insulin and problems with patient self-management	N	0	23	11	45	115
	%	0.0	11.9	5.7	23.2	59.3
Difficulty to control because of lack of necessary knowledge about insulin therapy (storage, injection sites and titration)	N	0	14	18	117	44
	%	0.0	7.3	9.3	60.6	22.8
Difficulty to control because of lack of confidence in using insulin regimens and how to intensify therapy for patients not reaching glycemic control	N	0	3	17	126	47
	%	0.0	1.6	8.8	65.3	24.4

Figure 3 showed the barriers score. High barriers represented 66.2% (129) of participants' respondents, while fair barriers represented 33.8% (66) of participants' respondents.

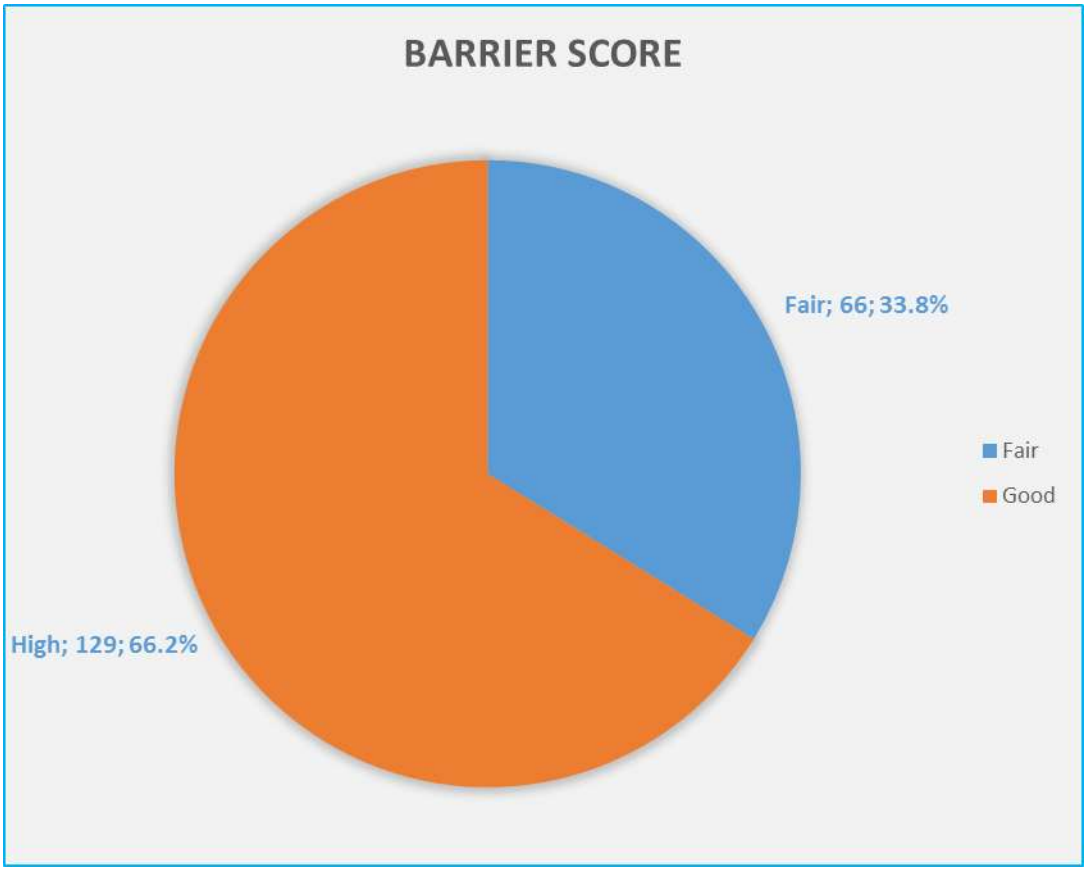

Figure 3. Barrier scores according to participants' response

Distribution of sociodemographic features of participants according to the score of barriers against glycemic control was elucidated in table 3. Unemployed participants and irregular access to care units were significantly associated with high barriers, (P=0.018 and P<0.001, respectively).

Distribution of disease features and PMH of participants according to the score of barriers against glycemic control was elucidated in table 4. Participants that not monitoring blood glucose by themselves, with comorbidities, hypertension, and dyslipidemia were significantly associated with high barriers, (P=0.001, P=0.015, P=0.043, and P0.008), respectively.

Table 3. Distribution of sociodemographic features of participants according to the score of barriers against glycemic control

Sociodemographic features		Barrier score				P value
		Fair		High		
		N	%	N	%	
Age group/ years	30-49	20	39.2	31	60.8	0.22
	50-69	44	33.8	86	66.2	
	≥70	2	14.3	12	85.7	
Sex	Male	17	32.7	35	67.3	0.49
	Female	49	34.3	94	65.7	
Residency	Urban	54	37.0	92	63.0	0.12
	Rural	12	24.5	37	75.5	
Marital status	Single	2	28.6	5	71.4	0.56
	Married	53	36.6	92	63.4	
	Divorced	2	20.0	8	80.0	
	Widow	9	27.3	24	72.7	
Occupation	Employed	32	45.1	39	54.9	0.018
	Unemployed	34	27.4	90	72.6	
Education	Illiterate	13	21.7	47	78.3	0.08
	Primary	16	32.7	33	67.3	
	Intermediate	16	41.0	23	59.0	
	Secondary	11	39.3	17	60.7	
	Higher EDUCATION	10	52.6	9	47.4	
Cigarette smoking	EX-smoker	2	22.2	7	77.8	0.7
	Current smoker	20	32.8	41	67.2	
	None	44	35.2	81	64.8	
Duration of smoking/years	≤10 years	11	34.4	21	65.6	0.8
	>10 years	11	28.9	27	71.1	
No. of Cigarette smoking/day	≤20	5	27.8	13	72.2	0.48
	>20-40	13	29.5	31	70.5	
	>40-60	4	50.0	4	50.0	
Access to care	Regular	35	72.9	13	27.1	<0.001
	irregular	31	21.1	116	78.9	
BMI	Normal	25	36.2	44	63.8	0.31
	Overweight	25	38.5	40	61.5	
	Obese	16	26.2	45	73.8	

Table 4. Distribution of disease features and PMH of participants according to the score of barriers against glycemic control

Parameters		Barrier score				P value
		Fair		High		
		N	%	N	%	
Age at diagnosis of DM/ years	<40	5	35.7	9	64.3	0.09
	40-49	30	32.6	62	67.4	
	50-59	28	37.3	47	62.7	
	60-69	3	21.4	11	78.6	
Duration of DM/year	≤5 years	34	43.0	45	57.0	0.06
	6-10 years	22	25.6	64	74.4	
	11-20 years	10	33.3	20	66.7	
Family history	Positive	48	31.2	106	68.8	0.14
	negative	18	43.9	23	56.1	
DM Treatment	Insulin	2	40.0	3	60.0	0.05
	Oral antidiabetic drug	50	34.5	95	65.5	
	Combined	13	31.7	28	68.3	
	diet and life style modification	1	25.0	3	75.0	
Self-monitoring of Bl. Glucose	Yes	27	52.9	24	47.1	0.001
	No	38	26.6	105	73.4	
Comorbidities	Without comorbidity	17	53.1	15	46.9	0.015
	With comorbidities	49	30.1	114	69.9	
Hypertenstion	Yes	42	29.6	100	70.4	0.043
	No	24	45.3	29	54.7	
Cardiovascular disease	Yes	12	25.5	35	74.5	0.22
	No	54	36.5	94	63.5	
Stroke/Transient ischemic attack	Yes	5	27.8	13	72.2	0.79
	No	61	34.5	116	65.5	
Dyslipidemia	Yes	42	28.6	105	71.4	0.008
	No	24	50.0	24	50.0	

Discussion

The high rate of poorly controlled type-2 DM is found to be associated with many negative outcomes among patients [10]. Despite the vast research findings that have focus on the importance of diet, physical activity, drug treatment, blood glucose monitoring and care of foot, the level of glycemic control is still unsatisfactory among patients worldwide. The findings of this research showed factors that influence diabetes self-glycemic control [11]. Furthermore, this study highlighted the influence of barriers, and lifestyles as determinants of patients control over the glycemic level.

These sociodemographic and disease criteria reported in the current study are slightly different from a Canadian study sample in which patients were of HbA1c ≥10% had an average age of 57.1 years, but the majority were males and employed and most of them were on insulin. A large proportion of the participants with high HbA1c in this study were significantly more likely to report fair or poor health status, have chronic illnesses like hypertension and cardiac diseases, be obese, and have depressive symptoms, and be taking

anti-hypertensive and/or cardio protective medications [12].

Participants' unemployment status and their lack of regular access to care facilities were found to be significantly associated with high barriers. Previous research has suggested a link between stress and the development of type 2 DM; however, the current study lacks sufficient data to draw any firm conclusions. Stress and economic deprivation likely activate the hypothalamic-pituitary-adrenal (HPA) axis and cause increased cortisol production, which contributes to this phenomenon physiologically [13]. Behavioral factors may also be playing a role. Occurrence peaks in those between 40-64 years of age and it has been hypothesized that employment-related stressors and the impact of shift work could underlie this progression. Access to care was also reported as a factor aiding in diabetic control in a USA study by Zhang et al. [14]. The study measured by patient health insurance coverage, area, and the number of times health care was received. Those who reported no health care visits were more likely to have an HbA1c of more than 9%, in comparison with those who reported four or more health care visits in the previous year. This is significant given that 6.0% of known diabetic adults in the USA were uninsured [14]. The Iraqi healthcare system offers in its guidelines universal healthcare coverage and patients' socio-economic characteristics should not be a barrier for access. However, little is known about the extent to which patients achieve equal opportunities to access the standardized care services, and how this associated with patient characteristics [15].

People with type 2 DM with poor glycemic control had a range of barriers that lead to reduced their self-management of the diabetes condition. Main barriers reported in this study are lack of confidence in using insulin regimens, lack of necessary knowledge about insulin therapy, difficulty to perform exercise therapy influence of complications or other chronic disease and some psychosocial factors. Some of these challenges are shared with other studies conducted in New Zealand, such as the findings of Chepulis et al. in 2021, who found that despite the availability of diabetes education resources, the limited resources provided to people with DM in New Zealand may be a direct barrier to good glycemic control and self-management over their condition. Participants in that study stated that their health care providers often failed to provide adequate or updated information specially those who were trying to control their health condition and diet had reported difficulty in finding information that was useful to them [16].

Patients from another study conducted by Tang et al. were more likely to identify financial hurdles, such as the lack of prescription insurance or the inability to purchase a healthy diet. In addition, their findings suggest a link between cost and glycemic control. Financial barriers found to increase the risk of non-compliance and are associated with poor utilization of care services among patients with chronic disease like diabetics [17]. Patients with poor HbA1c control were more likely to be elderly, who may have limited access to affordable, healthy food, as well as limited ability to prepare good foods items and maintain adequate meal spacing. This phenomenon was attributed by Chan et al. to the increased need for medication insurance and programs of nutritional assistance in these communities in order to overcome financial barriers [18]. In Arab societies, the elderly is typically cared for at home by their children and other relatives, hence these considerations are rarely put into practice. Weak understanding of the importance of healthy habits, peer pressure, and calorie-dense food all play a part. The high prevalence of overweight and obesity may be traced back to a number of factors, including the increasing urbanization of the population and the accompanying shifts in lifestyle, which include higher rates of food intake and less time spent [19].

On the other hand, in the latter study in New Zealand many participants also indicated that the side effects of medication formed as a barrier to good glycemic control [16]. As a result, these participants didn't adhere to their medication, or actively changed their medication, or tried to find alternate

medicines, which is usually remedies or herbs that fit with lifestyle like prolonged periods of stopping their diabetic medication, using cinnamon to help control their blood glucose level. However, the complications of these alternatives resulting in substantial increase in HbA1c levels and reduced quality of life like teasing from friends and disappointment.

Essential factors that influence adherence to the diabetes glycemic control were identified, and they extend beyond the level of the individual and the patient to include cultural, organizational, social, and policy influences such as local food habits, religious beliefs and traditions, and a lack of appropriate medical care [20]. Health care practitioners should expand the reach of health promotion to influence social norms and attitudes, leading to better patient outcomes, by identifying the factors that affect the health of individuals and diabetes patients.

In conclusion, patients with type 2 DM who participated in this study reported that a lack of education about the disease and its treatment, as well as social factors such as unemployment and limited access to medical care, were the most significant obstacles to achieving and maintaining good glycemic control.

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Author contribution

Dr. Rashid: Data collection and editing. Dr. Naji: Study design and writing. Dr. Ismaeel: Editing and Statistical analysis.

Conflict of interest

None.

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References

1. Meo SA, Usmani AM, Qalbani E. Prevalence of type 2 diabetes in the Arab world: impact of GDP and energy consumption. Eur Rev Med Pharmacol Sci. 2017; 21(6): 1303-12.
2. Iraqi Research Foundation for analysis. Health in Iraq. Available at: http://www.irfad.org/?s=health. accessed at December 2022
3. Mansour AA, Al-Maliky AA, Kasem B, et al. Prevalence of diagnosed and undiagnosed diabetes mellitus in adults aged 19 years and older in Basrah, Iraq. Diabetes Metab Syndr Obes. 2014; 7: 139-44. doi: 10.2147/DMSO.S59652.
4. Meo SA, Sheikh SA, Sattar K, et al. Prevalence of type 2 diabetes mellitus among men in the Middle East: A Retrospective Study. Am J Mens Health. 2019; 13(3): 1557988319848577. doi: 10.1177/1557988319848577.
5. Blonde L, Aschner P, Bailey C, et al. Gaps and barriers in the control of blood glucose in people with type 2 diabetes. Diab Vasc Dis Res. 2017; 14(3): 172-83. doi: 10.1177/1479164116679775.
6. Abusaib M, Ahmed M, Nwayyir HA, et al. Iraqi experts consensus on the management of type 2 diabetes/prediabetes in adults. Clin Med Insights Endocrinol Diabetes. 2020; 13: 1179551420942232. doi: 10.1177/1179551420942232.
7. Mohammad SH, Abu-Raghif AR, Fadhil NN. Effects of metformin alone, metformin with flaxseeds oil on serum 1,5 anhydroglucitol, adiponectin and insulin resistance in patients with type 2 diabetes mellitus. Iraqi J Med Sci. 2016, 14(1): 67-74.
8. Inzucchi SE, Bergenstal RM, Buse JB, et al. Management of hyperglycemia in type 2 diabetes, 2015: a patient-centered approach: update to a position statement of the American Diabetes Association and the European Association for the Study of Diabetes. Diabetes Care. 2015; 38(1): 140-9. doi: 10.2337/dc14-2441.
9. Yoshioka N, Ishii H, Tajima N, et al. Differences in physician and patient perceptions about insulin therapy for management of type 2 diabetes: the DAWN Japan study. Curr Med Res Opin. 2014; 30(2): 177-83. doi: 10.1185/03007995.2013.855187.
10. Arakawa S, Watanabe T, Sone H, et al. The factors that affect exercise therapy for patients with type 2 diabetes in Japan: A nationwide survey. Diabetol Int. 2015; 6: 19-25.
11. International Diabetic Federation. IDF diabetes atlas. 8th ed. 2017.
12. Sharara E, Akik C, Ghattas H, et al. Physical inactivity, gender and culture in Arab countries: A systematic assessment of the literature. BMC Public Health. 2018; 18(1): 639. doi: 10.1186/s12889-018-5472-z.
13. McBrien KA, Naugler C, Ivers N, et al. Barriers to care in patients with diabetes and poor glycemic control-A cross-sectional survey. PLoS One. 2017; 12(5): e0176135. doi: 10.1371/journal.pone.0176135.
14. Zhang X, Bullard KM, Gregg EW, et al. Access to health care and control of ABCs of diabetes. Diabetes Care. 2012; 35(7): 1566-71. doi: 10.2337/dc12-0081.
15. Mikhael EM, Hassali MA, Hussain SA, et al. Self-management knowledge and practice of type 2 diabetes mellitus patients in Baghdad, Iraq: a

qualitative study. Diabetes Metab Syndr Obes. 2018; 12: 1-17. doi: 10.2147/DMSO.S183776.

16. Chepulis L, Tamatea JAU, Wang C, et al. Glycaemic control across the lifespan in a cohort of New Zealand patients with type 1 diabetes mellitus. Intern Med J. 2021; 51(5): 725-31. doi: 10.1111/imj.14816.

17. Tang KL, Ghali WA, Manns BJ. Addressing cost-related barriers to prescription drug use in Canada. CMAJ. 2014; 186(4): 276-80. doi: 10.1503/cmaj.121637.

18. Chan J, DeMelo M, Gingras J, et al. Challenges of diabetes self-management in adults affected by food insecurity in a large urban centre of Ontario, Canada. Int J Endocrinol. 2015; 2015: 903468. doi: 10.1155/2015/903468.

19. Tremblay MS, Aubert S, Barnes JD, et al. Sedentary Behavior Research Network (SBRN) - Terminology Consensus Project process and outcome. Int J Behav Nutr Phys Act. 2017; 14(1): 75. doi: 10.1186/s12966-017-0525-8.

20. American Diabetes Association. What we recommend; types of activity; 2020. Available from: https://diabetes.org/healthy-living/fitness. Accessed February 15, 2023

Correspondence to Dr. Aseel A. Naji
E-mail: Aseelayad32@gmai.com
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The Association between Human Epididymal Protein 4 and Exposure to Poly Aromatic Hydrocarbons in Samples of Al-Daura Refinery Workers-Baghdad

Ashwak W. Shaker[1] *PhD*, **Estabraq A. Al-Wasiti**[2] *PhD*

[1]Dept. of Medical Laboratory Technique, Al-Maamoon University College, Baghdad, Iraq, [2]Dept. of Chemistry and Clinical Biochemistry, Al-Nahrain University College, Baghdad, Iraq

Abstract

Background Exposure to polycyclic aromatic hydrocarbons (PAHs) can cause many genetic alterations, which may contribute to cancers because PAHs derivatives can bound to DNA induces mutations and tumorigenesis. Some cancer-related proteins differentially expressed in exposed workers such as Human epididymal protein 4 (HE4) therefore, it considers a good biomarker for PAHs adverse effects that can progressed to tumors.

Objective To estimate HE4 level in refinery workers to predict related adverse effect of PAHs that can developed to occupational cancer.

Methods This study included 168 participants divided in three groups (control, office workers and in field workers). PAHs and HE4 were measured for each participant by GC/MS, ELISA respectively.

Results PAHs were negative in controls blood whereas they were elevated in office and field workers. Concentration of HE4 was significantly higher in field than in office workers and controls, as well as significant correlation between concentration of PAHs and HE4 levels with duration of exposure was found.

Conclusion PAHs and HE4 levels were significantly increased in refinery exposed workers (both field and office workers).

Keywords Polycyclic aromatic hydrocarbon, air pollution, Human epididymal protein4, mutation, tumor marker

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List of abbreviations: B[A]P = Benzo(a)pyrene, ELISA = Enzyme-linked immunosorbent assay, GC/MS = Gas chromatography coupled with mass spectrometry, HE4 = Human epididymal protein 4, PAHs = Polycyclic aromatic hydrocarbons

Introduction

One of the most important issues facing humanity and other life forms on our planet today is pollution. Pollutants can be substances that arise naturally or synthetically [(1)]. Toxic exposure alters essential cellular functions and is associated to the etiology of a number of chronic diseases that cause irreversible epigenetic alterations [(2)]. Environmental exposures, such as diet, tobacco, alcohol, stress, genetic factors, infectious agents, and environmental carcinogens like poly aromatic hydrocarbons (PAHs), all influence epigenetic marks such as [acetylation, DNA hypo or hypermethylation and micro-RNA expression]. Some epigenetic alterations alter the expression of tumor suppressor genes and oncogenes, potentially

leading to carcinogenesis [3]. Poly aromatic hydrocarbons like benzo(a) pyrene and its metabolites, known as epoxides, are highly poisonous, mutagenic and carcinogenic to microorganisms and people, thus they've gotten a lot of attention in recent years [4]. They have a poor water solubility, but are extremely lipophilic [5], as a result, they are easily absorbed from animals' gastrointestinal tracts and readily deposited in a range of tissues, including body fat. Because of their high lipophilicity, these chemicals have a high bioavailability after ingestion and inhalation. According to a previous study, detectable levels of PAHs can be found in almost all internal organs, particularly those with a lot of adipose tissue. These organs can act as storage depots for the hydrocarbons, allowing them to be released gradually [6].

Once PAHs enter the body, they must be activated by a multistep metabolic process involving certain enzymes. The mixed-function oxidase system is the enzyme system that is principally responsible for PAH metabolism [7], so to detoxify these chemicals, the initial reaction is an epoxidation, followed by PAHs epoxides coupled with glutathione. Epoxides that are not glutathione conjugated are transformed to phenols and diols as shown in figure (1). PAHs metabolites are not always polar enough to be excreted. As a result, they must be conjugated with glucuronic or sulfuric acids in order to be excreted. The majority of PAHs metabolites are eliminated in the feces and urine [8].

Figure 1. Formation of toxic and mutagenic epoxide from Benzo(a)pyrene (B[A]P)

A number of suspected cancer-related proteins have been shown to be differently expressed in PAHs-exposed workers [9]. According to pathway and gene ontology studies, cell movement, cell migration and cell adhesion were the most common biological processes linked with differentially expressed cancer-related proteins [such as human epididymal protein 4(HE4)] between exposed employees and non-exposed persons [10]. This secretory protein, HE4, is abundant in the human epididymis [11], it is a protease inhibitor that is found in the epididymis epithelium and plays a role in sperm maturation and it is an antimicrobial peptide that plays an important role in the immune system [12,13]. PAHs have recently been found to cause apoptosis in a variety of cell types. Apoptosis is a natural and active method of cell death marked by chromatin condensation in the nucleus and DNA fragmentation [14]. Intracellular cysteine proteases, such as HE4, are thought to be critical components of the intracellular apoptotic signaling pathways generated by PAHs, which result in the accumulation of the tumor suppressor protein p53, which may be important for apoptosis induction [14,15].

The objectives of this study was to estimate HE4 level in refinery workers to predict related adverse effect of PAHs.

Methods

One hundred and twelve (112) Iraqi male workers at Midland Refineries Company-Daura Refinery-Baghdad with age range (25-65 years) and fifty-six 56 healthy individuals who have been lived in distant areas that away from Al-Dura Refinery, with matched age range were enrolled in this study, during the period from 16 July to 2 September 2020.

Subjects were divided into three groups as the following:

Group1: 56 workers in the oil refining field.

Group2: 56 workers in the office (around field).

Group3: 56 apparent healthy individuals as controls, these controls have been lived in distant areas away from Al-Dura Refinery.

Serum concentration of HE4 was determined by enzyme-linked immunosorbent assay (ELISA) kit (Catalog Number: MBS771364), which is designed for the quantitative measurement of HE4 concentrations in serum, plasma, saliva, urine, tissue homogenate, cell culture supernates and other biological fluids, while PAHs concentration in serum was determined by gas chromatography mass spectrometry (GC-MS) after the extraction of these compounds from the serum specimens by liquid - liquid extraction technique [16].

The statistical analysis was performed with SPSS Statistics software (version 24.0, Chicago, USA). Chi squared test was performed to study the correlation between variables and student T test to compare between two groups while analysis of variance (ANOVA) test was used to compare between more than two groups. The level of Statistical significance was set when the p-value was less than 0.05.

Results

There is no statistically difference in age among the groups (controls, office workers and field workers) as shown in table (1).

Table 1. Comparison of the mean age among the groups

Group	N.	Age (Mean±SD)	P value
Control	56	33.96±3.33	
Office	56	38.81±4.00	0.976
Field	56	40.86±3.12	

P value by ANOVA

Mean concentration of PAHs and HE4 for the three groups (controls, office workers and field workers) shown in table (2), it was found that PAHs concentration for field workers was significantly higher than office workers (p=0.0001) as well as HE4 concentration(p=0.012). However, PAHs was not detected in controls

Table 2. The mean concentration of PAHs and HE4 among the groups

Parameter	Group	Mean±SE	P value
PAHs (ppm)	Control	-	0.0001*
	Office	0.35±0.05	
	Field	2.14±0.09	
HE4 (pmol/l)	Control	17.45±0.81	0.012**
	Office	18.21±0.75	
	Field	20.67±0.82	

* p value by unpaired ttest, **p value by ANOVA, PAHs: Polycyclic aromatic hydrocarbons, HE4: Human epididymal protein 4

PAH and HE4 levels was compared according to the duration of exposure for (≤10 years) and (>10 years) for field workers as shown in table (3), it was found that PAHs and HE4 levels were significantly higher in (>10 years group) than (≤10 years group) (p= 0.003 and p= 0.006 respectively). While in office workers, it was found that HE4 level was significantly higher in (>10 years group) than (≤10 years group) (p= 0.001), whereas there was no significant difference in PAHs in the two different duration groups (p=0.09).

Table 3. Levels of PAHs and HE4 correlated with duration of exposure for field workers

Group	Parameter	Duration of exposure	N	Mean±SE	p value
Field workers	PAHs (ppm)	≤10 years	28	1.54±0.06	0.003
		>10 years	28	2.75±0.08	
	HE4 (pmol/l)	≤10 years	28	18.45±1.24	0.006
		>10 years	28	22.89±0.91	
Office workers	PAHs (ppm)	≤10 years	28	0.03±0	0.09
		>10 years	28	0.67±0.06	
	HE4 (pmol/l)	≤10 years	28	15.7±0.81	0.001
		>10 years	28	20.72±1.09	

P value by unpaired ttest, PAHs: Polycyclic aromatic hydrocarbons, HE4: Human epididymal protein 4

Correlation between PAHs and HE4 for field and office workers together was positive but this correlation was not significant (r= 0.159 and p value=0.093) as shown in figure (2).

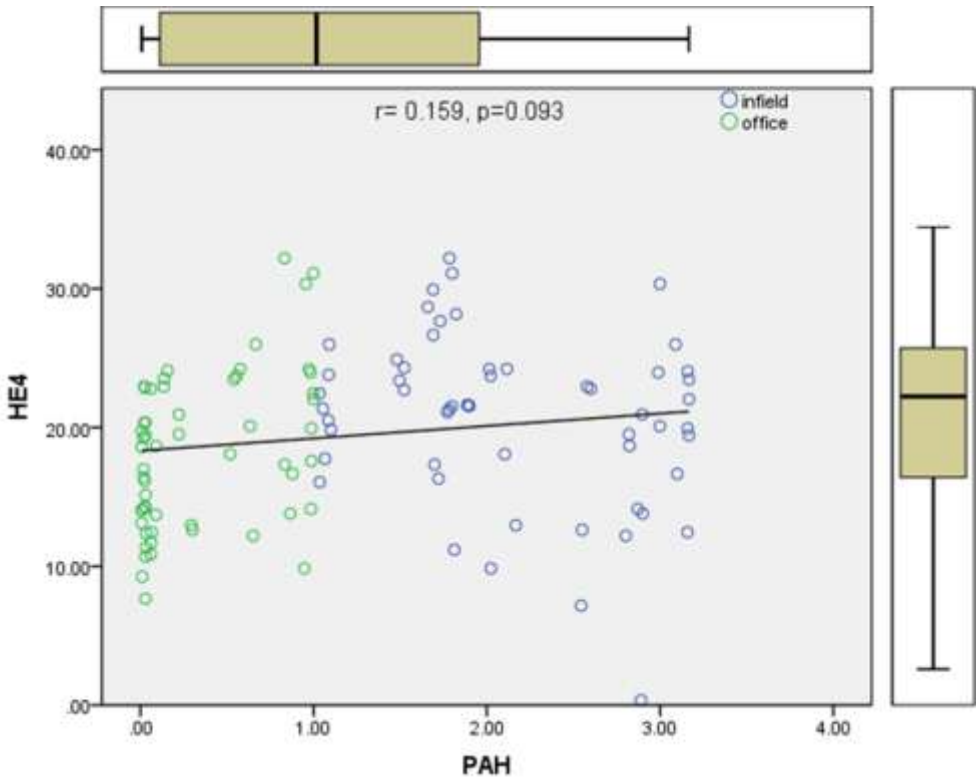

Figure 2. Correlation between PAHs and HE4 in field and office groups

Discussion

Workers' occupational exposure to PAHs, such as during coke manufacture, roofing with bituminous materials, oil refining, and coal gasification, has frequently resulted in human exposure to toxicants and associated carcinogenicity [17]. PAHs have been linked to malignancies of the skin, lungs, bladder, liver, and stomach [18], but at first, they must be converted by enzymes to electrophilic metabolites that can bind to nucleophilic DNA sites to exert their mutagenic or carcinogenic effects [19]. A range of unstable and reactive intermediates, such as diol epoxides and o-quinones, are generated during PAHs metabolism by xenobiotic metabolizing enzymes, which can assault DNA and cause cell toxicity and transformation [20,21] as well as modulate the gene expression through the induction of mutations [22]. This study results showed that PAHs concentration was negative in controls blood while field and office refinery workers have elevated blood concentration of these pollutants and their derivatives, these results are in line with Al-Ani and Al-Wasiti who found the same results [23]. Also, current study found that HE4, which has been used as a biomarker in this study for the adverse effects of PAHs is higher in field and office refinery workers than in controls, this is agree with Talhout et al. who found that there is a number of putative cancer-related proteins differentially expressed in workers exposed to PAHs like HE4 [9] because it regulates cell movement, cell migration and cell adhesion, according to gene ontology analyses [10]. Duration of exposure has a significant correlation with HE4 levels as shown by current results, which revealed that field and office workers who spent more than ten years in the refinery have higher levels of HE4 than those who spent less than ten years and these results are in line with Bingle et al. who suggested that HE4 elevated levels in exposed workers might be involved in the innate immunity defenses of the respiratory tract, nasal and oral cavities to prevent the development of lung adenocarcinoma [24], also, current results agree with LeBleu et al, Moore et al and Grondin et al. who reported that HE4 was increased upon chronic exposure to chemical pollutants such as benzo(a)anthracen, benzo(a) pyren and their metabolites as well as smoke and because its encoding gene was the most upregulated gene in fibrosis-associated myofibroblasts therefore, over expression of this gene and high levels of HE4 according to Solhaug et al. and Chhikara et al. considered to be essential components of the intracellular apoptotic signaling pathways that induced by PAHs and resulting in accumulation of the tumor suppressor protein p53 that may be important for the induction of apoptosis [14,15] and prevent tumorigenesis through interacts with insulin-like growth factor receptor (IGF1R) to arrest the invasion, metastasis and angiogenesis of cancer, so because IGF1R signal promotes non-cancerous cells to malignantly transform and possesses anti-apoptotic and mitogenic activity, therefor, the elevation of HE4 and it's interacting with IGF1R have a beneficial effect in tumorigenesis inhibition [25-27].

In conclusions, PAHs as one of the air pollutants was higher in refinery workers whether they were office or field workers. HE4 levels were significantly increased in refinery exposed workers (both field and office workers). It is recommended to do more investigations to find out the cause of HE4 elevation whether due to tumorigenesis or inflammation.

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Author contribution

Shaker and Dr. Al-Wasiti: designed the study and contributed to the acquisition of data. Shaker: contributed to sample preparation and was the main person in writing the manuscript. Both authors provided critical feedback and helped shape the research, interpreted the data and read and approved the final manuscript.

Conflict of interest

No potential conflict of interest was reported by the authors.

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References

1. Tan C, Lu S, Wang Y, et al. Long-term exposure to high air pollution induces cumulative DNA damages in traffic policemen. Sci Total Environ. 2017; 593-594: 330-6. doi: 10.1016/j.scitotenv.2017.03.179.
2. Shukla A, Bunkar N, Kumar R, et al. Air pollution associated epigenetic modifications: Transgenerational inheritance and underlying molecular mechanisms. Sci Total Environ. 2019; 656: 760-77. doi: 10.1016/j.scitotenv.2018.11.381.
3. Mathers JC, Strathdee G, Relton CL. Induction of epigenetic alterations by dietary and other environmental factors. Adv Genet. 2010; 71: 3-39. doi: 10.1016/B978-0-12-380864-6.00001-8.
4. Rengarajan T, Rajendran P, Nandakumar N, et al. Exposure to polycyclic aromatic hydrocarbons with special focus on cancer. Asian Pacific J Tropical Biomed. 2015; 5(3): 182-9. doi: https://doi.org/10.1016/S2221-1691(15)30003-4.
5. Srogi K. Monitoring of environmental exposure to polycyclic aromatic hydrocarbons: a review. Environ Chem Lett. 2007; 5(4): 169-95. doi: 10.1007/s10311-007-0095-0.
6. Abdel-Shafy HI, Mansour MSM. A review on polycyclic aromatic hydrocarbons: source, environmental impact, effect on human health and remediation. Egyptian J Petroleum. 2016; 25(1): 107-23. doi: https://doi.org/10.1016/j.ejpe.2015.03.011.
7. Höner A. Polycyclic aromatic hydrocarbon (PAH) metabolite. In: Kleiböhmer W. (ed). Handbook of analytical separations. 2001; 3: 99-121.
8. Campo L, Rossella F, Pavanello S, et al. Urinary profiles to assess polycyclic aromatic hydrocarbons exposure in coke-oven workers. Toxicol Lett. 2010; 192(1): 72-8. doi: 10.1016/j.toxlet.2008.12.018.
9. Talhout R, Schulz T, Florek E, et al. Hazardous compounds in tobacco smoke. Int J Environ Res Public Health. 2011; 8(2): 613-28. doi: 10.3390/ijerph8020613.
10. Alhamdow A, Tinnerberg H, Lindh C, et al. Cancer-related proteins in serum are altered in workers occupationally exposed to polycyclic aromatic hydrocarbons: a cross-sectional study. Carcinogenesis. 2019; 40(6): 771-81. doi: 10.1093/carcin/bgz022.
11. Shu Y, Wang W. serum human epididymal protein 4 is associated with depressive symptoms in patients with chronic obstructive pulmonary disease. Int J Chron Obstruct Pulmon Dis. 2020; 15: 3417-22. doi: 10.2147/COPD.S282214.
12. Kirchhoff C, Habben I, Ivell R, et al. A major human epididymis-specific cDNA encodes a protein with sequence homology to extracellular proteinase inhibitors. Biol Reprod. 1991; 45(2): 350-7. doi: 10.1095/biolreprod45.2.350.
13. Hua L, Liu Y, Zhen S, et al. Expression and biochemical characterization of recombinant human epididymis protein 4. Protein Expr Purif. 2014; 102: 52-62. doi: 10.1016/j.pep.2014.08.004.
14. Solhaug A, Refsnes M, Låg M, et al. Polycyclic aromatic hydrocarbons induce both apoptotic and anti-apoptotic signals in Hepa1c1c7 cells. Carcinogenesis. 2004; 25(5): 809-19. doi: 10.1093/carcin/bgh069.
15. Chhikara N, Saraswat M, Tomar AK, et al. Human epididymis protein-4 (HE-4): a novel cross-class protease inhibitor. PLoS One. 2012; 7(11): e47672. doi: 10.1371/journal.pone.0047672.
16. Oluremi OI, Idowu AO, Adedoyin KM, et al. Determination of polycyclic aromatic hydrocarbons in blood plasma of neurology patients. J Toxicol EnvironmentHealth Sci. 2019; 11(1): 1-8.
17. Zhang Y, Ding J, Shen G, et al. Dietary and inhalation exposure to polycyclic aromatic hydrocarbons and urinary excretion of monohydroxy metabolites--a controlled case study in Beijing, China. Environ Pollut. 2014; 184: 515-22. doi: 10.1016/j.envpol.2013.10.005.
18. Boström CE, Gerde P, Hanberg A, et al. Cancer risk assessment, indicators, and guidelines for polycyclic aromatic hydrocarbons in the ambient air. Environ Health Perspect. 2002; 110 Suppl 3(Suppl 3): 451-88. doi: 10.1289/ehp.110-1241197.
19. Melendez-Colon VJ, Luch A, Seidel A, et al. Cancer initiation by polycyclic aromatic hydrocarbons results from formation of stable DNA adducts rather than apurinic sites. Carcinogenesis. 1999; 20(10): 1885-91. doi: 10.1093/carcin/20.10.1885.
20. Arenas-Huertero F, Apátiga-Vega E, Miguel-Pérez G, et al. Molecular markers associated with the biological response to aromatic hydrocarbons from urban air in humans. Air Pollution: New Developments. 2011: 87-112. doi: 10.5772/17999.
21. Shimada T, Takenaka S, Murayama N, et al. Oxidation of pyrene, 1-hydroxypyrene, 1-nitropyrene and 1-acetylpyrene by human cytochrome P450 2A13. Xenobiotica. 2016; 46(3): 211-24. doi: 10.3109/00498254.2015.1069419.
22. Valavanidis A, Vlachogianni T, Fiotakis K, et al. Pulmonary oxidative stress, inflammation and cancer: respirable particulate matter, fibrous dusts and ozone as major causes of lung carcinogenesis through reactive oxygen species mechanisms. Int J Environ Res Public Health. 2013; 10(9): 3886-907. doi: 10.3390/ijerph10093886.
23. Al-Ani RH, Al-Wasiti EA. 7, 8-dihydro-8-oxoguanine (8-oxoG) as biomarker of oxidative DNA damage caused by air pollution with PAH in Midland Refineries Company-Daura Refinery workers.

Materials Today: Proceedings. 2021. doi: https://doi.org/10.1016/j.matpr.2021.05.446.

24. Bingle L, Cross SS, High AS, et al. WFDC2 (HE4): a potential role in the innate immunity of the oral cavity and respiratory tract and the development of adenocarcinomas of the lung. Respir Res. 2006; 7(1): 61. doi: 10.1186/1465-9921-7-61.

25. LeBleu VS, Teng Y, O'Connell JT, et al. Identification of human epididymis protein-4 as a fibroblast-derived mediator of fibrosis. Nat Med. 2013; 19(2): 227-31. doi: 10.1038/nm.2989. Epub 2013 Jan 27. PMID: 23353556; PMCID: PMC4457508.

26. Moore RG, Hill EK, Horan T, et al. HE4 (WFDC2) gene overexpression promotes ovarian tumor growth. Sci Rep. 2014; 4: 3574. doi: 10.1038/srep03574.

27. Grondin CJ, Davis AP, Wiegers TC, et al. Accessing an Expanded Exposure Science Module at the comparative toxicogenomics database. Environ Health Perspect. 2018; 126(1): 014501. doi: 10.1289/EHP2873.

Correspondence to Ashwak W. Shaker
E-mail: waheebashwak@gmail.com
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Evaluation of the Level and Duration of SARS-Cov-2 Neutralizing Antibodies in COVID-19 Convalescent Healthcare Workers

Zeena S. Mousa[1] *MSc*, **Ahmed S. Abdulamir**[2] *PhD*

[1]Baghdad Veterinary Hospital, Baghdad, Iraq, [2]Dept. of Microbiology, College of Medicine, Al-Nahrain University, Baghdad, Iraq

Abstract

Background The neutralizing antibodies (nAbs) are the main factor for the protective immunity against viruses.

Objective To evaluate the level and duration of anti-receptor-binding domain (RBD) nAbs for 8 months in convalescent Coronavirus disease-2019 (COVID-19) patients with a history of mild-moderate and severe disease.

Methods Up to 160 sera from COVID-19 convalescent hospital healthcare workers (HCWs) with a history of mild-moderate and severe disease at 1-, 3-, 5-, and 8-month post-recovery using in-house developed severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-RBD neutralizing competitive enzyme-linked immunosorbent assay (ELISA).

Results SARS-CoV-2 nAbs persisted till 8 months post-recovery in most convalescent patients, the level of nAbs in severe disease group was higher than in mild-moderate disease group; moreover, anti-RBD nAbs mean concentrations in COVID-19 convalescent HCWs tend to increase over time after infection. Similarly, frequency of samples with positive anti-RBD nAbs also tends to increase over time post-recovery.

Conclusion The protective immunity to SARS-CoV-2 in HCWs was found to last for a long time exceeding 8 months after recovery. The increase in the mean level of nAbs over time in COVID-19 convalescent HCWs might be attributed to the frequent asymptomatic re-exposures in HCWs.

Keywords COVID-19, neutralizing antibodies, anti-RBD nAbs, long-term immunity, SARS-COV-2-RBD neutralizing ELISA assay

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List of abbreviations: ACE2 = Angiotensin-converting enzyme 2, BMI = Body mass index, COVID-19 = Coronavirus disease-2019, cVNT = Conventional virus neutralization test, ELISA = Enzyme-linked immunosorbent assay, HCWs = Healthcare workers, HRP = Horse-reddish peroxidase, nAbs = Neutralizing antibodies, pVNT = Pseudovirus-based virus neutralization test, RBD = Receptor-binding domain, PCR = Polymerase chain reaction, SARS-CoV-2 = Severe acute respiratory syndrome coronavirus 2, S = Spike, TMPRSS2 = Transmembrane protease serine 2, TMB = Tetramethylbenzene

Introduction

The coronavirus disease-2019 (COVID-19) pandemic is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a member of the SARS-related coronavirus species [1-4]. COVID-19 includes a variety of clinical syndromes, from asymptomatic cases to mild flu-like illness to severe illness that demands hospitalization, primarily due to pulmonary complications [3,5,6]. Despite the fact that SARS-CoV-2 primarily affects the respiratory system, new evidence suggests that COVID-19 also affects the vascular system, causing thrombotic microangiopathy and thrombosis in a variety of organs, including the lungs [7-9]. As a result, it is

no surprise that patients with pre-existing cardiovascular diseases, hypertension, and other comorbidities are at a higher risk [10]. For entry into target cells, SARS-CoV-2 uses angiotensin-converting enzyme 2 (ACE2) as a receptor, and for activation of the viral spike (S) protein, it uses transmembrane protease serine 2 (TMPRSS2), a cellular serine protease [11,12]. Antibodies that target the receptor-binding domain (RBD) of the S protein are particularly interesting because they can prevent virus infection and spread by blocking virus entry into cells. Additionally, these neutralizing antibodies (nAbs) may be used in passive antibody therapy [13-15]. As a result, it is not surprising that antibody responses to SARS-CoV-2 have gotten a lot of attention as a way to accurately assess infection prevalence [13,16]. Regarded anti-SARS-CoV-2 antibody responses are not known to be long-lasting, especially when considering evidence that antibody responses to other coronaviruses are changeable and transient [17-20]. It is also unclear whether all COVID-19 patients, particularly those with mild disease, will produce enough SARS-CoV-2 nAbs to prevent re-infection in comparison with those with severe COVID-19. Therefore, in the present study, it was applied in-house developed SARS-COV-2 RBD neutralizing ELISA assay to qualitatively and quantitatively assess the level and duration of nAbs against SARS-CoV-2 infection primarily in sera collected at different time points post-recovery from convalescent individuals' cohorts with mild-moderate and severe infection with COVID-19.

Methods

Study population

This is a cross-sectional study conducted at the Microbiology Department, College of Medicine, Al-Nahrain University during the period between December 2020 and September 2021. The study included a recovered COVID-19 frontline Healthcare workers (HCWs) at many hospitals in Baghdad. A total of 160 serum samples were collected randomly from convalescent COVID-19 HCWs that volunteered to donate. All donors had previously polymerase chain reaction (PCR)-confirmed SARS-CoV-2 infection. Serum samples were collected at different time points (1-, 3-, 5-, and 8-months post-recovery), each time point covered 20 individuals with a history of mild-moderate disease and 20 individuals with a history of severe disease.

Inclusion criteria

(1) Ex COVID-19 patients who had previously positive SARS-CoV-2 PCR
(2) Informed consent
(3) Age >18 years.

Exclusion criteria

(1) Refusal to give informed consent
(2) Contraindication to venipuncture
(3) Vaccinated individuals.

Ethical approval

This study was approved by the Institutional Review Board, College of Medicine, Al-Nahrain University (No. 202011112 on 8/12/2020). After obtaining the consent, participants filled out a baseline questionnaire, and blood samples were drawn. The study was conducted by COVID-19 contact restrictions using appropriate measures for infection prevention.

Data collection

Participants were asked to provide information about their age, sex, weight, height, general health status (e.g. cardiovascular or respiratory diseases, diabetes mellitus, pregnancy, any autoimmune diseases, etc.); moreover, patients were asked for the history of the previous COVID-19 infection including the severity of COVID-19 signs and symptoms, minimum level of oxygen saturation (SpO_2), hospital admission, date of the positive COVID-19 PCR test result, date of enrolment and the date of recovery or negative COVID-19 PCR test result.

SARS-CoV-2 RBD neutralizing enzyme-linked immunosorbent assay (ELISA) assay

Using an in-house developed in vitro SARS-CoV-2 RBD neutralizing ELISA assay prepared in a recent study [21], the qualitative and quantitative detection of circulating neutralizing/blocking antibodies in serum samples was done in an isotype-independent manner. For quantitative assay, standards preparation was performed by a serial dilution of the stock purified recombinant concentrated anti-SARS-CoV-2 Spike nAbs to get four points for the standard curve to calculate the unknown nAbs concentration in the samples. Thus, the anti-SARS-CoV-2 spike nAbs standards concentration was 10000 ng/ml, 1000 ng/ml, 100 ng/ml, and 0 ng/ml. Sample diluent served as the zero standard (0 ng/ml). For qualitative assay, the prepared anti-Human SARS-CoV-2 highest standard of 10000 ng/ml as positive control and the sample diluent as negative control, and the optimal established cut-off value of the assay were used.

To perform this competitive assay, a calculated volume of receptor-binding domain labeled with horse-reddish peroxidase (RBD-HRP) working solution to fit 10 ng per well was mixed with a volume ratio of 1:1 with serum samples at a final dilution of 1:10 and each the freshly prepared standards. The mixture was then incubated at 37°C for 1 hour to allow the neutralization reaction to occur. For binding reaction, 100 µL of each standard mixture and each sample mixture were transferred to the wells of the human ACE2 coated microplate and incubated in dark at 37°C for 1 hr. The unbound HRP-RBD, as well as any HRP-RBD bound to non-nAb, will be captured on the plate by binding to hACE2, whereas the circulating nAb- RBD-HRP complexes remain in the supernatant and are washed away 4 times with wash buffer. That is, if nAbs are present in the serum, the interaction of ACE2-RBD can be neutralized (inhibited/blocked) by specific nAbs in patient serum, just like in Conventional virus neutralization test (cVNT) or Pseudovirus-based virus neutralization test (pVNT). For substrate reaction, one-component tetramethylbenzene (TMB) substrate was added to each well and incubated in the dark at 37°C for 20 minutes. Then, stop solution was added to each well to quench the reaction. The absorbance was read in the microtiter plate reader (BioTek) at 450 nm immediately after adding the stop solution.

Depending on the amount of nAbs present in convalescent sera, the binding of SARS-CoV-2 S RBD to ACE2 would be blocked to various degrees that should correlate with the optical density of this enzyme-linked immune sorbent-based assay. The serum samples with more nAbs show a lower signal intensity.

Statistical analysis

The data were processed using statistical package for social sciences (SPSS) version 16.0.0, Microsoft Excel 2010, and Graphpad Prism version 7.04. The data of the current study were scrutinized carefully in terms of being parametric or non-parametric using normality tests. Accordingly, the proper statistical tests were used. Student t-test and analysis of variance (ANOVA) test were used for parametric data to measure the significance of difference in means taking into account whether variables of analysis sharing different or equal variance. For qualitative nominal data, Pearson's chi-square test, with or without Yate's correction, Fisher Exact test, and McNemar test were used to measure significance of hypothesis for association. Correlation coefficient tests, odd ratio, correlation coefficient, among variables were used to assess the nature of correlation in terms of positive, negative or indifference.

Results

Descriptive data of study population

To estimate the level and persistence of the response of anti-SARS-CoV-2-RBD nAbs in a sample of Iraqi convalescent COVID-19 HCWs, the validated in-house neutralizing ELISA assay was used; a total of 160 sera of hospital's COVID-19 convalescent HCWs who had previously PCR-confirmed SARS-CoV-2 infection were collected at various intervals of post-recovery time (1, 3, 5, and 8 months post-recovery); each time interval included 20 HCWs

with a history of mild-moderate disease and 20 HCW with a history of severe disease. Up to 62.5% (100/160) of participants were females. Up to 19.4% (31/160), 48.1% (77/160), and 32.5% (52/160) of participants were normal weight, overweight and obese, respectively. Of note, 66.2% (106/160) of participants were without medical co-morbidities while 33.8% (54/160) were with different types of co-morbidity. Among the co-morbidities reported by the participants, diabetes mellitus (DM) of 11.2% (18/160), cardiovascular disease of 11.9% (19/160), asthma of 6.2% (10/160), and others of 4.4% (7/160). Following the serological testing by in-house SARS-CoV-2 RBD neutralizing ELISA kit, it was demonstrated that 71.9% (115/160) of the COVID-19 convalescent HCWs in all groups were positive to anti-RBD nAbs presence, while 28.1% (45/160) of HCWs were negative.

Descriptive qualitative data of the study

The descriptive statistics of the age, body mass index (BMI), and anti-SARS-CoV-2-RBD nAbs concentration is demonstrated in table 1. The mean, median, and range of age was 37.1 and 35, (21-72) years, respectively, and the mean, median, and range of BMI was 28.44, 28.05, (15.59-42.02) kg/m^2, respectively. Also, the mean concentration of the anti-SARS-CoV-2-RBD nAbs in HCWs (n=160) was 5370.3 ng/ml while, the median concentration was 1546.3 ng/ml.

Table 1. The descriptive statistics of the age, body mass index and anti-SARS-CoV-2-RBD neutralizing antibodies concentration

Variable		Statistic	Std. Error
Age (yr)	Mean	37.11	0.87
	Median	35.00	
	Std. Deviation	11.00	
	Minimum	21.00	
	Maximum	72.00	
BMI (kg/m^2)	Mean	28.45	0.34
	Median	28.05	
	Std. Deviation	4.33	
	Minimum	15.59	
	Maximum	42.02	
Anti-RBD neutralizing IgG antibodies (ng/ml)	Mean	5370.3	2337.82
	Median	1546.3	
	Std. Deviation	29571.3	
	Minimum	0.03	
	Maximum	293000	

Age and BMI in the different groups of study

The mean age of COVID-19 convalescent HCWs has no statistical significance when compared with different COVID-19 severity groups at different time points (p-value >0.05). Nevertheless, HCWs with a history of severe COVID-19 disease were a bit older than those with a history of mild-moderate COVID-19

disease. The mean BMI with different disease severity was found to be significant (p<0.023). It was found that the mean BMI in groups of convalescent HCWs with a history of severe COVID-19 disease was higher than in convalescent HCWs groups with a history of mild-moderate COVID-19 disease as shown in table 2.

Table 2. Mean body mass index values of HCWs in all different disease severity groups at the different times points

		BMI (kg/m^2)		
Study groups	**N**	**Mean**	**Std. Deviation**	**P value**
Mild/moderate-1-month	20	27.87	5.16	0.023
Severe-1-month	20	28.37	4.07	
Mild/moderate-3-month	20	28.13	4.49	
Severe-3-month	20	29.23	3.08	
Mild/moderate-5-month	20	25.95	3.13	
Severe-5-month	20	30.19	2.67	
Mild/moderate-8-month	20	27.49	4.38	
Severe-8-month	20	30.36	5.70	
Total	160	28.45	4.33	

The relationship of level of anti-SARS-COV-2-RBD nAbs and the disease severity in different study groups

The statistically significant relationship between the mean concentration of anti-SARS-CoV-2-RBD nAbs and the different groups of convalescent HCWs with different disease severity was proven (p=0.016; p<0.05). It was observed that the mean level of anti-SARS-CoV-2-RBD nAbs in convalescent HCWs groups with a history of severe disease was higher than in convalescent HCWs groups with a history of mild-moderate disease when comparing without looking at the difference between the post-recovery time points of each group (Table 3).

The changing over time of the level of anti-SARS-CoV-2-RBD nAbs among the HCW groups recovered from the mild-moderate disease

The mean concentration of anti-RBD nAbs in COVID-19 convalescent HCWs groups with a history of mild-moderate disease significantly changed with different time points post-recovery (p<0.05). The mean level of nAbs in mild-moderate groups of COVID-19 convalescent HCWs were in a trend of increasing over time (1-, 3-, 5-, and 8-months post-recovery) as shown in figures 1. That mean level of nAbs did not wane over time in COVID-19 convalescent HCW groups, even for those recovering from the mild-moderate disease.

Table 3. Mean level of anti-SARS-COV-2-RBD neutralizing antibodies in convalescent HCW groups with different disease severity and different time points post-recovery

Study groups	N	Anti-RBD neutralizing IgG antibodies (ng/ml) Mean	Std. Deviation	P value
Mild/moderate-1-month	20	301.46	857.81	
Severe-1-month	20	1897.60	2782.02	
Mild/moderate-3-month	20	779.56	1224.13	
Severe-3-month	20	2571.60	1819.75	0.016
Mild/moderate-5-month	20	1634.00	1951.96	
Severe-5-month	20	2484.70	2115.46	
Mild/moderate-8-month	20	2694.30	1723.07	
Severe-8-month	20	30599.00	80759.85	
Total	160	5370.30	29571.31	

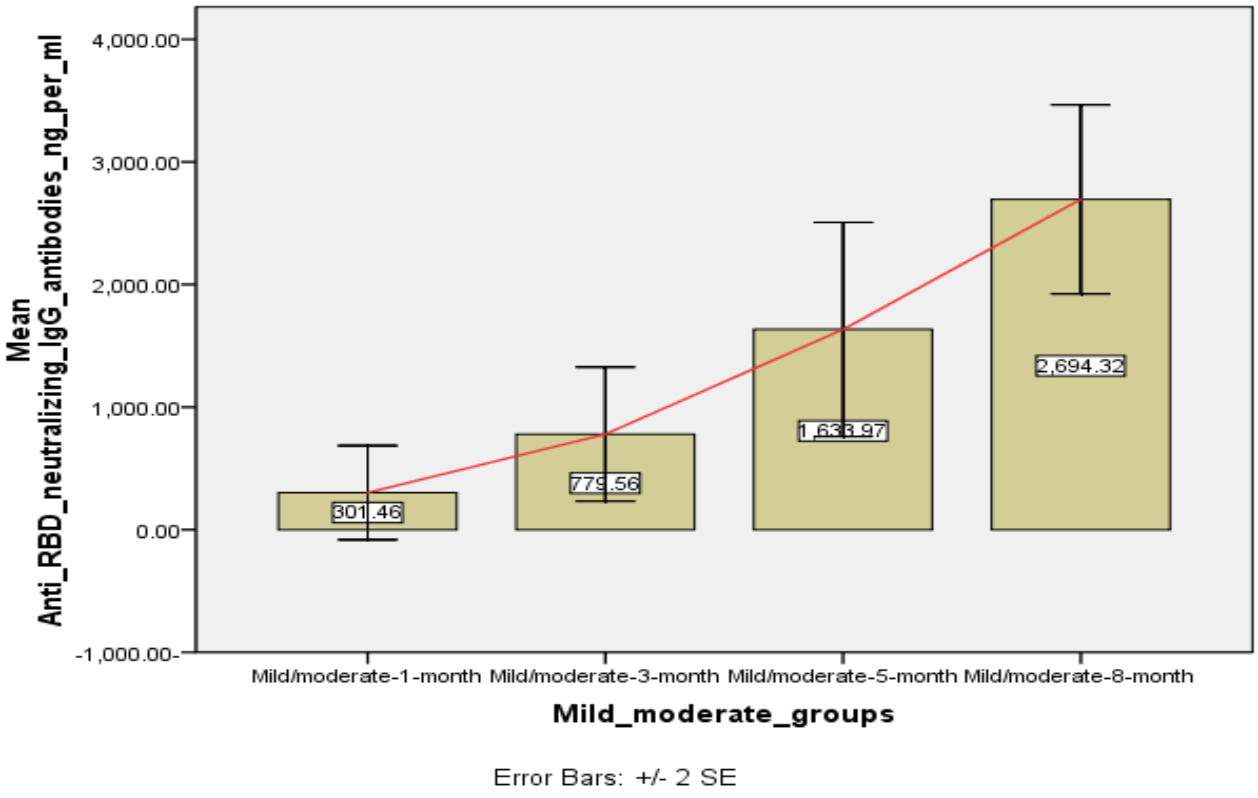

Figure 1. The changes of the mean level of nAbs in mild-moderate groups of COVID-19 convalescent HCWs over time

The changing over time of the level of anti-SARS-CoV-2-RBD nAbs among the HCW groups recovered from the severe disease

Despite the mean concentration of anti-RBD nAbs in COVID-19 convalescent HCWs groups with a history of severe disease had no statistically significant difference among different time points post-recovery (p=0.07; p>0.05), the mean level of nAbs in severe groups of COVID-19 convalescent HCWs tends to increase over time (1, 3, 5, and 8 months post-recovery) (Figure 2).

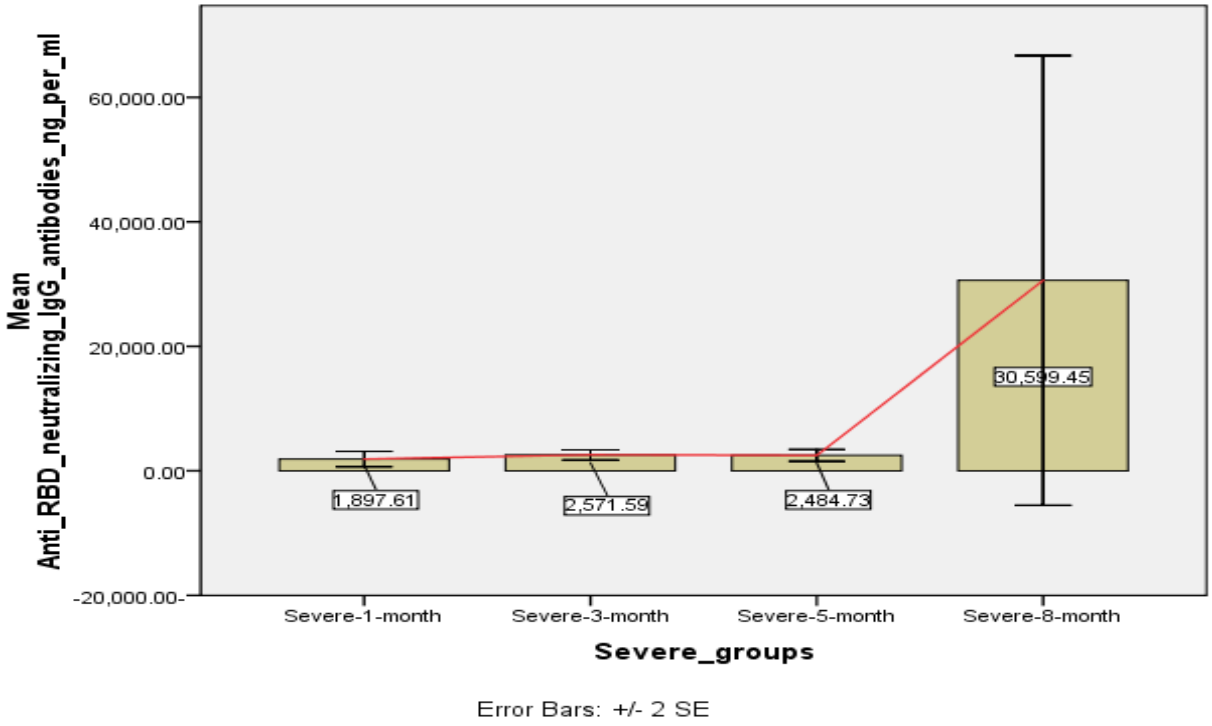

Figure 2. The changes of the mean level of nAbs in severe groups of COVID-19 convalescent HCWs over time

The association of the positive anti-SARS-CoV-2-RBD nAbs with the post-recovery time in COVID-19 convalescent HCWs groups with a history of mild -moderate disease

The evaluation of the association between the presence of detectable anti-SARS-CoV-2-RBD nAbs and time intervals post-recovery was performed using the chi-square test. For COVID-19 convalescent HCWs groups with a history of the mild-moderate disease, a total of 80 sera grouped according to the time interval post-recovery were assessed for the presence of detectable anti-RBD nAbs using the in-house SARS-CoV-2-RBD neutralizing ELISA assay used in this study. The frequencies of samples with positive anti-RBD nAbs were found to increase over time as shown in figure 3, reflecting the increasing level of anti-SARS-CoV-2-RBD nAbs in mild-moderate groups of COVID-19 convalescent HCWs over time and indicating the persistence of the nAbs until the eight-month post-recovery. The frequencies of convalescent HCWs with positive anti-RBD nAbs were 4/20 (20%), 11/20 (55%), 12/20 (60%), and 19/20 (95%) at 1, 3, 5, and 8 months after recovery, respectively (P = 0.0001).

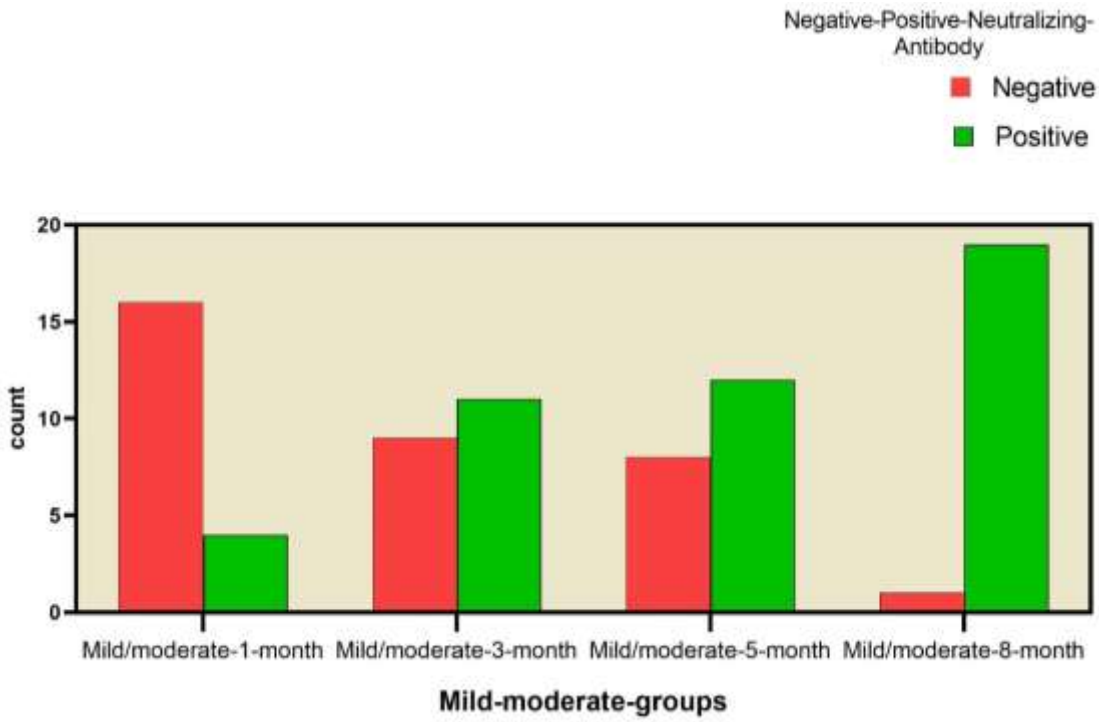

Figure 3. Illustration for the association of the positive anti-SARS-COV-2-RBD nAbs with the post-recovery time in COVID-19 convalescent HCW groups with a history of mild-moderate disease by displaying the increasing frequencies of samples with positive anti-RBD nAbs versus the negative

The association of the positive anti-SARS-CoV-2-RBD nAbs with the post-recovery time in COVID-19 convalescent HCWs groups with a history of severe disease

Of a total of 80 sera collected at different time intervals post-recovery from COVID-19 convalescent HCWs with a history of the severe disease, the frequencies of samples reading a positive anti-RBD nAbs were determined for each time group using the in-house neutralizing ELISA kit. The frequencies of samples reading positive anti-RBD nAbs in the different severe groups of COVID-19 convalescent HCWs were relatively close to each other and slightly tend to increase over time as shown in figure 4, reflecting a fluctuating level of anti-SARS-CoV-2-RBD nAbs but generally mildly increasing over time. The frequencies of convalescent HCWs with positive anti-RBD nAbs were 12/20 (60%), 20/20 (100%), 18/20 (90%), and 19/20 (95%) at 1, 3, 5, and 8 months after recovery, respectively (P = 0.001).

The association of the sex of the study population with the positive anti-SARS-CoV-2-RBD nAbs post-recovery and with the presence of co-morbidities

It was shown that sex grouping has no association with the presence of detectable anti-SARS-CoV-2-RBD nAbs post-recovery (p=0.683) or the presence of co-morbidities (p=0.546). The frequency of COVID-19 convalescent HCWs with positive detectable nAbs by sex was 73/100 (73%) and 42/60 (70%) for females and males, respectively. The frequency of COVID-19 convalescent HCWs with co-morbidities by sex was 32/100 (32%) and 22/60 (36.6%) for females and males, respectively.

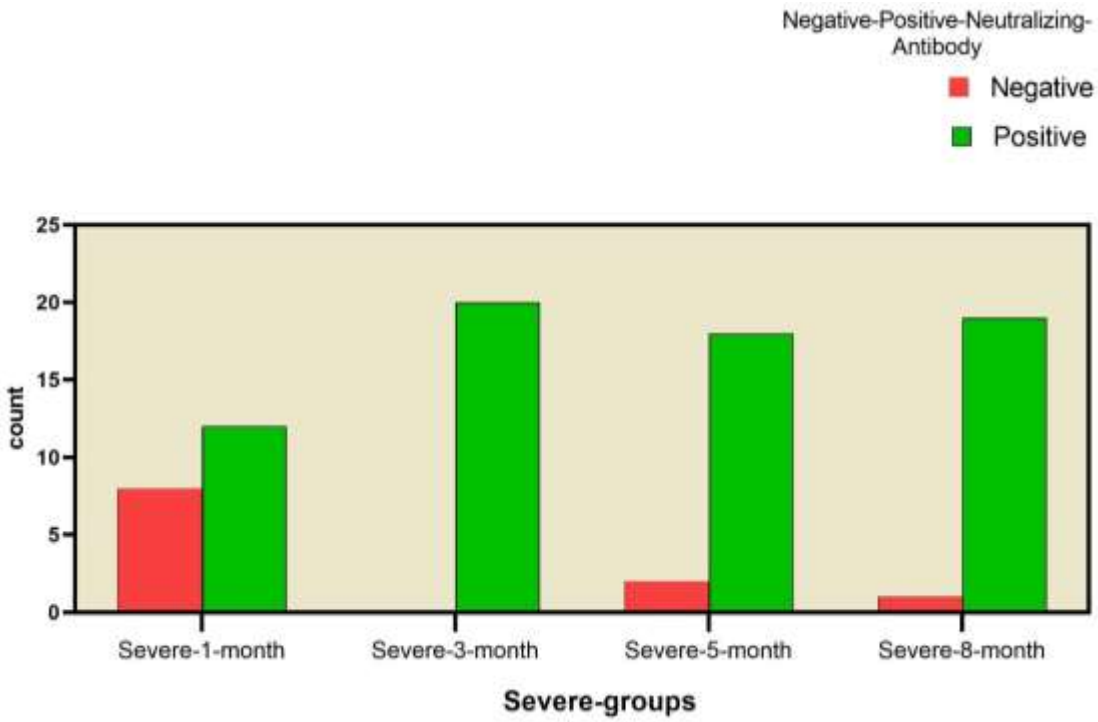

Figure 4. Illustration for the association of the positive anti-SARS-COV-2-RBD neutralizing antibodies with the post-recovery time in COVID-19 convalescent HCW groups with a history of severe disease by displaying the increasing frequencies of samples with positive anti-RBD nAbs versus the negative

The association of obesity, co-morbidities, and type of co-morbidity of the study population with the positive detection of anti-SARS-CoV-2-RBD nAbs post-recovery

The current study revealed that no significant difference in the frequencies of COVID-19 convalescent HCWs with positive detectable nAbs by obesity (p=0.798), co-morbidities (p=0.5), or type of co-morbidity (p=0.909). The frequency of COVID-19 convalescent HCWs with positive detectable nAbs by obesity was 21/31 (67.7%), 57/77 (74%), and 37/52 (71%) for normal and overweight, and obese, respectively. The frequency of COVID-19 convalescent HCWs with positive detectable nAbs by co-morbidities was 78/106 (73.5%) for those who had no co-morbidities and 37/54 (68.5%) for those who had co-morbidities. The frequency of COVID-19 convalescent HCWs with positive detectable nAbs by type of co-morbidity was 13/18 (72.2%), 13/19 (68.4%), 7/10 (70%), and 4/7 (57.1%) for DM and cardiovascular disease, asthma, and other, respectively.

Relationship between the level of anti-SARS-CoV-2-RBD nAbs and the co-morbidities of COVID-19 convalescent HCWs

The mean concentrations of anti-SARS-CoV-2-RBD nAbs were borderline significantly different between ones with comorbidities versus those without comorbidities (p=0.055). It was observed that the mean concentration level of anti-SARS-CoV-2-RBD nAbs of COVID-19 convalescent HCWs having co-morbidities was higher than those having no co-morbidities as illustrated in figure 5.

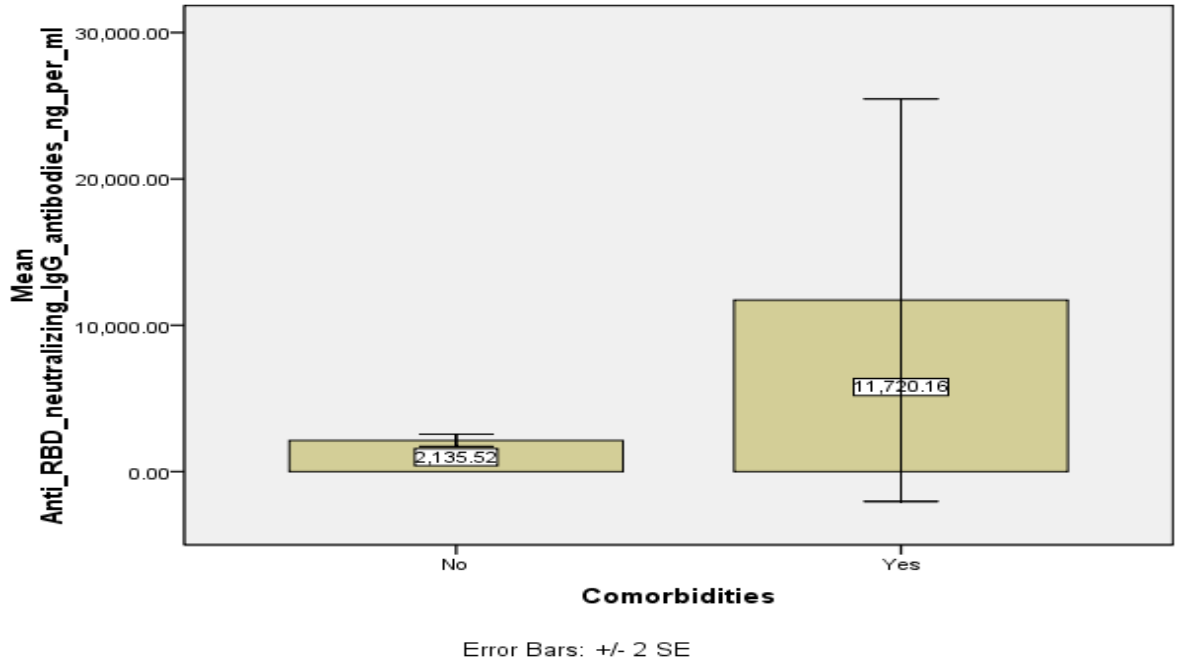

Figure 5. Relationship between the level of anti-SARS-CoV-2-RBD nAbs and co-morbidities of the COVID-19 convalescent HCW in different study groups

Relationship between the level of anti-SARS-CoV-2-RBD nAbs and type of co-morbidity of the COVID-19 convalescent HCWs

The mean concentrations of anti-SARS-CoV-2-RBD nAbs were not statistically significantly different when compared among different groups of COVID-19 convalescent HCWs who grouped according to the type of co-morbidity ($p>0.05$).

Relationship between the level of anti-SARS-CoV-2-RBD nAbs and obesity of the COVID-19 convalescent HCWs

There was no statistically significant difference in the mean concentrations of anti-SARS-CoV-2-RBD nAbs among the different obesity groups of COVID-19 convalescent HCWs ($p>0.05$).

Discussion

Better diagnostic tests and treatment, as well as the development of effective vaccines are necessary to help control COVID-19 pandemic; moreover, clarifying the neutralizing memory arsenal against SARS-CoV-2 will enable a clear explanation of immune responses and the needed strategies to combat this viral infection. In this study, the anti-RBD nAbs levels and their persistence in 160 COVID-19 convalescent HCWs with a history of mild-moderate and severe disease were monitored at various intervals (1, 3, 5, and 8 months) of post-recovery time. Similar to a previous study [22], convalescent HCWs with high BMI were associated with more severe disease and were more likely to have an increased level of anti-RBD nAbs.

In the terms of the relationship between the level of nAbs and severity, this study found that the mean level of anti-SARS-CoV-2-RBD nAbs in convalescent HCWs groups with a history of severe disease was higher than in convalescent HCWs groups with a history of mild-moderate disease, as reported by others [23-26]. It is possible that disease severity as measured by a symptom density score, influences the initial magnitude of antibodies, resulting in weaker humoral responses in mildly ill patients but instead strong stimulation of short-lived plasmablasts by inflammatory prolonged disease in those with severe disease. Following infection or immunization, an initial peak, as well as an early decline of antibodies, is usual, since most short-lived antibody secreting

plasmablasts concerned for the early antibody peak has dropped dead by month three [27]. However, findings in this study showed that anti-RBD nAbs persisted until the eight months post recovery in most convalescent individuals and this indicates that protective immunity against SARS-CoV-2 may relatively be long-lasting enough for reasonable annual vaccination programs especially in front line HCWs, as described in previous studies [24,28], but contrasted with another study that suggested unlikely long term protective immunity on-line with the other coronaviruses [29]. As was stated before, long-lived plasma cells are responsible for the longer-term retention of anti-RBD nAbs during months 8 and thereafter [29].

In addition, this study revealed that the mean level of nAbs tends to increase over time among all mild-moderate groups as well as severe groups of the COVID-19 convalescent HCWs. A similar study demonstrated the increase of anti-RBD titer over time [28] but other studies revealed contrasting findings with a decrease in anti-RBD nAbs over post-recovery time [30,31]. The observed increase in anti-RBD nAbs possibly belongs to the reboots from asymptomatic re-exposure that occurred in individuals at high risk such as HCWs despite the development of an efficient humoral immune response after the symptomatic initial infection. The mean concentration of anti-RBD nAbs in HCWs with mild-moderate and severe disease at eight months post-recovery might be exposed more than once to SARS-CoV-2 resulting in multiple folds higher the mean concentration of nAbs in 8-month than in 1-month post-recovery as a baseline. Recent studies have indicated that asymptomatic SARS-CoV-2 reinfections [24,32,33], as well as, symptomatic COVID-19 re-infections in HCWs are frequent when compared to the general population even though with the presence of high protective immunity [29,32].

Other investigations showed that antibodies to SARS-CoV-2 have been linked to a lower incidence of SARS-CoV-2 reinfection in healthcare workers for up to seven months following infection [34,35]. Notably, a study with similar results explained the increase in the level of nAbs over time linked to the preferential detection of increasing higher-affinity antibodies and considered the secondary re-exposure unlikely due to low circulation of the virus [35]. But another research suggested that despite herd protection from vaccination or infection, SARS-CoV-2 may continue to circulate throughout community populations [36].

In addition, the current study showed that the frequency of samples with positive anti-RBD nAbs from HCWs with mild-moderate and severe diseases tends to increase over time, confirming the boosting effect caused by re-infections, which also occurred in HCWs with waning or seroconverted nAbs levels, explaining the increased proportion of positive samples. Therefore, even for HCWs who already have recovered from a mild COVID-19 disease and have antibodies detected in routine serologic tests, continue the use of personal protective equipment to support infection prevention and control, at least until strong indicators of immunological protection are established.

In conclusions, anti-RBD nAbs persisted until the eight-months post-recovery in most of convalescent patients. The mean level of nAbs in mild-moderate and severe COVID-19 convalescent HCWs groups tends to increase over time, this possibly belongs to the reboots from asymptomatic re-exposure that occurred in individuals at high risk such as HCWs despite the development of an efficient humoral immune response after the symptomatic initial infection. Frequency of samples with positive anti-RBD nAbs in COVID-19 convalescent HCWs tends to increase over time, confirming the boosting effect caused by re-infection, which also occurred in those with waning nAbs levels, explaining the increased proportion of positive samples. Severe cases tend to develop higher level of nAbs to SARS-CoV-2.

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Author contribution

Mousa: Did the laboratory work and wrote the article. Dr. Abdulamir: Supervision of the study.

Conflict of interest

The authors declare that there is no conflict of interest.

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References

1. Abebe EC, Dejenie TA, Shiferaw MY, et al. The newly emerged COVID-19 disease: A systemic review. Virol J. 2020; 17(1): 96. doi: 10.1186/s12985-020-01363-5.
2. Chen N, Zhou M, Dong X, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: A descriptive study. Lancet. 2020; 395(10223): 507-13. doi: 10.1016/S0140-6736(20)30211-7.
3. Zhu N, Zhang D, Wang W, et al. A novel Coronavirus from patients with pneumonia in China, 2019. N Engl J Med. 2020; 382(8): 727-33. doi: 10.1056/NEJMoa2001017.
4. Peiris JS, Lai ST, Poon LL, et al. Coronavirus as a possible cause of severe acute respiratory syndrome. Lancet. 2003; 361(9366): 1319-25. doi: 10.1016/s0140-6736(03)13077-2.
5. Zhu J, Ji P, Pang J, et al. Clinical characteristics of 3062 COVID-19 patients: A meta-analysis. J Med Virol. 2020; 92(10): 1902-14. doi: 10.1002/jmv.25884.
6. Oran DP, Topol EJ. Prevalence of asymptomatic sars-cov-2 infection: A narrative review. Ann Intern Med. 2020; 173(5): 362-7. doi: 10.7326/M20-3012.
7. Liu PP, Blet A, Smyth D, et al. The science underlying COVID-19: Implications for the cardiovascular system. Circulation. 2020; 142(1): 68-78. doi: 10.1161/CIRCULATIONAHA.120.047549.
8. Ackermann M, Verleden SE, Kuehnel M, et al. Pulmonary vascular endothelialitis, thrombosis, and angiogenesis in Covid-19. N Engl J Med. 2020; 383(2): 120-8. doi: 10.1056/NEJMoa2015432.
9. Menter T, Haslbauer JD, Nienhold R, et al. Postmortem examination of COVID-19 patients reveals diffuse alveolar damage with severe capillary congestion and variegated findings in lungs and other organs suggesting vascular dysfunction. Histopathology. 2020; 77(2): 198-209. doi: 10.1111/his.14134.
10. Ruan Q, Yang K, Wang W, et al. Clinical predictors of mortality due to COVID-19 based on an analysis of data of 150 patients from Wuhan, China. Intensive Care Med. 2020; 46(5): 846-8. doi: 10.1007/s00134-020-05991-x.
11. Hoffmann M, Kleine-Weber H, Schroeder S, et al. SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. Cell. 2020; 181(2): 271-80.e8. doi: 10.1016/j.cell.2020.02.052.
12. Ou X, Liu Y, Lei X, et al. Characterization of spike glycoprotein of SARS-CoV-2 on virus entry and its immune cross-reactivity with SARS-CoV. Nat Commun. 2020; 11(1): 1620. doi: 10.1038/s41467-020-15562-9.
13. Özçürümez MK, Ambrosch A, Frey O, et al. SARS-CoV-2 antibody testing-questions to be asked. J Allergy Clin Immunol. 2020; 146(1): 35-43. doi: 10.1016/j.jaci.2020.05.020.
14. Casadevall A, Pirofski LA. The convalescent sera option for containing COVID-19. J Clin Invest. 2020; 130(4): 1545-8. doi: 10.1172/JCI138003.
15. Rasheed AM, Fatak DF, Hashim HA, et al. The therapeutic potential of convalescent plasma therapy on treating critically-ill COVID-19 patients residing in respiratory care units in hospitals in Baghdad, Iraq. Infez Med. 2020; 28(3): 357-66.
16. Petherick A. Developing antibody tests for SARS-CoV-2. Lancet. 2020; 395(10230): 1101-2. doi: 10.1016/S0140-6736(20)30788-1.
17. Alshukairi AN, Khalid I, Ahmed WA, et al. Antibody response and disease severity in healthcare worker MERS survivors. Emerg Infect Dis. 2016; 22(6): 1113–5. doi: 10.3201/eid2206.160010.
18. Tang F, Quan Y, Xin ZT, et al. Lack of peripheral memory B cell responses in recovered patients with severe acute respiratory syndrome: a six-year follow-up study. J Immunol. 2011; 186(12): 7264-8. doi: 10.4049/jimmunol.0903490.
19. Liu W, Fontanet A, Zhang PH, et al. Two-year prospective study of the humoral immune response of patients with severe acute respiratory syndrome. J Infect Dis. 2006; 193(6): 792-5. doi: 10.1086/500469.
20. Wu LP, Wang NC, Chang YH, et al. Duration of antibody responses after severe acute respiratory syndrome. Emerg Infect Dis. 2007; 13(10): 1562-4. doi: 10.3201/eid1310.070576.
21. Mousa ZS, Abdulamir AS. Application and validation of SARS-CoV-2 RBD neutralizing ELISA assay. Arch Razi Inst. 2022; 77(1): 391-402. doi: 10.22092/ARI.2021.356677.1890.
22. Fafi-Kremer S, Bruel T, Madec Y, et al. Serologic responses to SARS-CoV-2 infection among hospital staff with mild disease in eastern France. EBioMedicine. 2020; 59: 102915. doi: 10.1016/j.ebiom.2020.102915.
23. Bruni M, Cecatiello V, Diaz-Basabe A, et al. Persistence of anti-SARS-CoV-2 antibodies in non-hospitalized COVID-19 convalescent health care workers. J Clin Med. 2020; 9(10): 3188. doi: 10.3390/jcm9103188.
24. Haveri A, Ekström N, Solastie A, et al. Persistence of neutralizing antibodies a year after SARS-CoV-2 infection in humans. Eur J Immunol. 2021; 51(12): 3202-13. doi: 10.1002/eji.202149535.
25. Zhao J, Yuan Q, Wang H, et al. Antibody responses to SARS-CoV-2 in patients with novel Coronavirus

disease 2019. Clin Infect Dis. 2020; 71(16): 2027-34. doi: 10.1093/cid/ciaa344.

26. Wu F, Wang A, Liu M, et al. Neutralizing antibody responses to SARS-CoV-2 in a COVID-19 recovered patient cohort and their implications. medRxiv 2020. 20047365; doi: https://doi.org/10.1101/2020.03.30.20047365.

27. Smith KG, Hewitson TD, Nossal GJ, et al. The phenotype and fate of the antibody-forming cells of the splenic foci. Eur J Immunol. 1996; 26(2): 444-8. doi: 10.1002/eji.1830260226.

28. L'Huillier AG, Meyer B, Andrey DO, et al. Antibody persistence in the first 6 months following SARS-CoV-2 infection among hospital workers: A prospective longitudinal study. Clin Microbiol Infect. 2021; 27(5): 784.e1–8. doi: 10.1016/j.cmi.2021.01.005.

29. Selhorst P, van Ierssel SH, Michiels J, et al. Symptomatic severe acute respiratory syndrome coronavirus 2 reinfection of a healthcare worker in a Belgian nosocomial outbreak despite primary neutralizing antibody response. Clin Infect Dis. 2021; 73(9): e2985-91. doi: 10.1093/cid/ciaa1850.

30. Iyer AS, Jones FK, Nodoushani A, et al. Persistence and decay of human antibody responses to the receptor binding domain of SARS-CoV-2 spike protein in COVID-19 patients. Sci Immunol. 2020; 5(52): eabe0367. doi: 10.1126/sciimmunol.abe0367.

31. Ibarrondo FJ, Fulcher JA, Goodman-Meza D, et al. Rapid decay of anti-SARS-CoV-2 antibodies in persons with mild Covid-19. N Engl J Med. 2020; 383(11): 1085-7. doi: 10.1056/NEJMc2025179.

32. Ahmadian S, Fathizadeh H, Shabestari Khiabani S, et al. COVID-19 reinfection in a healthcare worker after exposure with high dose of virus: A case report. Clin Case Rep. 2021; 9(6): e04257. doi: 10.1002/ccr3.4257.

33. Shastri J, Parikh S, Aggarwal V, et al. Severe SARS-CoV-2 breakthrough reinfection with delta variant after recovery from breakthrough infection by alpha variant in a fully vaccinated health worker. Front Med (Lausanne). 2021; 8: 737007. doi: 10.3389/fmed.2021.737007.

34. Hall VJ, Foulkes S, Charlett A, et al. SARS-CoV-2 infection rates of antibody-positive compared with antibody-negative health-care workers in England: A large, multicentre, prospective cohort study (SIREN). Lancet. 2021; 397(10283): 1459-69. doi: 10.1016/S0140-6736(21)00675-9.

35. Lumley SF, Wei J, O'Donnell D, et al. The duration, dynamics, and determinants of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) antibody responses in individual healthcare workers. Clin Infect Dis. 2021; 73(3): e699-e709. doi: 10.1093/cid/ciab004.

36. To KK, Hung IF, Ip JD, et al. Coronavirus disease 2019 (COVID-19) re-infection by a phylogenetically distinct severe acute respiratory syndrome coronavirus 2 strain confirmed by whole genome sequencing. Clin Infect Dis. 2021; 73(9): e2946-51. doi: 10.1093/cid/ciaa1275.

Correspondence to Zeena S. Mousa
E-mail: z.s.mousa1991@gmail.com
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Assessment of Vitamin A versus Vitamin E effect on Motility and DNA Integrity of Human Cryopreserved Sperms

Samir A. Al-Anbari[1] *MSc*, **Mustafa M. Ibraheem**[2] *PhD*, **Thaer M. Farhan**[2] *FIBMS*

[1]High Health Institute, Medical City Directorate, Ministry of Health, Baghdad, Iraq, [2]Dept. of Human Anatomy, College of Medicine, University of Al-Mustansiriyah, Baghdad, Iraq, [3]Dept. of Human Anatomy, College of Medicine, Al-Nahrain University, Baghdad, Iraq

Abstract

Background It is well known that cryopreservation procedure may result in negative impact on spermatozoa function throughout relative overproduction of reactive oxygen species (ROS). However, ROS overproduction can be corrected by antioxidants.

Objective To find out the effect of vitamin A on cryopreserved spermatozoa throughout measuring their motility rate and DNA fragmentation and compare it with vitamin E effect.

Methods Forty seminal fluid specimens were individually collected from forty healthy, non-drug and non-alcohol consumers, normozoospermic males in Um Al-Baneen Center for Infertility Management and In Vitro Fertilization in Baghdad. To facilitate comparing between used vitamins and experimenting each one alone, those specimens were distributed into 4 groups of 10 specimens each. Following deriving their own controls without any vitamin treatment, specimens of these groups, in general, were treated with two concentrations of vitamin A (20 µg/dl and 30 µg/dl), and two concentrations of vitamin E (10 µmol/l and 20 µmol/l). Then, each specimen was incubated for 1 hour before being cryopreserved in liquid nitrogen for 14 days. Motility percentage and DNA fragmentation were assessed following cryopreservation and thawing of spermatozoa.

Results Results revealed that there were significant statistically differences in post-thawing motility and DNA fragmentation means between specimens treated with vitamin A and their relevant control; between specimens treated with vitamin E and their relevant control; and between specimens treated with vitamin E and those treated with vitamin A among all groups of the study.

Conclusion These results lead to the conclusion that both of vitamin A and E play an important role in improving and protecting sperm motility and DNA integrity following cryopreservation, and vitamin E is more effective than vitamin A.

Keywords Seminal fluid analysis, vitamin A, vitamin E, reactive oxygen species, sperm cryopreservation, sperm DNA fragmentation, sperm motility assessment

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List of abbreviations: ART = Assisted reproductive technology, dUTP = deoxyuridine triphosphate, ROS = Reactive oxygen species, SCSA = Sperm Chromatin Structure Assay, SCD = Sperm chromatin dispersion; SFA = Seminal fluid analysis TdT= Terminal deoxynucleotidyl transferase, TUNEL = Terminal deoxynucleotidyl transferase – Mediated deoxyuridine triphosphate Nick-End Labeling

Introduction

In spite of the advances in the field of assisted reproductive technology (ART) nowadays [1-7], infertility still constitutes a big challenge in medicine since it affects about 48 million couples and about 186 million individuals live with infertility all over the world

[8]. It has many variable classifications [8-13], and has multiple etiological factors [14-17]. One of the most common etiological factors of male infertility is relative overproduction of reactive oxygen species (ROS) in relation to antioxidant activity since it accounts for 30-80% of male infertility cases [18-20]. ROS overproduction leads to increased oxidative stress, which can adversely affect various types of molecules within spermatozoa such as nucleic acids, lipids, and proteins resulting in DNA fragmentation, which subsequently lead to their apoptosis, lipid peroxidation in sperm plasma membrane, which accounts for motility defect represented by asthenozoospermia, and denaturation of the enzymes that finally lead to abnormal spermatogenesis represented by teratozoospermia [19,21,22]. Cryopreservation is considered as one of the extrinsic physical stress factors that account for overproduction of ROS [23-25]. In the same time, cryopreservation constitutes an important procedure used in ART since it keeps family chance to acquire children when the male partner requires chemotherapy, radiotherapy, or radical surgery; or when the male partner is azoospermic and exposed to high risk of testicular damage following testicular sperm extraction [5,25]. This urges researchers to experiment adding antioxidants into seminal fluid and follow up its effect on sperm parameters specifically following cryopreservation. It has been found that vitamin E and D improve and protect motility and DNA integrity of sperms following cryopreservation [26-28].

Regarding to vitamin A, it has been found that sperms of human ejaculate retain high levels of retinyl palmitate and stearate besides retaining retinyl hydrolase enzyme [29,30]. It has been shown that seminal plasma level of vitamin A in normozoospermic men constitutes about 19.1 (±2.5) µg/dl, while level of vitamin A in seminal plasma of oligozoospermic men constitutes about 10.4 (±1.8) µg/dl [31]. These collectively indicates to the role of vitamin A in sperm function. However, little studies were done to investigate the role of vitamin A in improving sperm parameters. These studies, in general, depended the oral route to supplement this vitamin to seminal fluid to improve sperm parameters and did not experiment the possibility of being added directly into it that facilitates its usage in ART. Besides what is preceded, these studies did not assess the role of vitamin A in improving sperm parameters following cryopreservation [32-34].

Methods of DNA fragmentation assessment can be categorized into: direct assessment methods such as TUNEL test and COMET test, and indirect assessment methods such as Sperm Chromatin Structure Assay (SCSA) and Sperm Chromatin Dispersion (SCD) [35,36]. However, TUNEL test is widely used because of its high valuable indication to DNA fragmentation and, in turn, to male infertility [36]. The word 'TUNEL' represents the abbreviation of Terminal deoxynucleotidyl transferase (TdT) – Mediated deoxyuridine triphosphate (dUTP) Nick-End Labeling [35]. Its principle is based on activation of endonuclease enzymes between nucleosomes when cells begin to undergo apoptosis. This results in genomic DNA cleavage which, in turn, exposes the cleaved site (3'-OH) to react with a fluorescein deoxyuridine triphosphate as a result of the catalytic effect of TdT. Then, cells are examined by fluorescence microscopy [37].

Undergoing seminal fluid analysis (SFA), motility can be assessed using light microscope with magnification power of (X200) or (X400) (38). The four – grade system is considered as the most convenient system to classify sperms according to velocity of their motility. It consists of four grades of sperm movement. Grade (A) represents rapidly progressive spermatozoa, which move actively either linearly or in a large circle, covering a distance, from the starting point to the end point, of at least 25 µm (equal to ½ tail length) in one second. Grade (B) represents slowly progressive spermatozoa, which move actively either linearly or in a large circle, covering a distance, from the starting point to the end

point, of 5 to <25 µm (or at least one head length to less than ½ tail length) in one second. Grade (C) represents non-progressive spermatozoa that include all other patterns of active tail movements that are associated with an absence of progression; i.e., swimming in small circles, such as the flagellar force displacing the head less than 5 µm (one head length), from the starting point to the end point, in one second. While, grade (D) represents immotile spermatozoa, which reveal no active tail movement. Summation of grade (A), (B), and (C) represents the motility rate [38-41].

The of objective of this study was trying to find a clinical basis to overcome stressful effect of cryopreservation and, thus, improve ART in cases requiring sperm cryopreservation, objectives of this study aim to find out the effect of vitamin A on cryopreserved spermatozoa throughout measuring their motility rate and DNA fragmentation and compare it with vitamin E effect.

Methods

This study was intended to be a prospective experimental analytic study. Its plan was determined to collect a number of specimens of seminal fluid from a number of randomly selected participants, in which each participant gave only one specimen following an abstinence of 2-5 days. Forty participants in this study were randomly selected from Um Al-Baneen Center for Infertility Management and In Vitro Fertilization, Al-Imamein Al-Kadhimein Medical City in Baghdad. Inclusion criteria of participants in this study included being less than 50 years old, healthy, non-smokers, non-drug consumers, and non-alcohol consumers, and having normozoospermic seminal fluid criteria with volume more than 2 ml for each specimen.

All seminal fluid specimens were subjected to same conditions. Following assessing their first SFA, all specimens then were incubated at room temperature (22-27˚C) preparing for experimental groups derivation. The collected specimens were subdivided according to their volumes into two categories. Using vitamin A (Central Drug House (P) Ltd.; India) and vitamin E (HIMedia Laboratories Pvt. Ltd.; India), each category was managed according to possibility to derive the main experimental groups of this study from it.

Representing the first category, the collected specimens with volume ranging between >2 ml and 2.5 ml were subjected to procedures by which vitamin A treated group (Group I) and vitamin E treated group (Group II) were derived. These procedures included dissolving a quantity of vitamin A or vitamin E in about 500 µl seminal plasma derived from centrifugation of seminal fluid to form vitamin A solution with a concentration approximately equal to 0.1 µg/µl or vitamin E solution with a concentration approximately equal to 4 µg/µl respectively. Then, 1 µl and 1.5 µl of vitamin A solution from each specimen were well-mixed with two partitions of about 500 µl of seminal fluid each preparing for two specimens with 20 µg/dl and 30 µg/dl concentrations of vitamin A respectively; while, 0.5 µl and 1 µl of vitamin E solution from each specimen were well-mixed with two partitions of about 500 µl seminal fluid each preparing for two specimens with 10 µmol/l and 20 µmol/l concentrations of vitamin E respectively.

Representing the second category, the collected specimens with volume >2.5 ml were subjected to procedures by which low concentrations vitamin A and E treated group (Group III) and high concentrations vitamin A and E treated group (Group IV) were derived. These procedures were similar to those deriving Group (I) and group (II) except that preparation of vitamin solution in each of group III and IV required derivation of two partitions of seminal plasma of about 500 µl each, which were derived from each specimen in the group in order to dissolve each vitamin individually.

Following vitamin supplemented subgroup derivation, each experimental group would be consisted of three subgroups: one of them representing the control, which was not supplemented with vitamins and the other two were supplemented with vitamins.

Table (1) briefly demonstrates vitamin treated subgroups preparation, while table (2) demonstrates the finally formed experimental groups.

Table 1. Preparation of vitamin supplemented subgroups

Procedure			Vitamin type	
			Vitamin A	**Vitamin E**
Concentration of vitamin used in the beginning of preparation			100 µg/µl	400 µg/µl (approximately equivalent to 1000 µmol/ml)
Preparation of vitamin solutions	Volume of solute (vitamin) taken		0.4 – 0.5 µl	4 – 5 µl
	Volume of solvent (seminal plasma) taken		399.6 – 499.5 µl	396 – 495 µl
	Concentration of vitamin solution formed		0.1 µg/µl (approximately)	4 µg/µl (approximately equivalent to 10 µmol/ml)
Preparation of vitamin treated subgroups	Lower vitamin concentration subgroups	Recommended volume of vitamin solution	1 µl	0.5 µl
		Recommended volume of seminal fluid	499 µl	499.5 µl
		Final vitamin concentration	0.0002 µg/µl (approximately equal to 20 µg/dl)	0.004 µg/µl (approximately equivalent to 10 µmol/l)
	Higher vitamin concentration subgroups	Recommended volume of vitamin solution	1.5 µl	1 µl
		Recommended volume of seminal fluid	498.5 µl	499 µl
		Final vitamin concentration	0.0003 µg/µl (approximately equal to 30 µg/dl)	0.008 µg/µl (approximately equivalent to 20 µmol/l)

Table 2. Finally formed experimental groups

Experimental group	**Group I: vitamin A treated group**	**Group II: vitamin E treated group**	**Group III: Low concentrations vitamin A and vitamin E treated group**	**Group IV: High concentrations vitamin A and vitamin E treated group**
Its constituting subgroups	Control (500 µl)	Control (500 µl)	Control (500 µl)	Control (500 µl)
	Low vitamin A concentration (500 µl)	Low vitamin E concentration (500 µl)	Low vitamin A concentration (500 µl)	High vitamin A concentration (500 µl)
	High vitamin A concentration (500 µl)	High vitamin E concentration (500 µl)	Low vitamin E concentration (500 µl)	High vitamin E concentration (500 µl)

Following their derivation, these experimental groups were subjected to 1 hour incubation at room temperature (22-27°C) before being cryopreserved for 14 days. Following cryopreservation and thawing of sperms, assessment of sperm motility rate, and DNA fragmentation were done for all specimens in all experimental groups.

Motility was assessed by undergoing seminal fluid analysis, and examining spermatozoa by light microscope at (X400) magnification power. While DNA fragmentation was assessed by TUNEL test depending fluorescein isothiocyanate (FITC) stain as the main fluorescent stain, which stains fragmented DNA green, and 4,6-diamidino-2-phenylindole (DAPI) as the contrast stain [37].

Statistical analysis was done using GraphPad Prism 9.3.1 released on 2021. ANOVA and Multiple Comparisons Tuckey's tests were

applied to statistically evaluate results. Significant P value was specified to be <0.05 [28].

Results

Applying ANOVA test and Tukey's multiple comparisons test, statistical studies revealed the following according to each experimental group.

In group (I) as shown in tables (3) and (4), in addition to graphs (1) and (2), it has been shown that there are significant statistical differences in motility rate and DNA fragmentation means between vitamin A treated subgroups and their relevant controls. However, there is no significant statistical difference between lower and higher vitamin A treated subgroups (adjusted P value < 0.05).

This means that motility and DNA preservation is significantly more in vitamin A treated subgroups than in control; while, the difference in motility and DNA preservation between low and high vitamin A subgroups is of no significance in spite of being higher in high vitamin A subgroup.

Table 3. Multiple comparison of post-thawing motility of vitamin A group

Tukey's multiple comparisons test	Mean Diff.	95% CI of diff.	Below threshold?	Summary	Adjusted P Value
Control (post) vs. low vit. A (post)	-13.5	-20.94 to -6.062	Yes	***	0.0003
Control (post) vs. high vit. A (post)	-18.5	-25.94 to -11.06	Yes	****	<0.0001
Low vit. A (post) vs. high vit. A (post)	-5	-12.44 to 2.438	No	ns	0.2362

(***) refers to P value <0.001; while, (****) refers to P value <0.0001

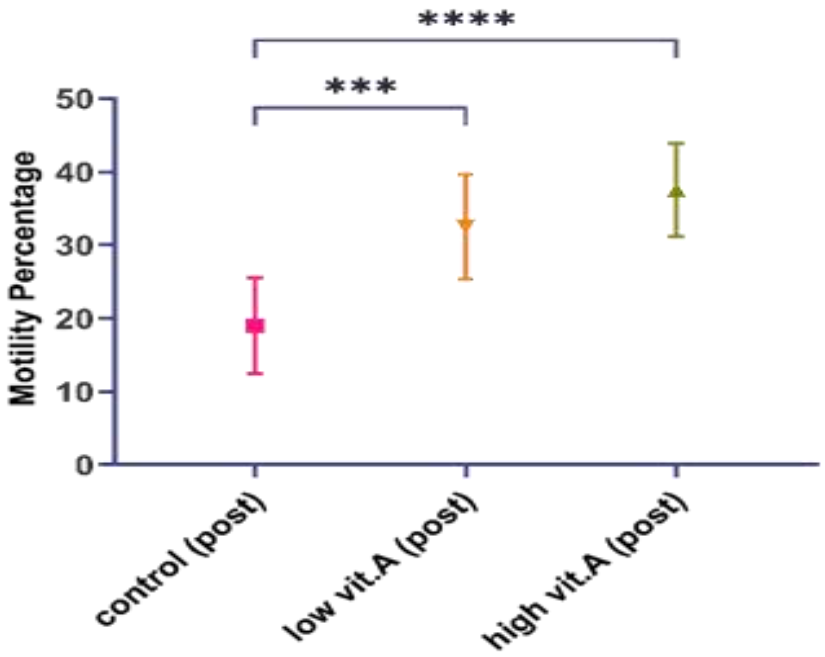

Figure 1. Post-thawing motility percentage assessment and statistical analysis of vitamin A experimental group. It reveals that there are significant differences among its subgroups. The symbol (***) refers to P value <0.001; while, (****) refers to P value <0.0001

Table 4. Multiple comparison test of post-thawing DNA fragmentation of vitamin A group

Tukey's multiple comparisons test	Mean Diff.	95% CI of diff.	Below threshold?	Summary	Adjusted P Value
Control (post) vs. low vit. A (post)	16.6	1.783 to 31.42	Yes	*	0.0258
Control (post) vs. high vit. A (post)	15.4	0.5825 to 30.22	Yes	*	0.0404
Low vit. A (post) vs. high vit. A (post)	-1.2	-16.02 to 13.62	No	ns	0.978

(*) refers to P value <0.05

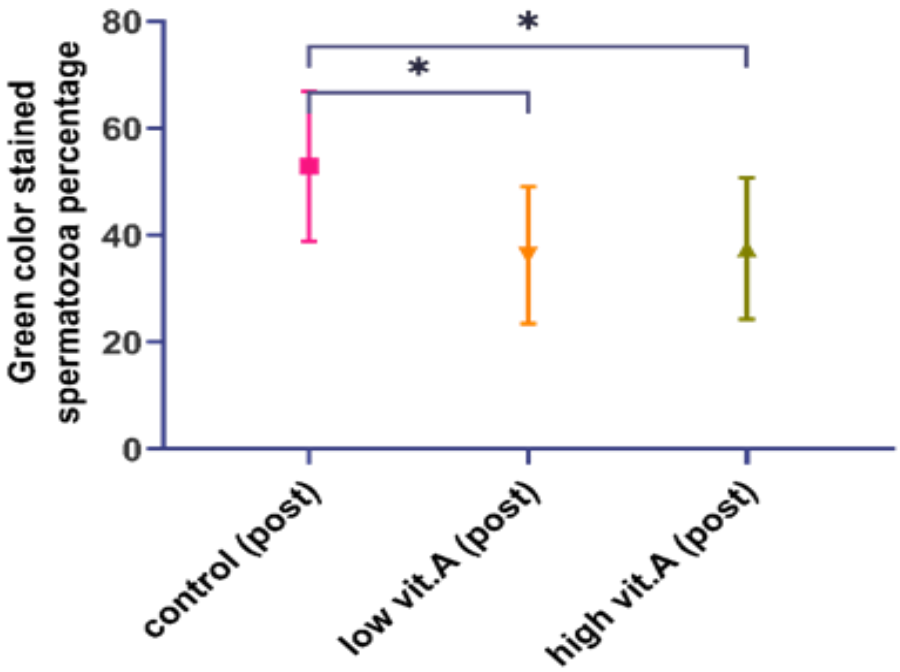

Figure 2. Post-thawing DNA fragmentation assessment and statistical analysis of vitamin A experimental group. It reveals that there are significant differences among its subgroups. The symbol (*) refers to P value <0.05

In group (II) as shown in table (5) and (6), in addition to graph (3) and (4), it has been shown that there are significant statistical differences in motility rate and DNA fragmentation means between vitamin E treated subgroups and their relevant controls (adjusted P value <0.05). However, there is no significant statistical difference between lower and higher vitamin E treated subgroups.

This means that motility and DNA preservation is significantly more in vitamin E treated subgroups than in control; while, the difference in motility and DNA preservation between low and high vitamin E subgroups is of no significance in spite of being higher in high vitamin E subgroup.

Table 5. Multiple comparison test of post-thawing vitamin E group motility

Tukey's multiple comparisons test	Mean Diff.	95% CI of diff.	Below threshold?	Summary	Adjusted P Value
Control (post) vs. low vit. E (post)	-13.5	-20.13 to -6.871	Yes	****	<0.0001
Control (post) vs. high vit. E (post)	-15.5	-22.13 to -8.871	Yes	****	<0.0001
Low vit. E (post) vs. high vit. E (post)	-2	-8.629 to 4.629	No	ns	0.7374

(***) refers to P value <0.001; while, (****) refers to P value <0.0001

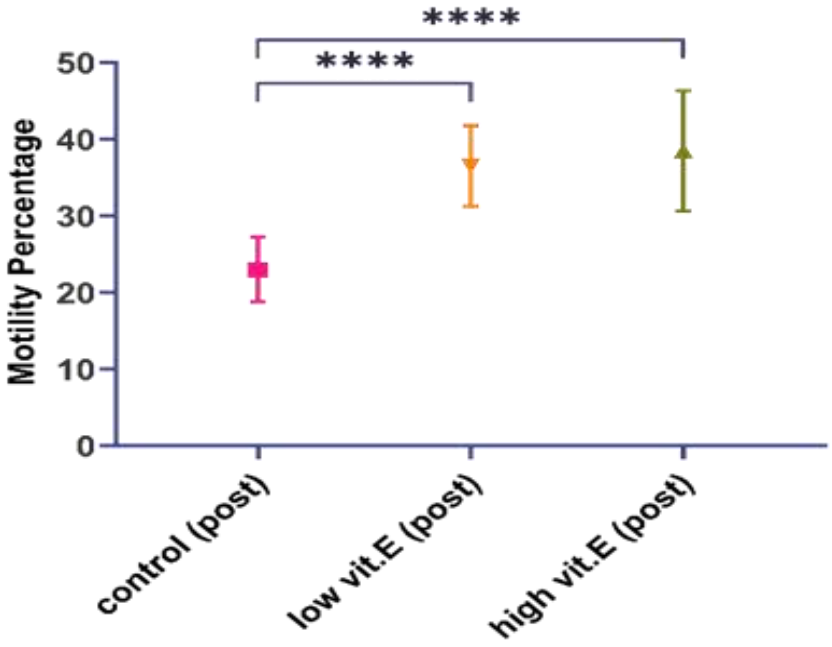

Figure 3. Post-thawing motility assessment and statistical analysis of vitamin E experimental group. It reveals that there are significant differences among its subgroups. The symbol (****) refers to P value <0.0001

Table 6. Multiple comparison test of post-thawing vitamin E group DNA fragmentation assessment

Tukey's multiple comparisons test	Mean Diff.	95% CI of diff.	Below threshold?	Summary	Adjusted P Value
Control (post) vs. low vit. E (post)	22.5	13.25 to 31.75	Yes	****	<0.0001
Control (post) vs. high vit. E (post)	23	13.75 to 32.25	Yes	****	<0.0001
Low vit. E (post) vs. high vit. E (post)	0.5	-8.746 to 9.746	No	ns	0.9901

(***) refers to P value <0.001; while, (****) refers to P value <0.0001

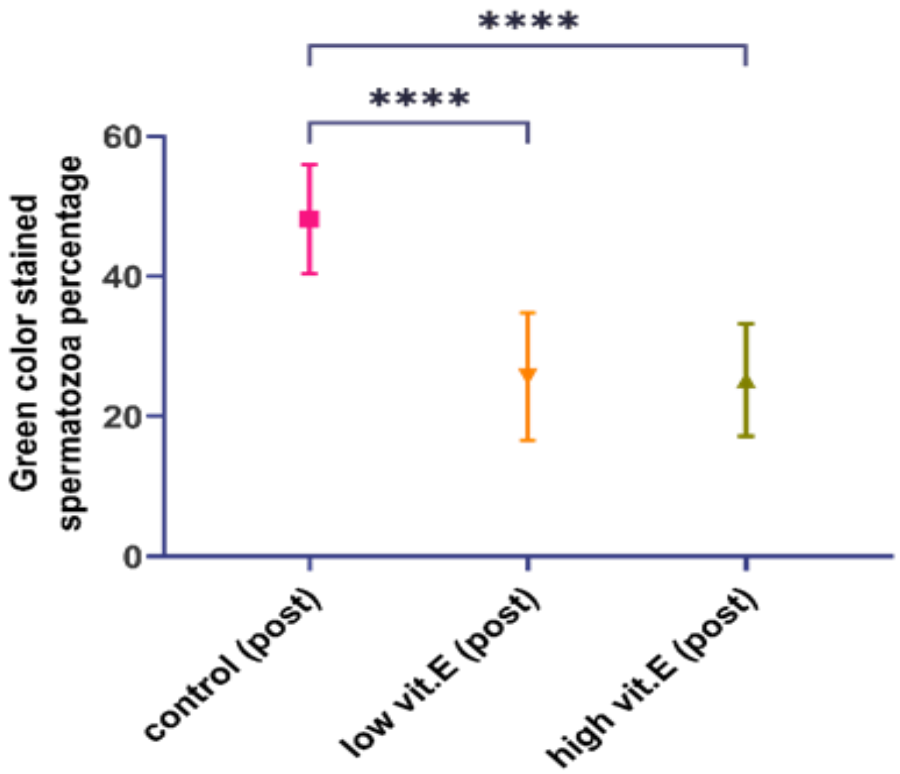

Figure 4. Post-thawing DNA fragmentation assessment and statistical analysis of vitamin E experimental group. It reveals that there are significant differences among its subgroups. The symbol (****) refers to P value <0.0001

In group (III) as shown in table (7) and (8), in addition to graph (5) and (6), it has been shown that there are significant statistical differences in motility rate and DNA fragmentation means between lower concentrations vitamin A and E treated subgroups and their relevant control, and between motility rate means of vitamin A treated subgroup and vitamin E treated subgroup (adjusted P value <0.05). However, there is no significant statistical difference between DNA fragmentation means of vitamin A treated subgroup and vitamin E treated subgroup.

This means that motility and DNA preservation is significantly more in low vit. E and low vit. A treated subgroups than in control, and significantly more in low vit. E treated subgroups than in low vitamin A treated subgroups.

Table 7. Multiple comparison of post-thawing low concentration vitamin A and E treated group motility assessment

Tukey's multiple comparisons test	Mean Diff.	95% CI of diff.	Below threshold?	Summary	Adjusted P Value
Control (post) vs. low vit. A (post)	-7.8	-14.74 to -0.8635	Yes	*	0.0252
Control (post) vs. low vit. E (post)	-16.5	-23.44 to -9.564	Yes	****	<0.0001
Low vit. A (post) vs. low vit. E (post)	-8.7	-15.64 to -1.764	Yes	*	0.0118

(***) refers to P value <0.001; while, (****) refers to P value <0.0001

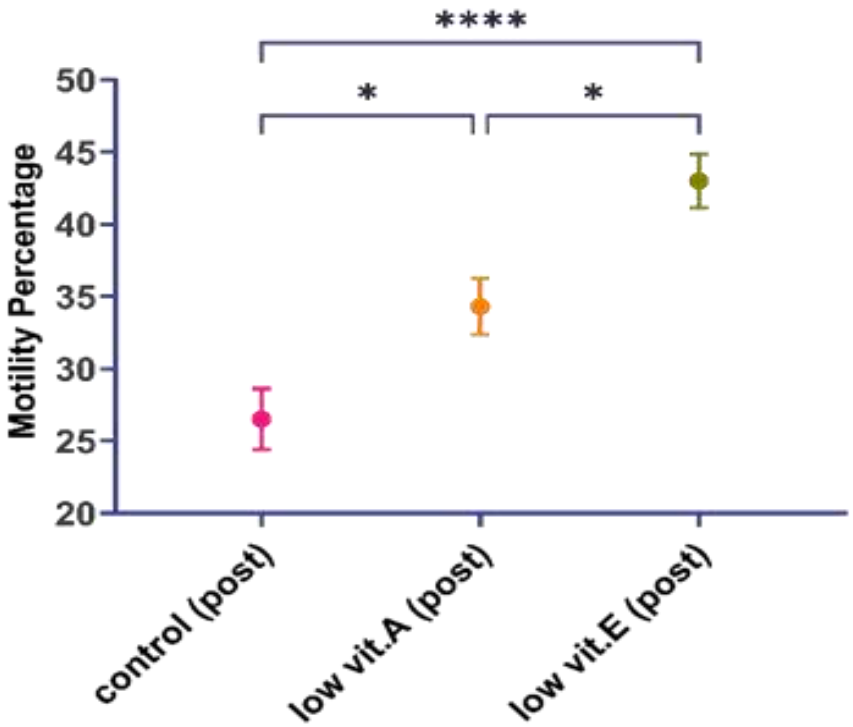

Figure 5. Post-thawing motility assessment and statistical analysis of low concentration vitamin A and E treated experimental group. It reveals that there are significant differences among its subgroups. The symbol (*) refers to P value <0.05; while, (****) refers to P value <0.0001

Table 8. Multiple comparison test of post-thawing low concentration vitamin A and E treated group DNA fragmentation

Tukey's multiple comparisons test	Mean Diff.	95% CI of diff.	Below threshold?	Summary	Adjusted P Value
Control (post) vs. low vit. A (post)	12.1	1.382 to 22.82	Yes	*	0.0245
Control (post) vs. low vit. E (post)	22.7	11.98 to 33.42	Yes	****	<0.0001
Low vit. A (post) vs. low vit. E (post)	10.6	-0.1178 to 21.32	No	ns	0.053

The symbol (*) refers to P value <0.05; while, (****) refers to P value <0.0001

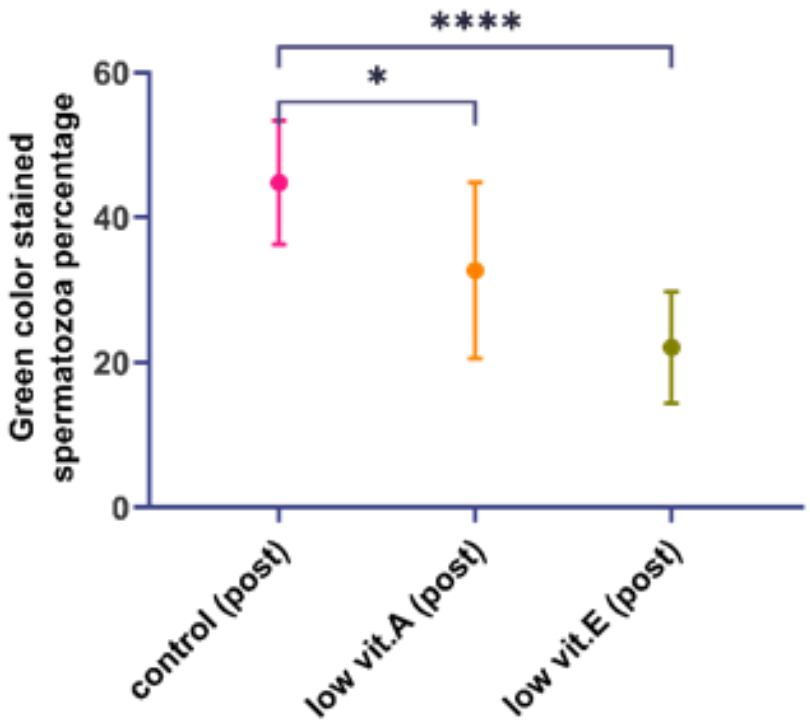

Figure 6. Post-thawing DNA fragmentation assessment and statistical analysis of low concentration vitamin A and E treated group. It reveals that there are significant differences among its subgroups. The symbol (*) refers to P value <0.05; while, (****) refers to P value <0.0001

In group (IV) as shown in tables (9) and (10) in addition to graphs (7) and (8), it has been shown that there are statistically significant differences in motility rate and DNA fragmentation means between higher concentrations vitamin E treated subgroup and their relevant control (adjusted P value < 0.05); and between motility means of vitamin A treated subgroup and its relevant control (adjusted P value <0.05). However, there is no statistically significant differences in motility rate and DNA fragmentation means between vitamin A treated subgroup and vitamin E treated subgroup, and between DNA fragmentation means of vitamin A treated subgroup and its relevant control.

This means that motility and DNA preservation is significantly more in high vitamin E and high

vitamin A treated subgroups than in control, and significantly more in low vitamin E treated subgroups than in high vit A treated subgroups.

Table 9. Multiple comparison test of post-thawing motility of high concentration vitamin A and E group

Tukey's multiple comparisons test	Mean Diff.	95% CI of diff.	Below threshold?	Summary	Adjusted P Value
Control (post) vs. high vit. A (post)	-8.8	-16.09 to -1.513	Yes	*	0.0156
Control (post) vs. high vit. E (post)	-15.5	-22.79 to -8.213	Yes	****	<0.0001
High vit. A (post) vs. high vit. E (post)	-6.7	-13.99 to 0.5870	No	ns	0.076

The symbol (*) refers to P value <0.05; while, (****) refers to P value <0.0001

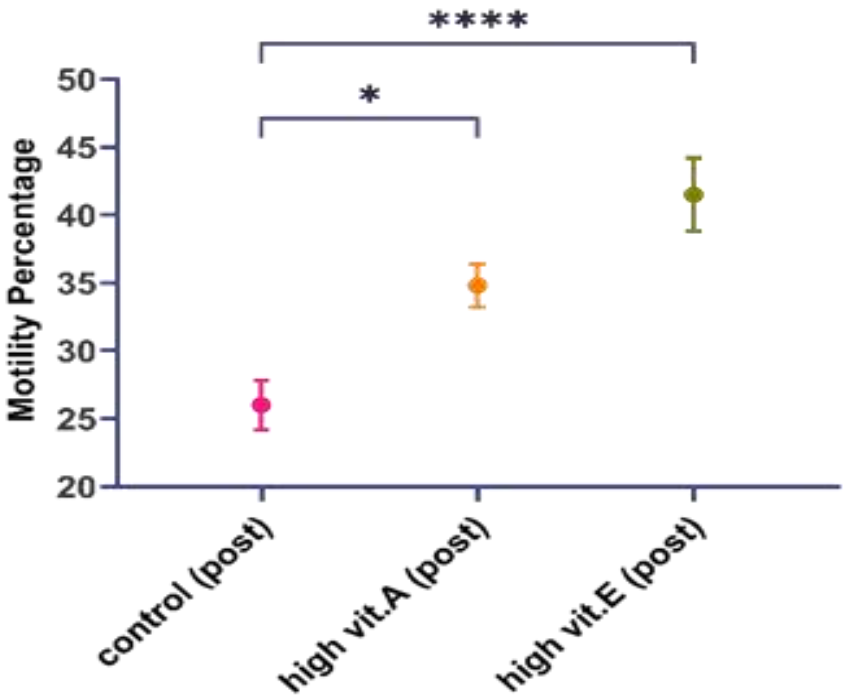

Figure 7. Post-thawing motility assessment and statistical analysis of high concentration vitamin A and E treated experimental group. It reveals that there are significant differences among its subgroups. The symbol (*) refers to P value <0.05; while, (****) refers to P value <0.0001

Table 10. Multiple comparison test of post thawing high concentration vitamin A and E treated group DNA fragmentation

Tukey's multiple comparisons test	Mean Diff.	95% CI of diff.	Below threshold?	Summary	Adjusted P Value
Control (post) vs. high vit. A (post)	9.6	-4.494 to 23.69	No	ns	0.2277
Control (post) vs. high vit. E (post)	16.1	2.006 to 30.19	Yes	*	0.0227
High vit. A (post) vs. high vit. E (post)	6.5	-7.594 to 20.59	No	ns	0.4964

The symbol (*) refers to P value < 0.05

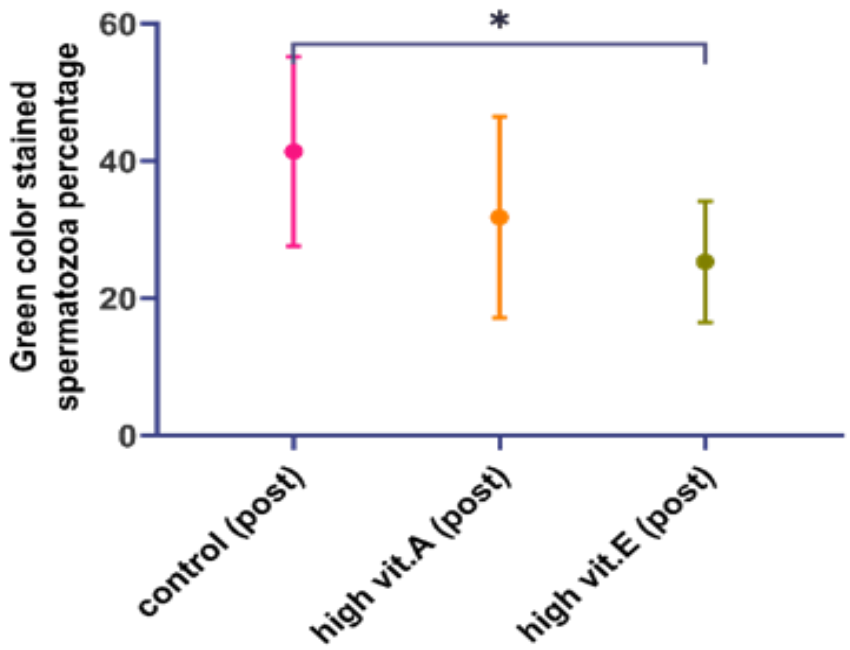

Figure 8. Post-thawing DNA fragmentation assessment and statistical analysis of high concentration vitamin A and E treated group. It reveals that there is significant difference among its subgroups. The symbol (*) refers to P value < 0.05

Figures (9), (10), (11), (12), (13), (14), and (15) reveal TUNEL test showing DNA fragmentation of spermatozoa of different subgroups.

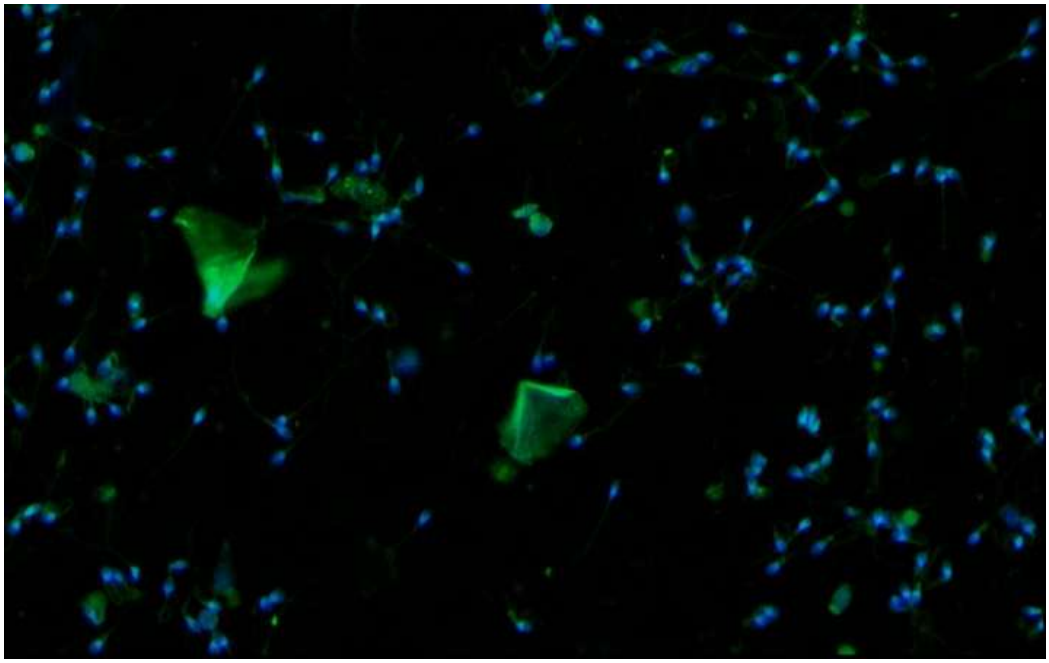

Figure 9. This figure represents demonstration for TUNEL test, in which the seminal fluid sample was stained with the main fluorescent DNA stain (FITC) and the counter stain (DAPI); and reveals the presence of spermatozoa, round cells and epithelial cells. Magnification power was (X200)

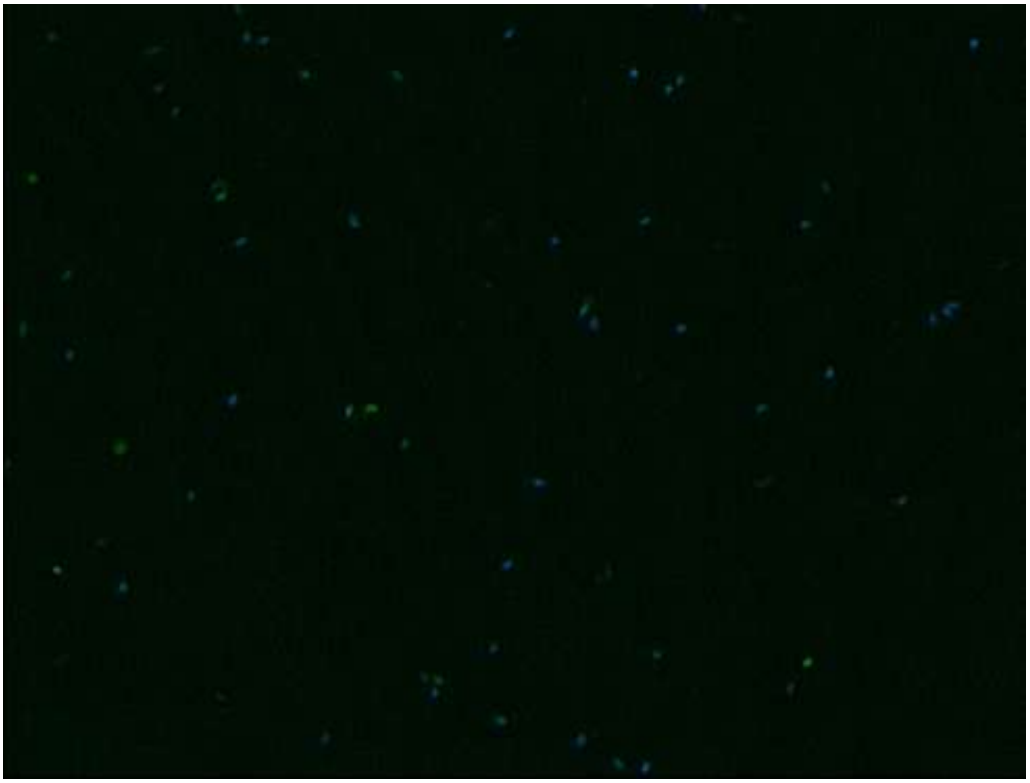

Figure 10. This figure demonstrates TUNEL test undergone for a post-thawing control sample. Magnification power was (X100)

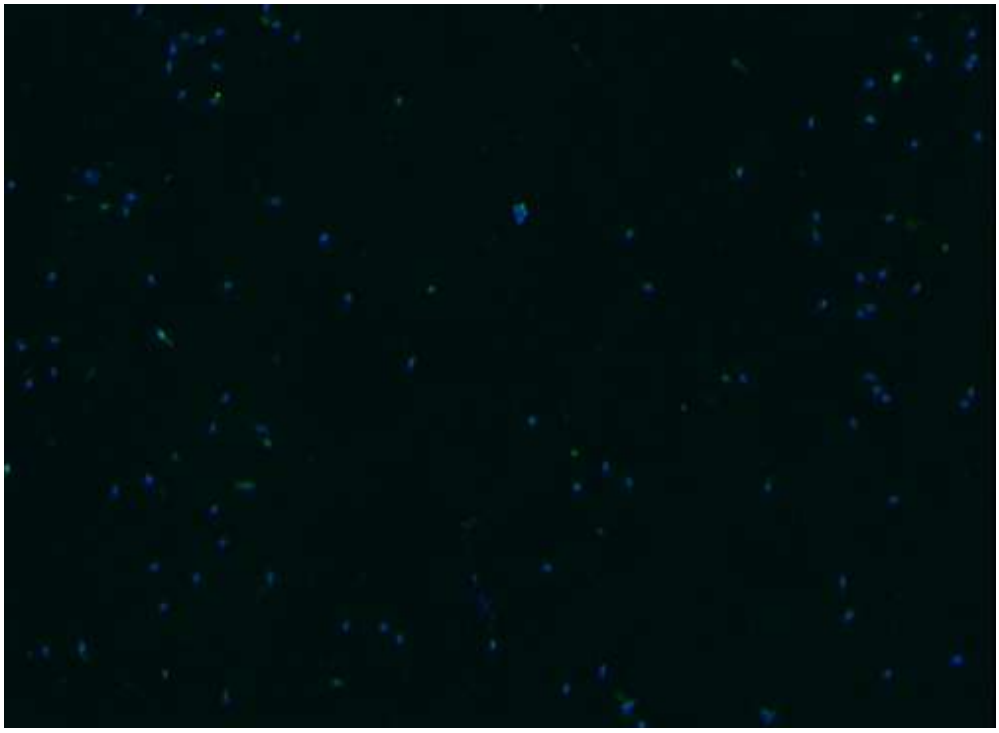

Figure 11. This figure demonstrates TUNEL test undergone for a sample from post-thawing low concentration vitamin A treated group. Magnification power was (X100)

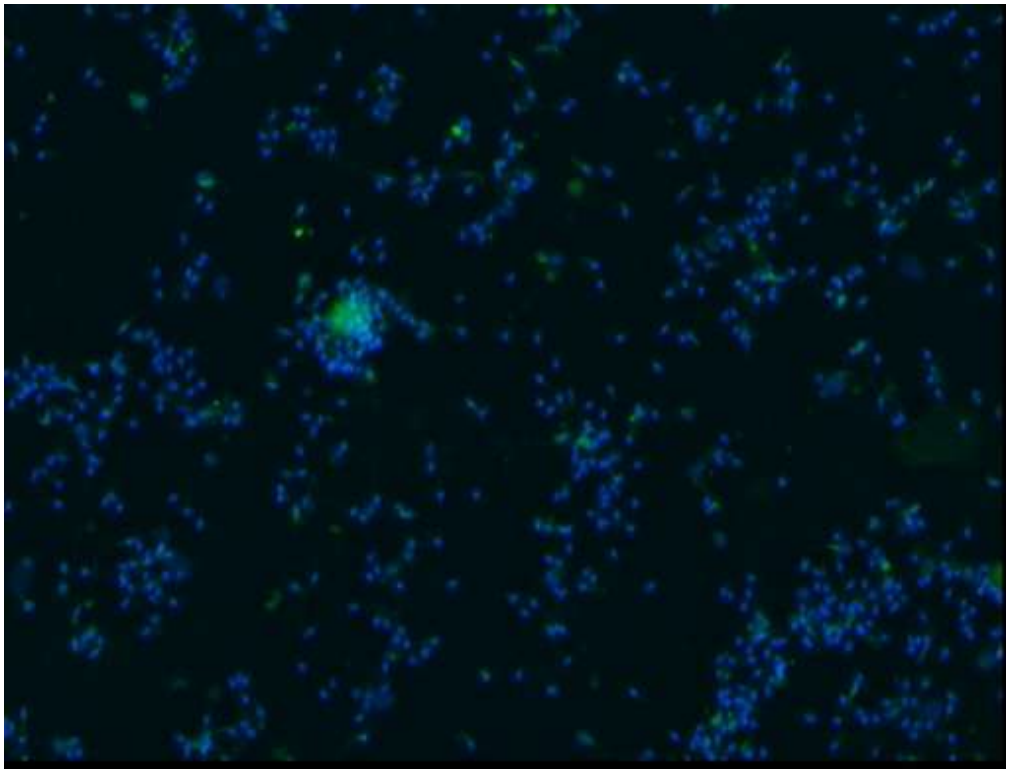

Figure 12. This figure demonstrates TUNEL test undergone for a sample from post-thawing low concentration vitamin E treated group. This sample reveals high number of spermatozoa. Magnification power was (X100)

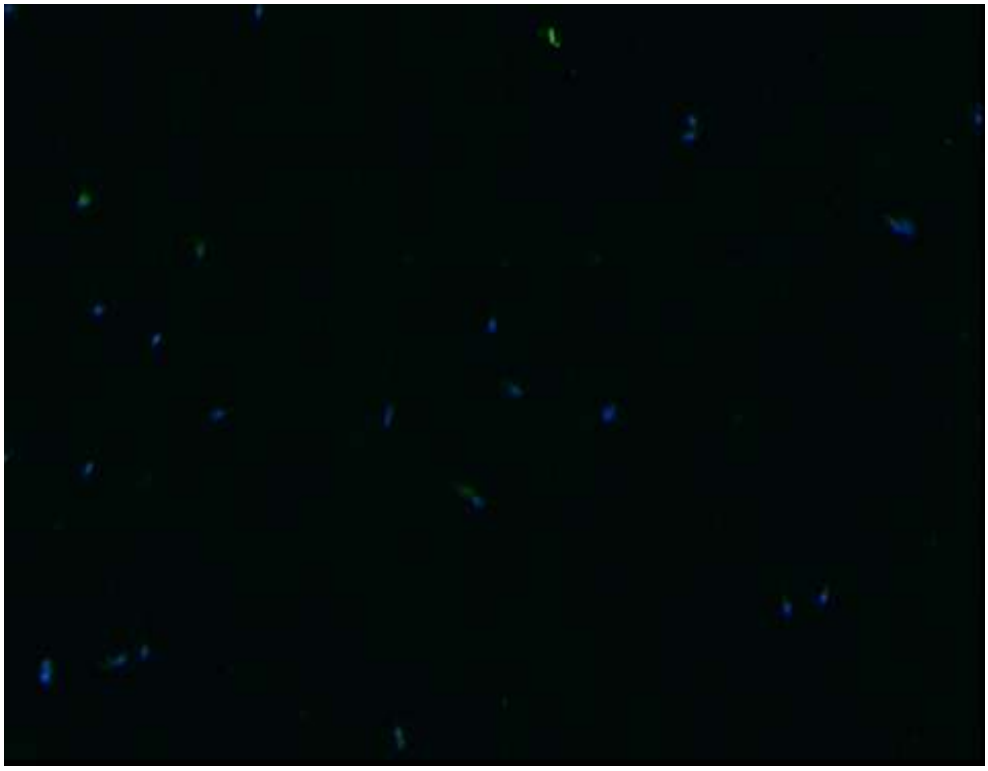

Figure 13. This figure demonstrates TUNEL test undergone for a sample from post-thawing high concentration vitamin A treated group. Magnification power was (X100)

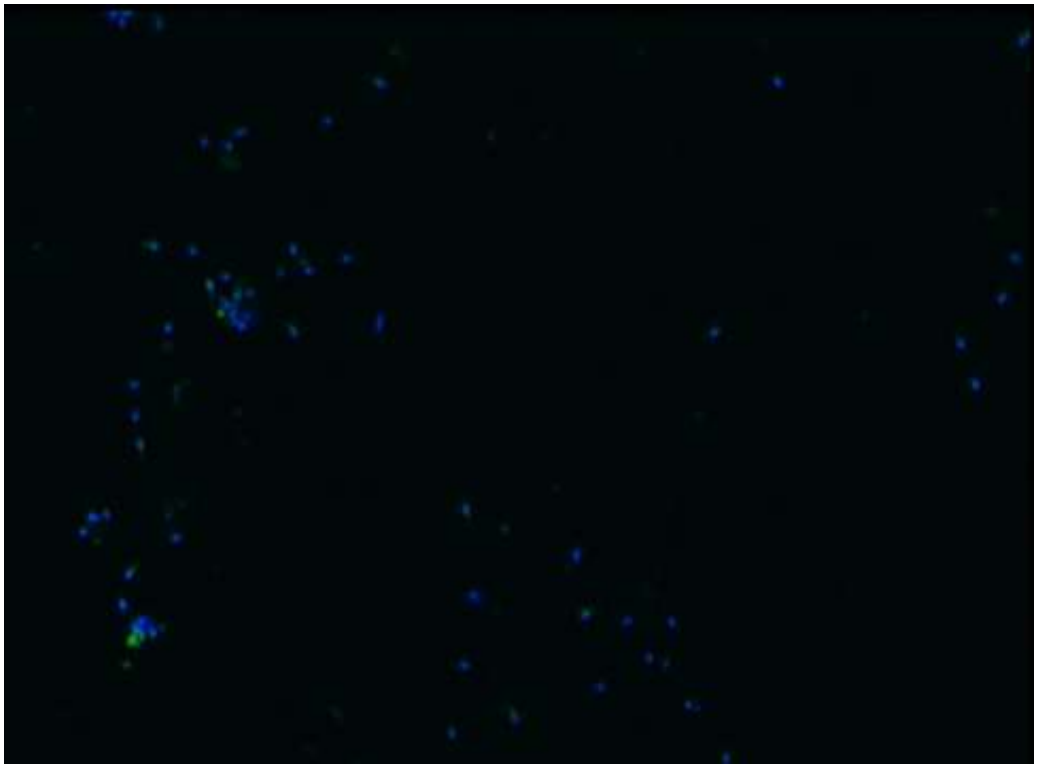

Figure 14. This figure demonstrates TUNEL test undergone for a sample from post-thawing high concentration vitamin E treated group. Magnification power was (X100)

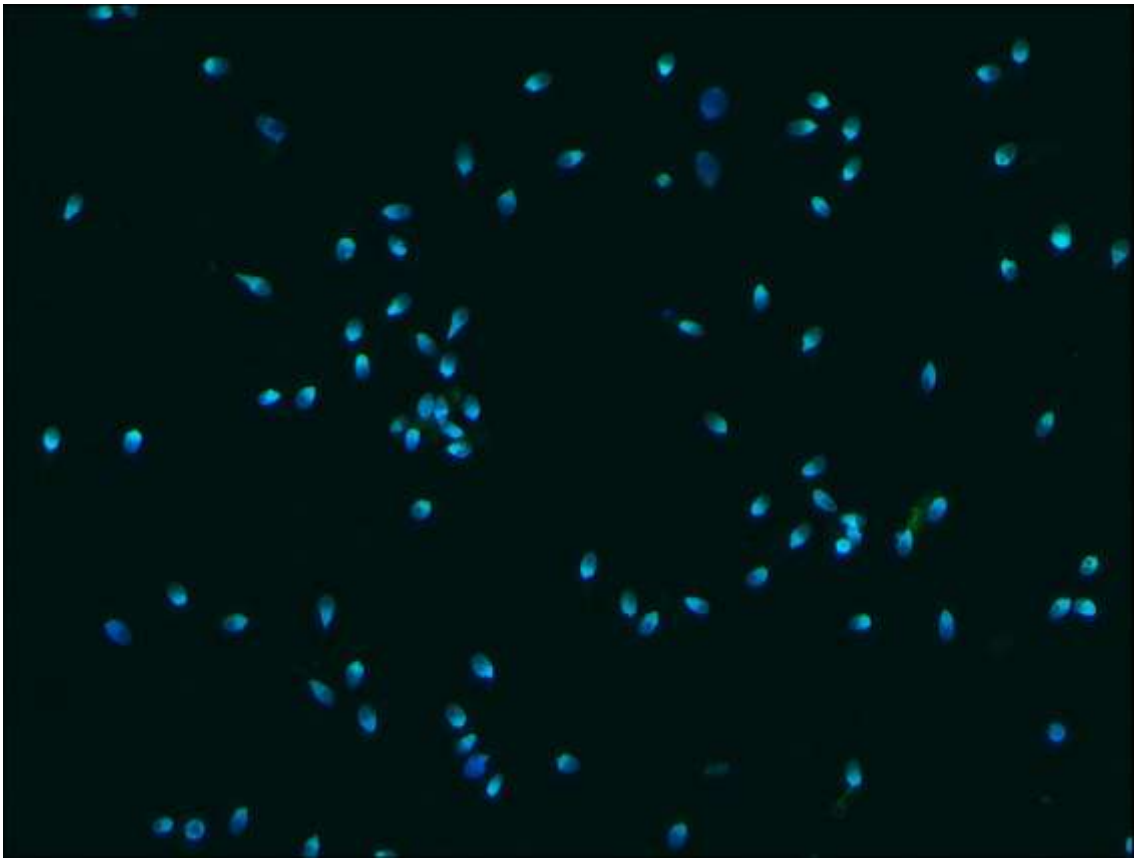

Figure 15. This figure demonstrates TUNEL test undergone for a sample of post-thawing lower concentration vitamin A group, in which spermatozoa were stained by FITC as the main fluorescent stain and DAPI as the counter stain. Magnification power was (X400)

Discussion

In spite of being more possible to be conducted on animal models, this study was not intended to be done on animal models but on human-being since animal model experiments depend on prediction to meet reality while studies done on human-being reflect the real effect of antioxidants on human sperm quality. Depending normozoospermic men in this study, this is in order to minimize the effect of any factor other than ROS to properly assess antioxidant role [28].

This study was designed to investigate the role of vitamin A in maintaining and improving sperm function and to compare it with the role of one of the previously experimented antioxidants such as vitamin E in maintenance of sperm activity, specifically following thawing of cryopreserved sperms since cryopreservation has a negative impact on spermatozoa.

Regarding to their concentrations used in this study, each of vitamin A and vitamin E was experienced with two concentrations, which are in close relation to their normal seminal plasma levels since the concentrations of 20 µg/dl and 10 µmol/L, which represented the lower concentrations used in this study, were approximately equal to the normal upper limits of vitamin A and vitamin E concentrations in seminal plasma of normozoospermic men according to Singer et al. (1982), and Omu et al. (1999) respectively [31,42]. This was intended in order to study the effect of normal upper limits of these vitamins and their slight increment on sperm motility, and DNA fragmentation before and after cryopreservation.

Comparing results of vitamin A treated subgroups with those of their relevant controls,

statistical differences in means of motility rate and DNA fragmentation between them, in general, reveal the effect of vitamin A in improving motility rate and reducing DNA fragmentation of sperms. This agrees with Singer et al. (1982), Pappas et al. (1993), Schreiber et al. (2012), and Ghyasvand et al. (2015), who collectively indicate the role of vitamin A in maintaining and improving sperm function [29-31,34]. However, these significant differences show variable extent among all groups taking their P values in consideration. This is convenient with Nallella et al. (2004), whose findings revealed that the process of cryopreservation could produce significant increment in inter-sample variability in post-thawing sperm parameters in comparison to pre-cryo parameters [43]. Besides that, small sample size could be an additional reason behind this increased variability since it is associated with increased impact of random error, which is in accordance with what is stated by Lee et al. (2015), Thiese et al. (2016) and Andrade et al. (2020) [44-46]. Added to what is preceded, the findings of Le et al. (2019), which revealed that DNA fragmentation index is not strongly correlated with other conventional semen parameters, may also bring for increased variability in statistical differences among groups [47].

Comparing results of vitamin E treated subgroups with those of their relevant controls, statistical differences in means of motility rate and DNA fragmentation between them, in general, reveal the effect of vitamin E in improving motility rate and reducing DNA fragmentation of sperms. This agrees with the findings of Maruoka et al. (2008), Zhu et al. (2011), and Howard et al. (2015), which revealed that vitamin E has a potent antioxidant that scavenges ROS, repairs and protects plasma membrane, and stabilizes it [26,48,49].

Comparing between vitamin A treated subgroups and vitamin E treated subgroups, statistical differences in means of motility rate and DNA fragmentation between them, in general, reveal that vitamin E has more effect in improving sperm function than that of vitamin A. This could be due to the potent dual action of vitamin E as both an ROS scavenger and plasma membrane stabilizer, which agrees with Maruoka et al (2008), and Howard et al. (2011) [48,49].

Comparing between lower concentration vitamin A and higher concentration vitamin A treated subgroups, statistical results revealed that there are no significant differences in motility rate and DNA fragmentation means between them. This may be due to individual baseline plasma level differences in their vitamin A contents as stated by singer et al. (1982) [31], which make variable responses toward addition of the high concentration of vitamin A that either result in improvement of spermatozoa function or suppression in their function due to the toxic effect of vitamin A. This, in turn, make the statistical differences between means of these parameters in the two subgroups small and not significant. This requires performing the study on larger size sample to reach the proper conclusion.

Comparing between lower concentration vitamin E and higher concentration vitamin E treated subgroups, statistical results reveal that there are no significant differences in motility rate and DNA fragmentation means between them. This may be due to individual baseline plasma level differences in their vitamin E contents as stated by Omu et al. (1999) [42], which make, upon addition of high concentration vitamin E, variable responses that either result in improvement of spermatozoa function or relative suppression in their function due to the toxic effect of vitamin E. This, in turn, make the statistical differences between means of the parameters in the two subgroups small and not significant. This requires performing the study on larger size sample to reach the proper conclusion.

Taking these results in consideration, it has been concluded that both concentrations of vitamin A and vitamin E have a vital role in improving and protecting spermatozoa motility and DNA integrity parameters following cryopreservation. However, differences between the low and high concentrations of each vitamin used in this study need to be further assessed; and it is recommended both in terms of safety and efficacy to use low levels

for both vitamin A and vitamin E since both vitamin A and E can be potentially toxic at high concentrations taking in consideration that there is no significant difference in motility and DNA fragmentation between low and high concentrations of these vitamins. In addition to that, vitamin E is shown to be more effective than vitamin A.

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Author contribution

The authors, altogether, conceived and planned the study. The experiment was done by Samir A. Al-Anbari under supervision of Dr. Ibraheem and Dr. Farhan.

Conflict of interest

There is no conflict of interest.

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References

1. Sadler TW. Langman's Medical Embryology. 12th ed. Lippincott Williams & Wilkins; 2012. p. 29-42.
2. Deep JP. Assisted reproductive technology. J Chitwan Med Coll. 2014; 4(7). 1-10. doi: 10.3126/jcmc.v4i1.10840.
3. Southern California Reproductive Center (SCRC). The evolution of fertility treatments and development of IVF. 2016 Cited from: https://blog.scrcivf.com/ivf-the-history-and-evolution-fertility-treatments.
4. Foote RH. The history of artificial insemination: Selected notes and notables. Am Society Animal Sci; 2002. cited from: https://www.asas.org/docs/default-source/midwest/mw2020/publications/footehist.pdf?sfvrsn=59da6c07_0 in 4 Feb 2021.
5. Pegg DE. The history and principles of cryopreservation. Semin Reprod Med. 2002; 20(1): 5-13. doi: 10.1055/s-2002-23515.
6. Gook DA. History of oocyte cryopreservation. Reprod Biomed Online. 2011; 23(3): 281-9. doi: 10.1016/j.rbmo.2010.10.018.
7. Sztein JM, Takeo T, Nakagata N. History of cryobiology, with special emphasis in evolution of mouse sperm cryopreservation. Cryobiology. 2018; 82: 57-63. doi: 10.1016/j.cryobiol.2018.04.008.
8. World Health Organization (WHO). Infertility. 2020. Cited from: https://www.who.int/news-room/fact-sheets/detail/infertility in 5 Feb 2021.
9. Jenkins J, Daya S, Kremer J, et al. European Classification of Infertility Taskforce (ECIT) response to Habbema et al., 'Towards less confusing terminology in reproductive medicine: A proposal. Hum Reprod. 2004; 19(12): 2687-8. doi: 10.1093/humrep/deh499.
10. Aflatoonian A, Baghianimoghadam B, Partovi P, et al. A new classification for female infertility. Clin Exp Obstet Gynecol. 2011; 38(4): 379-81.
11. Lee HD, Lee HS, Park SH, et al. Causes and classification of male infertility in Korea. Clin Exp Reprod Med. 2012; 39(4): 172-5. doi: 10.5653/cerm.2012.39.4.172.
12. Naz M, Kamal M. Classification, causes, diagnosis and treatment of male infertility: A review. Oriental Pharm Experiment Med; 2017; 17, 89-109. https://doi.org/10.1007/s13596-017-0269-7.
13. Sharma A. Male infertility; evidences, risk factors, causes, diagnosis and management in human. Ann Clin Lab Res. 2017; 5(3): 188. doi: 10.21767/2386-5180.1000188.
14. Wong TW, Straus FH, Jones TM, et al. Pathological aspects of the infertile testis. Urol Clin North Am. 1978; 5(3): 503-30.
15. Eisenberg ML, Lipshultz LI. Male infertility. In: Bieber EJ, Sanfilippo JS, Horowitz IR, et al (eds). Clinical gynecology; Section 8 – Infertility and reproductive endocrinology. Cambridge University Press; 2015. p. 898-917.
16. Nargund VH. Effects of psychological stress on male fertility. Nat Rev Urol. 2015; 12(7): 373-82. doi: 10.1038/nrurol.2015.112.
17. Dimitriadis F, Adonakis G, Kaponis A, et al. Pre-testicular, testicular, and post-testicular causes of male infertility. In: Simoni M, Huhtaniemi I (eds). Endocrinology of the testis and male reproduction. Endocrinology. Springer, Cham. 2017. https://doi.org/10.1007/978-3-319-29456-8_33-2 .
18. Ahmadi S, Bashiri R, Ghadiri-Anari A, et al. Antioxidant supplements and semen parameters: An evidence based review. Int J Reprod Biomed. 2016; 14(12): 729-36.
19. Sabeti P, Pourmasumi S, Rahiminia T, et al. Etiologies of sperm oxidative stress. Int J Reprod Biomed. 2016; 14(4): 231-40.
20. Tvrdá E, Massanyi P, and Lukáč N. Physiological and pathological roles of free radicals in male

reproduction. In: Meccariello R (ed). Spermatozoa - Facts and perspectives. InTechl 2018. doi: 10.5772/intechopen.70793.
21. Phaniendra A, Jestadi DB, Periyasamy L. Free radicals: Properties, sources, targets, and their implication in various diseases. Indian J Clin Biochem. 2015; 30(1): 11-26. doi: 10.1007/s12291-014-0446-0.
22. Guthrie HD, Welch GR. Effects of reactive oxygen species on sperm function. Theriogenology. 2012; 78(8): 1700-8. doi: 10.1016/j.theriogenology.2012.05.002.
23. Kim S, Lee YJ, Kim YJ. Changes in sperm membrane and ROS following cryopreservation of liquid boar semen stored at 15 °C. Anim Reprod Sci. 2011; 124(1-2): 118-24. doi: 10.1016/j.anireprosci.2011.01.014.
24. Kim S, Agca C, Agca Y. Effects of various physical stress factors on mitochondrial function and reactive oxygen species in rat spermatozoa. Reprod Fertil Dev. 2013; 25(7): 1051-64. doi: 10.1071/RD12212.
25. Anger JT, Gilbert BR, Goldstein M. Cryopreservation of sperm: indications, methods and results. J Urol. 2003; 170(4 Pt 1): 1079-84. doi: 10.1097/01.ju.0000084820.98430.b8.
26. Zhu Z, Fan X, Lv Y, et al. Vitamin E Analogue Improves rabbit sperm quality during the process of cryopreservation through its antioxidative action. PLoS One. 2015; 10(12): e0145383. doi: 10.1371/journal.pone.0145383.
27. Kalthur G, Raj S, Thiyagarajan A, et al. Vitamin E supplementation in semen-freezing medium improves the motility and protects sperm from freeze-thaw-induced DNA damage. Fertil Steril. 2011; 95(3): 1149-51. doi: 10.1016/j.fertnstert.2010.10.005.
28. Taheri Moghadam M, Asadi Fard Y, Saki G, et al. Effect of vitamin D on apoptotic marker, reactive oxygen species and human sperm parameters during the process of cryopreservation. Iran J Basic Med Sci. 2019; 22(9): 1036-43. doi: 10.22038/ijbms.2019.36258.8634.
29. Pappas RS, Newcomer ME, Ong DE. Endogenous retinoids in rat epididymal tissue and rat and human spermatozoa. Biol Reprod. 1993; 48(2): 235-47. doi: 10.1095/biolreprod48.2.235.
30. Schreiber R, Taschler U, Preiss-Landl K, et al. Retinyl ester hydrolases and their roles in vitamin A homeostasis. Biochim Biophys Acta. 2012; 1821(1): 113-23. doi: 10.1016/j.bbalip.2011.05.001.
31. Singer R, Barnet M, Sagiv M, et al. Vitamin A and beta-carotene content of seminal fluid and sperm of normospermic and oligozoospermic men. Arch Androl. 1982; 8(1): 61-4. doi: 10.3109/01485018208987019.
32. Hogarth CA, Griswold MD. The key role of vitamin A in spermatogenesis. J Clin Invest. 2010; 120(4): 956-62. doi: 10.1172/JCI41303.
33. Ramadhan O, Ian FP. The Biological significance of vitamin a in humans: A review of nutritional aspects and clinical considerations. ScienceJet. 2012; 1: 1-13.
34. Ghyasvand T, Goodarzi MT, Amiri I, et al. Serum levels of lycopene, beta-carotene, and retinol and their correlation with sperm DNA damage in normospermic and infertile men. Int J Reprod Biomed. 2015; 13(12): 787-92.
35. Denton D, Kumar S. Terminal Deoxynucleotidyl Transferase (TdT)-Mediated dUTP Nick-End Labeling (TUNEL) for detection of apoptotic cells in Drosophila. Cold Spring Harb Protoc. 2015; 2015(6): 568-71. doi: 10.1101/pdb.prot086199.
36. Hassanen E, Elqusi K, Zaki H, et al. TUNEL assay: Establishing a sperm DNA fragmentation cut-off value for Egyptian infertile men. Andrologia. 2019; 51(10): e13375. doi: 10.1111/and.13375.
37. Elabscience. TUNEL assay kit (Enhanced FITC): Detection principle. 8th ed. Copyright©2020-2021 Elabscience Biotechnology Inc.; catalog no. E-CK-A334.
38. World Health Organization (WHO). Basic examination/ 2.4. Examination and post examination procedures. In: WHO laboratory manual for the examination and processing of human semen. 6th ed. World Health Organization; 2021. p. 15-63.
39. Vasan SS. Semen analysis and sperm function tests: How much to test? Indian J Urol. 2011; 27(1): 41-8. doi: 10.4103/0970-1591.78424.
40. Campbell MJ, Lotti F, Baldi E, et al. Distribution of semen examination results 2020 - A follow up of data collated for the WHO semen analysis manual 2010. Andrology. 2021; 9(3): 817-22. doi: 10.1111/andr.12983.
41. World Health Organization (WHO). Appendices/ 8.1. Interpretation of semen examination results. In: WHO laboratory manual for the examination and processing of human semen. 6th ed. World Health Organization; 2021. p. 211-3.
42. Omu AE, Fatinikun T, Mannazhath N, et al. Significance of simultaneous determination of serum and seminal plasma alpha-tocopherol and retinol in infertile men by high-performance liquid chromatography. Andrologia. 1999; 31(6): 347-54. doi: 10.1046/j.1439-0272.1999.00296.x.
43. Nallella KP, Sharma RK, Said TM, et al. Inter-sample variability in post-thaw human spermatozoa. Cryobiology. 2004; 49(2): 195-9. doi: 10.1016/j.cryobiol.2004.07.003.
44. Lee DK, In J, Lee S. Standard deviation and standard error of the mean. Korean J Anesthesiol. 2015; 68(3): 220-3. doi: 10.4097/kjae.2015.68.3.220.
45. Thiese MS, Ronna B, Ott U. P value interpretations and considerations. J Thorac Dis. 2016; 8(9): E928-E931. doi: 10.21037/jtd.2016.08.16.
46. Andrade C. Sample Size and its Importance in Research. Indian J Psychol Med. 2020; 42(1): 102-3. doi: 10.4103/IJPSYM.IJPSYM_504_19.
47. Le MT, Nguyen TAT, Nguyen HTT, et al. Does sperm DNA fragmentation correlate with semen parameters? Reprod Med Biol. 2019; 18(4): 390-6. doi: 10.1002/rmb2.12297.

48. Maruoka N, Murata T, Omata N, et al. Effects of vitamin E supplementation on plasma membrane permeabilization and fluidization induced by chlorpromazine in the rat brain. J Psychopharmacol. 2008; 22(2): 119-27. doi: 10.1177/0269881107078487.
49. Howard AC, McNeil AK, McNeil PL. Promotion of plasma membrane repair by vitamin E. Nat Commun. 2011; 2: 597. doi: 10.1038/ncomms1594.

Correspondence to Samir A. Al-Anbari
E-mail: samer_anbari@yahoo.com
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Evaluation the Levels of Melatonin, Glutathione Peroxidase and Superoxide Dismutase Enzymes in Prediabetic Individuals

Zeena A. Abd Ali *PhD*

Dept. of Chemistry and Biochemistry, College of Medicine, Al Nahrain University, Baghdad, Iraq

Abstract

Background Prediabetes is a serious health condition where blood sugar levels are higher than normal, but not high enough yet to be diagnosed as type 2 diabetes. Diabetes mellitus (DM) is a disease characterized by elevated blood glucose levels due to the inability of the body to yield or use insulin or both. It is believed that oxidative stress may play an important role in the development of vascular complications in patients with type 2 diabetes mellitus.

Objective To evaluate the serum levels of endogenous melatonin and its association with superoxide dismutase (SOD) and glutathione peroxidase (GPx) in prediabetes individuals in comparison with normal individuals as controls.

Methods Endogenous melatonin and SOD concentrations were measured in sera of 50 prediabetes individuals in comparison with 50 volunteers enlisted as normal controls aged from 20 to 65 using enzyme-linked immunosorbent assay (ELISA); volunteers enlisted as normal. Serum levels of lipid profile, urea, creatinine, GPx, fasting blood glucose and plasma level of glycated hemoglobin (HbA1c) were measured. All individuals were matched for body mass index and sex.

Results Serum levels of melatonin, SOD and GPx enzymes in prediabetic patients were significantly lower than those of controls (p=0.036, p=0.024, and p=0.044; respectively) in prediabetic individuals when compared with controls with a significant positive correlation of theses biomarker levels in prediabetic individuals as compared with the control group.

Conclusion Decreased levels of melatonin in prediabetic subjects may play an essential role by influencing decreased levels of SOD and GPx enzymes, which are considered major defense mechanisms against ROS that may lead to the development of diabetes (type 2).

Keywords Melatonin, superoxide dismutase, glutathione peroxidase, oxidative stress, prediabetes

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List of abbreviations: ADA = American Diabetic Association, BMI = Body mass index, CNS = Central nervous system, Chi2 = Chi-square test, FBS = Fasting blood sugar, WHO = World health organization

Introduction

Diabetes is a family of disorders that is characterized by hyperglycemia. It is caused by an absolute or relative insulin deficiency [1-3]. In diabetes mellitus type 2, there is a spectrum of disorders ranging from insulin resistance with relative insulin deficiency to a predominantly secretory defect with insulin resistance. The chronic complication of diabetes mellitus type 2 may lead to diabetic retinopathy, diabetic neuropathy, and diabetic nephropathy [4].

Prediabetes is a metabolic state lying between diabetes and normoglycemia. It can be classified according to World health organization (WHO) to impaired glucose

tolerance and impaired fasting glucose, it remains a state of high risk for developing diabetes with a yearly conversion rate of 5-10%. Observational evidence suggests as association between prediabetes and microvascular complications of diabetes and the risk of macrovascular disease [5].

Free radicals are highly reactive, short-lived, and unstable electrons that may contain one or more unpaired electrons. They can generate and be involved in the normal process of differentiation and migration. Accumulation of free radicals causes damage to cells by disrupting membranes and perhaps causing cancer and atherosclerosis [6-9].

Oxidative stress causes healthy cells of the body to lose their function and structure by attacking them. It is believed that oxidative stress plays important role in the development of vascular complications in diabetes particularly type 2 diabetes [10-12]. Increasing the levels of ROS in diabetes mellitus may be due to an increase in the destruction or a decrease in the production of catalase superoxide dismutase (SOD) and glutathione peroxidase (GPx) antioxidants. Fluctuations in the levels of these enzymes make tissues vulnerable to oxidative stress, leading to the development of diabetes complications. According to epidemiological studies, significantly more deaths from diabetes can be explained by an increase in vascular pathologies other than hyperglycemia [13-15].

The aim of the current work was to determine the serum concentrations of endogenous melatonin, GPx and SOD in cases of prediabetes and compare it with normal controls. The present work also aimed to assess the correlation between melatonin, GPx and SOD and determine the correlation of these markers with different variables like glycated hemoglobin (HbA1c), fasting blood and sugar (FBS).

Methods

Study design case-control study

The present study was done on 50 prediabetic patients according to American Diabetic Association (ADA) definition of prediabetes (Fasting blood glucose 6.1-6.9 mmol/l) or (110-125 mg/dl) and HbA1c (5.7% to 6.4%) with age ranged from 20-65 years old (mean±SD; 34.23±9.75) recruited from Al-Imamain Al-Kadhimain Medical City, Baghdad, Iraq who compared with 50 age, body mass index (BMI) and sex were matched with a healthy control group with age ranged from 18-57 year (mean±SD 36.2±12.71).

Inclusion criteria

Patients with prediabetes were included according to ADA definition of prediabetes [16].

Only healthy individuals (volunteers including medical staff, relatives, friends will be included within the (control) group free from diabetes mellitus disease confirmed by fast blood glucose level test (less than 6.1 mmol\l).

Exclusion criteria

- Patients with type 1 or 2 diabetes.
- Patients with liver or pancreatic inflammation.
- Patients with any type of cancer or tumor.
- Patients taking insulin, supplements, sedative medications (central nervous system depressants), birth control pills (contraceptive drugs), anticoagulant /antiplatelet drugs).

Blood sampling

Blood sample was collected from all 100 subjects from Al-Imamain Al-Kadhimain Medical City, Baghdad, Iraq with the approval of the Institutional Board Review (IRB) of College of Medicine, Al-Nahrain University.

In addition, an informed written consent for participation in the study was signed by the participant according to the Helsinki principles. Participants' consent was taken after explaining to them the nature and goals of our study that may help them and the community for better health care, promising the

participants to protect their private information, for which participants fully understood and agreed.

Serum levels of melatonin, GPx and SOD were measured by enzyme-linked immunosorbent assay (ELISA) technique. The ELISA kits used in the study for melatonin was (Sunlong, melatonin kit Catalog No. SL1169Hu), SOD kit No. (SL3490Hu), GPx kit No. (SL2786Hu). The glucose company kit was (Biosystem S.A.) and kit no. (15011). The HbA1c company kit was (Bio-Rad system) and kit no. (Variant II TURBO 270-2601A).

Statistical analysis

Statistical analysis was carried out by using SPSS version 23 and Microsoft excel 2013. The numerical data were expressed as mean±SD. Comparison between mean serum levels of melatonin, GPx, and SOD of cases and controls were performed by t-test. Pearson correlation test was done between parameters within each group (prediabetes and controls).

Results

Age and BMI of the studied groups were summarized in table (1) and figure (1). Table (1) showed non-significant differences in age and BMI between diabetic patients and controls

Table 1. Age and body mass index of the prediabetic patients in comparison with controls

Parameter	Group	N	Mean	Std. Deviation	P-value
Age (yr)	Cases	50	36.2	12.71	0.54
	Control	50	34.23	9.75	
BMI (kg/m^2)	Cases	50	26.6	7.16	0.8
	Control	50	28.95	7.89	

BMI: Body mass index

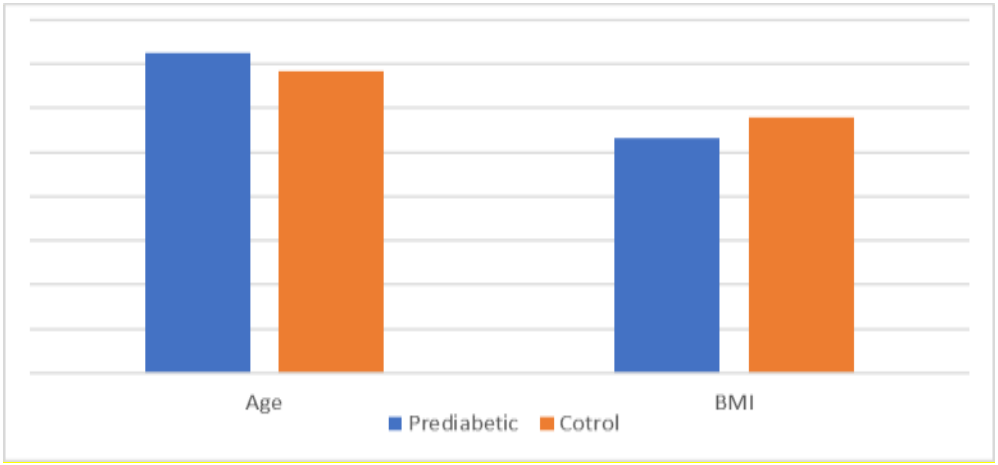

Figure 1. Age and body mass index of the prediabetic patients in comparison with controls

Results demonstrated in table (2) revealed that there were non-significant differences between the gender distribution and the smoking habit between cases and control as represented by the Chi-square test (Chi2) results obtained.

Results illustrated in table (3) revealed that the levels of FBS in prediabetic patients were non-

significantly differ from those of controls (p value 0.16). However, the levels of HbA1c in prediabetic patients were significantly higher than those of healthy controls' levels (5.84 vs 5.14 %) respectively; (p value <0.001).

Table (4) shows that the levels of melatonin, SOD and GPx enzymes were significantly lower (p=0.036, p=0.024 and p=0.044) respectively than those of controls.

Table 2. Comparison in the gender and smoking habit distribution between cases and controls

		Gender		Smoking	
		Male n (%)	Female n (%)	Smoker n (%)	Non-smoker n (%)
Cases		26 (52)	24 (48)	20 (50)	30 (60)
Control		27 (54)	23 (46)	22 (44)	28 (56)
Chi2	Phi	0.012		0.28	
	p-value	0.92		0.2	

Table 3. Comparison of fasting blood sugar and glycated hemoglobin between prediabetes with controls groups

Parameter	Group	N	Mean	Std. Deviation	P value
FBS (mg/dl)	Cases	50	89.2	18.66	0.16
	Control	50	83.8	12.91	
HbA1c (%)	Cases	50	5.84	0.36	<0.001
	Control	50	5.14	0.31	

Table 4. Comparison of melatonin, superoxide dismutase and glutathione peroxidase between prediabetes with controls groups

Parameter	Group	N	Mean	Std. Deviation	P value
Melatonin (pg/ml)	Cases	50	13.75	1.36	0.036
	Control	50	15.68	4.05	
SOD (ng/ml)	Cases	50	1.01	0.18	0.024
	Control	50	1.21	0.37	
GPx (pmol/ml)	Cases	50	5.27	1.37	0.044
	Control	50	6.92	3.56	

From the given data in the table (5), melatonin has no significant correlation with age, BMI, FBS and HbA1c in both groups. However, it has highly significant positive correlation with SOD and GPx in control group (p value <0.001), yet, in prediabetes group, significant positive correlation with SOD, and insignificant correlation with GPx (p value 0.034, 0.387) respectively.

Regarding correlation of SOD with other parameters that shown in table (6), like melatonin, it also has significant correlation

with melatonin and GPx in control group (p value <0.001) and insignificant correlation with GPx in prediabetes (p value 0.748). Additionally, it has significant negative correlation with HbA1c in prediabetes cases that is insignificant in control group (p value 0.025, 0.316) respectively.

Table 7 shows correlation of GPx with other parameters, and as mentioned above, it has highly significant correlation with melatonin and SOD just in control group but insignificant in control group. Also, like SOD, GPx has negative correlation with HbA1c only in prediabetes cases but not in control group (p value 0.028, 0.383) respectively.

Table 5. The correlation between melatonin and other parameters in prediabetes and controls groups

Parameter		Melatonin (pg/ml)	
		Cases	Controls
Age (yr)	r	0.398	0.125
	p	0.141	0.424
BMI (kg/m^2)	r	-0.284	-0.208
	p	0.585	0.496
SOD (ng/ml)	r	0.464	0.507
	p	**0.034**	**<0.001**
GPx (pmol/ml)	r	0.03	0.485
	p	0.387	**<0.001**
FBS (mg/dl)	r	0.032	-0.020
	p	0.892	0.888
HbA1c (%)	r	-0.267	0.192
	p	0.254	0.169

Table 6. The correlation between superoxide dismutase and other parameters in prediabetes and controls groups

Parameter		SOD (ng/ml)	
		Cases	Controls
Age (yr)	r	0.421	0.148
	p	0.118	0.343
BMI (kg/m^2)	r	-0.144	<0.001
	p	0.786	0.999
Melatonin (pg/ml)	r	0.464	0.507
	p	**0.034**	**<0.001**
GPx (pmol/ml)	r	0.045	0.636
	p	0.748	**<0.001**
FBS (mg/dl)	r	0.301	0.099
	p	0.198	0.478
HbA1c (%)	r	-0.498	0.140
	p	**0.025**	0.316

Table 7. The correlation between glutathione peroxidase and other parameters in prediabetes and controls groups

Parameter		GPx (pmol/ml)	
		Cases	Controls
Age (yr)	r	-0.126	0.071
	p	0.655	0.652
BMI (kg/m^2)	r	0.493	-0.220
	p	0.321	0.471
Melatonin (pg/ml)	r	0.03	0.485
	p	0.387	**<0.001**
SOD (ng/ml)	r	0.045	0.636
	p	0.748	**<0.001**
FBS (mg/dl)	r	0.034	0.181
	p	0.888	0.191
HbA1c (%)	r	-0.491	0.122
	p	**0.028**	0.383

Discussion

In the current work, all subjected individuals either patients or controls were non-significantly differed from each other in age, BMI, gender, and smoking habit to exclude any effect of these variables on the oxidative status of them in an attempt to elucidate the effect of the melatonin levels on the oxidative status of prediabetic patients that represented as a level of SOD and GPx enzymes.

The levels FBS were not significantly different between the two groups, indicating that FBS levels may not be a good predictor of prediabetes. However, the levels of HbA1c were significantly higher in prediabetic patients than in healthy controls, and within the range of prediabetes according to ADA definition of prediabetes suggesting that HbA1c may be a more reliable marker for prediabetes [16,17].

Melatonin is a hormone secreted by the pineal gland and is known to play an important role in regulating the sleep-wake cycle. Additionally, melatonin has been shown to have antioxidant and anti-inflammatory effects, which may be beneficial in the management of various health conditions such as diabetes and cardiovascular diseases. In this study, melatonin was significantly lower in prediabetic cases than controls. According to these results melatonin may be used as a biomarker of inflammation and it may have a role in the genesis of diabetes because it triggers a phase shift in insulin secretion. In contrast, impaired in the regulation of daily insulin secretion is an essential feature of prediabetes that may be ended with type 2 diabetes [18]. Melatonin may protect the individuals from being prediabetic and thus regulating insulin secretion and protecting against ROS since the β-cells of pancreas are objective for oxidative stress because they have low antioxidative capacity [19].

Melatonin can elevate electrical gradients between the two sides of the inner mitochondrial membrane leads to increase the production of adenosine triphosphate (ATP) by increasing the activity of the electron transport chain (ETC) and increased membrane fluidity, while reducing oxidative stress [20].

Results obtained in the current study revealed that the levels of SOD and GPx enzymes were significantly reduced in prediabetic patients in comparison with controls, which indicate that one of the most important defense mechanisms against ROS were defective. One of the explanations for decreased activity of SOD in these patients may be due to the accumulation of hydrogen peroxide (H_2O_2). The

Cu/Zn-SOD considered as primary catalytic cellular defense that protects cells and tissues against potentially destructive reactions of ROS. It has been observed that SOD can be rapidly induced in some conditions when cells or organisms are exposed to oxidative stress. The inhibition of Cu/Zn SOD by nonenzymatic glycation, which is the other cause for H_2O_2 production [17,21]. Fluctuations in the levels of these enzymes make tissues vulnerable to oxidative stress, leading to the development of diabetes complications prediabetes.

Strong positive correlation was found in this study between melatonin with SOD and GPx in control groups which was less significant or insignificant in prediabetes group, which indicate non-parallel reduction in their levels in prediabetes, which may contribute to the pathophysiology of prediabetes.

These results agree with another studies which demonstrated that melatonin antioxidant activity originates from its ability to improve the activities of antioxidant enzymes such as SOD [22,23]. Melatonin acts in multiple ways to reduce oxidative stress; while melatonin can remove toxic oxygen species directly or indirectly, it also has other means at its disposal to combat free radical damage. When a molecule like melatonin only transfers one of its unpaired electrons to neutralize free radicals, this action is accomplished without the receptor's involvement [24]. However, it is well documented that melatonin's ability to reduce oxidative stress sometimes also depends on its interaction with melatonin membrane receptors located on many, possibly all, cells [25,26]. These antioxidant actions of melatonin depend on interaction with transmembrane receptors located on the cell membrane or on intracellular organelles. Membrane receptors for melatonin may also be present in all living organisms. The receptor-mediated actions of melatonin are indirect and likely involve stimulation of antioxidant enzymes, for example, SOD, GPx, etc [27]. When melatonin acts via receptors to carry out its antioxidant actions, it can achieve this effect at concentrations much lower than those required when it acts as a direct scavenger of free radicals. This relates to the fact that the signal transduction pathways associated with the receptors amplify the response [22,27]. The above-mentioned mechanism of melatonin role as an antioxidant consistent with the correlation results obtained in the present work that showed a significant directly proportional relationship between melatonin and SOD and GPx enzymes [28].

In conclusion, melatonin levels decreased significantly in prediabetic individuals, which may play an important role in reducing the defense mechanism represented by the activity of SOD and GPx enzymes against producing of ROS, which may lead to a progression of prediabetes to type 2 diabetes mellitus.

It is important to note that this study has some limitations, such as small sample size and lack of control for confounding variables like diet and physical activity. Further research is necessary to confirm the findings and investigate other potential factors that may contribute to the development of prediabetes. Overall, the results of this study contribute to a better understanding of the biochemical markers associated with prediabetes and may help in the development of more effective prevention and treatment strategies.

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Conflict of interest

Author declares she has no conflict of interests.

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References

1. Panari H, Vegunarani M. Study on complications of diabetes mellitus among the diabetic patients. Asian J Nurs Edu Res. 2016; 6(2): 171-82. doi: 10.5958/2349-2996.2016.00032.X.
2. Parsa P, Ahmadinia-Tabesh R, Mohammadi Y. Assessment of the risk of coronary heart disease in diabetes patients type-II. Asian J Nurs Edu Res. 2019; 9(2): 267-70. doi: 10.5958/2349-2996.2019.00057.0.

3. Bhardwaj J, Choudhary R, Sharma P. A comparative study on quality of life among type 1 and type 2 diabetes mellitus clients at selected hospitals of district Mohali, Punjab. Asian J Nurs Edu Res. 2018; 8(1): 152-8. doi: 10.5958/2349-2996.2018.00032.0.
4. Banday MZ, Sameer AS, Nissar S. Pathophysiology of diabetes: An overview. Avicenna J Med. 2020; 10(4): 174-88. doi: 10.4103/ajm.ajm_53_20.
5. Bansal N. Prediabetes diagnosis and treatment: A review. World J Diabetes. 2015; 6(2): 296-303. doi: 10.4239/wjd.v6.i2.296.
6. Bansal AK, Bilaspuri GS. Impacts of oxidative stress and antioxidants on semen functions. Vet Med Int. 2010; 2010: 686137. doi: 10.4061/2011/686137.
7. Selvakumar K, Madhan R, Srinivasan G, et al. Antioxidant assays in pharmacological research. Asian J Pharm Tech. 2011; 1(4): 99-103.
8. Jaydeokar AV, Bandawane DD, Nipate SS, et al. Natural antioxidants: A review on therapeutic applications. Res J Pharmacol Pharmacodynamics. 2012; 4(1): 55-61.
9. Bright R, Raj PS, Amala K, et al. Free radical - A sword with two edges. Asian J Res Chem. 2021; 14(2): 139-44. doi: 10.5958/0974-4150.2021.00026.2.
10. Giacco F, Brownlee M. Oxidative stress and diabetic complications. Circ Res. 2010; 107(9): 1058-70. doi: 10.1161/CIRCRESAHA.110.223545.
11. Kushwaha SP, Rawat SK, Kumar P, et al. Coupling antioxidant and antidiabetic assets of 2, 4-thiazolidinedione derivatives. Asian J Pharm Ana 2011; 1(4): 71-3.
12. Lakshmana G, Chitra V, Rajesh Kumar D, et al. Study of antidiabetic and free radical scavenging activity of the methanolic and N-hexane extract of *Asystasia gangetica* Leaf in alloxan induced diabetic rats. Res J Pharmacol Pharmacodynamics. 2014; 6(2): 86-93.
13. Oguntibeju OO. Type 2 diabetes mellitus, oxidative stress and inflammation: examining the links. Int J Physiol Pathophysiol Pharmacol. 2019; 11(3): 45-63.
14. Pattabiraman K, Muthukumaran P. Antidiabetic and antioxidant activity of *Morinda tinctoria roxb* fruits extract in streptozotocin-induced diabetic rats. Asian J Pharm Tech. 2011; 1(2): 34-9.
15. Chakraborty P, Kumar S, Dutta D, et al. Role of antioxidants in common health diseases. Res J Pharm and Tech. 2009; 2(2): 238-44.
16. American Diabetes Association. Understanding A1c diagnosis. URL: https://diabetes.org/diabetes/a1c/diagnosis#:~:text=What%20is%20Prediabetes%3F,to%20be%20diagnosed%20as%20diabetes.
17. Luc K, Schramm-Luc A, Guzik TJ, Mikolajczyk TP. Oxidative stress and inflammatory markers in prediabetes and diabetes. J Physiol Pharmacol. 2019 Dec;70(6). doi: 10.26402/jpp.2019.6.01. Epub 2020 Feb 19. PMID: 32084643.
18. Awni N, Ahmed TS, Sarhat ER, et al. Altered serum levels of melatonin, antioxidant enzymes and oxidative stress in individuals with diabetes mellitus type 2. Revista Latinoamericana De Hipertensión, 2022; 17(2): 138–141. doi: http://doi.org/10.5281/zenodo.6672848.
19. Pham-Huy LA, He H, Pham-Huy C. Free radicals, antioxidants in disease and health. Int J Biomed Sci. 2008; 4(2): 89-96.
20. Asmat U, Abad K, Ismail K. Diabetes mellitus and oxidative stress-A concise review. Saudi Pharm J. 2016; 24(5): 547-53. doi: 10.1016/j.jsps.2015.03.013.
21. Madi M, Babu S, Kumari S, et al. Status of serum and salivary levels of superoxide dismutase in type 2 diabetes mellitus with oral manifestations: A case control study. Ethiop J Health Sci. 2016; 26(6): 523-32. doi: 10.4314/ejhs.v26i6.4.
22. Reiter RJ, Tan DX, Rosales-Corral S, et al. Mitochondria: Central organelles for melatonin's antioxidant and anti-aging actions. Molecules. 2018; 23(2):509. doi: 10.3390/molecules23020509.
23. Boutin JA. Quinone reductase 2 as a promising target of melatonin therapeutic actions. Expert Opin Ther Targets. 2016; 20(3): 303-17. doi: 10.1517/14728222.2016.1091882.
24. Galano A, Tan DX, Reiter RJ. Melatonin as a natural ally against oxidative stress: a physicochemical examination. J Pineal Res. 2011; 51(1): 1-16. doi: 10.1111/j.1600-079X.2011.00916.x.
25. Barberino RS, Menezes VG, Ribeiro AEAS, et al. Melatonin protects against cisplatin-induced ovarian damage in mice via the MT1 receptor and antioxidant activity. Biol Reprod. 2017; 96(6): 1244-1255. doi: 10.1093/biolre/iox053.
26. Deng SL, Sun TC, Yu K, et al. Melatonin reduces oxidative damage and upregulates heat shock protein 90 expression in cryopreserved human semen. Free Radic Biol Med. 2017; 113: 347-54. doi: 10.1016/j.freeradbiomed.2017.10.342.
27. Reiter RJ, Mayo JC, Tan DX, et al. Melatonin as an antioxidant: under promises but over delivers. J Pineal Res. 2016; 61(3): 253-78. doi: 10.1111/jpi.12360.
28. Ghosh AK, Naaz S, Bhattacharjee B, et al. Mechanism of melatonin protection against copper-ascorbate-induced oxidative damage in vitro through isothermal titration calorimetry. Life Sci. 2017; 180: 123-36. doi: 10.1016/j.lfs.2017.05.022.

E-mail: dr.zenaalsedi74@gmail.com
zeenaalsedi@colmed.ahrainuniv.edu.iq
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Assessment of Nutritional Status and Growth in Children with End Stage Renal Disease Undergoing Maintenance Hemodialysis: A Multicenter Study

Zina A. Rashid[1] *FIBMS, CABMS/FM*, **Besmah M. Ali**[2] *FIBMS/CM*, **Nariman F. A. Azat**[3] *FICMS, CAPB (Ped), FICMS (Ped Nephrology)*

[1]Fellowship of Clinical Nutrition, Kirkuk Health Dept, Kirkuk, Iraq, [2]Head of Scientific Council of Clinical Nutrition Fellowship Arab Board, Ghazy Al-Hariri Hospital for Surgical Specialties, Baghdad, Iraq, [3]Children Welfare Teaching Hospital, Baghdad, Iraq

Abstract

Background Chronic kidney disease is a worldwide health problem, with increasing prevalence and adverse outcomes. Children's nutritional status reflects the degree to which physiologic needs for nutrients are being met. Impairment of growth is associated with all stages of chronic renal failure

Objective To assess the nutritional status and the severity of malnutrition in children undergoing hemodialysis (HD) in all pediatrics dialysis centers in Baghdad.

Methods A total of 86 participants (41 males and 45 females), with end stage renal disease (ESRD) undergoing HD, from all pediatric HD centers in Baghdad City, recruited in this cross-sectional study with analytic components, among six months' period. A structured interview questionnaire was used with the children or their care givers, included: demographic and clinical characteristics of patients, nutritional assessment of studied children using pediatric subjective global nutritional assessment (SGNA) tool and also growth parameters to assess the growth status of the study group.

Results The overall proportion of estimated nutritional status was as follow: Thirty-three children (38.4%) were normal/well nourished, 39 patients (45.3%) were moderately malnourished and 14 patients (16.3%) were severely malnourished. Uremic symptoms were more significantly associated with severe state of nutritional deficiency (p=0.002). The duration of dialysis was significantly higher in children with severe nutritional deficiency in comparison with children with normal or moderate nutritional deficiency, their mean and interquartile range were 41.00 (76.00) months versus 24.00 (27.00) months and 24.00 (38.00) months, respectively (p = 0.001).

Conclusion Nutritional assessments are complex and critical in pediatric patients with ESRD. Using a comprehensive tool such as pediatric SGNA is more reflective of their nutritional status than when compared to other parameters.

Keywords Nutritional status, pediatrics end stage renal disease, pediatrics subjective global nutritional assessment (SGNA)

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List of abbreviations: BMI = Body mass index, CKD = Chronic Kidney Disease, ESRD = End stage renal disease, HD = Hemodialysis, MAC = Mid-arm circumference, SGNA = Pediatric subjective global assessment tool, TSFT = Triceps skin fold thickness

Introduction

Nutrition in pediatrics has always been one of the most important factors for optimal growth. Patient with chronic

kidney disease (CKD) need special consideration for better long-term outcomes, including nutritional status, optimal height, and cognitive function [1]. Malnutrition is an evident problem in children with end stage renal disease (ESRD) on hemodialysis (HD) [2].

Factors implicated in growth failure in children with CKD may include growth hormone insensitivity, electrolyte disturbances, metabolic acidosis and poor nutritional intake [3,4]. Some factors can be managed and are potentially amenable to correction such as mineral bone disorders, acidosis, electrolyte imbalance, hematological and metabolic disorders, hormonal abnormalities and nutritional deficiencies; however, others are difficult to be modified such as parental height, associated syndromes and birth parameters, which have significant impact on achieving normal longitudinal growth [5,6].

Data about growth and development of Iraqi children with ESRD are rarely available in published literature and the effect of dialysis on growth parameters in those children is incompletely understood. The poverty of such information has encouraged us to plan and conduct the current study aiming at assessment the nutritional status and the evidence of malnutrition and its related factors in association with growth parameters of children with ESRD undergoing hemodialysis, using the pediatric subjective global assessment tool (SGNA), anthropometric measurements, growth parameters and biochemical tests.

Methods

This is a descriptive cross-sectional study with analytic elements. The study was conducted in five governmental HD units from 1st of February 2022 to 31st of July 2022.

We included all patients with ESRD who were scheduled to undergo regular HD (at least one session per week) for more than 6 months. Age ranged from 6-18 years old.

Data collection

Data were collected by direct interviews using a structured interview questionnaire was designed and used to interview the children or their care givers. It included the demographic and clinical characteristics of patients, and nutritional assessment.

Nutritional assessment parameters It included six parts:

Part I: Biosocial and medical data:

- Biosocial data
- Family history of CRF
- The duration, frequency of HD among children
- Uremic symptom

Part II: Physical assessment sheet.

Part III: Anthropometric measurements sheet.

Part IV: Biochemical tests.

Part V: Dietary intake survey.

Part VI: Pediatrics SGNA tool [7], which was based on the history and physical examination of the participants.

Anthropometric data

- Height measurement; based on recently documented measures from the patient's record.
- Weight measurement, based on the documented last post dialysis weight (dry weight) measurements.
- Body mass index (BMI) was calculated by dividing the dry weight over the squared height in meters, and was classified according to the World Health Organization categorization for patients on HD [8].

Growth parameters

Patients over 2 years of age can be evaluated by monitoring their weight, height, BMI, and growth velocity using the Centers for Disease Control and Prevention (CDC) (2–20-years) growth charts [9], classified as:

- Underweight: Less than the 5th percentile.
- Healthy Weight: 5th percentile to less than the 85th percentile.

- Overweight: 85th to less than the 95th percentile.
- Obesity: Equal to or greater than the 95th percentile.
- Wasting (low weigh for-height) <3rd percentile (or <-2 SD), long-term undernutrition.
- Stunting (low height-for-age) <3rd percentile (or <-2 SD), short stature.

Biochemical variables

The following reference ranges were used for patients on HD during the data analysis: normal serum calcium range: 8.4-10.2 mg/dl, serum creatinine 0.6-1.2 mg/dl, serum phosphorus 3-4.5 mg/dl, parathyroid hormone (PTH) 230-630 pg/ml, total serum protein 6-7.8 mg/dl, and serum albumin 3.5-5.5 mg/dl [8].

Dialysis adequacy

Dialysis adequacy was estimated by calculating the Kt/V, according to Daugirdas formula [9]

Ethical consideration

Arab Board Committee approval will be obtained and the confidentiality of the data were explained to the participants. Written informed consents, were taken from each participant before the start of the study.

Data management and statistical analysis

Statistical analysis was performed using statistical package for social sciences (SPSS, version 26; Chicago, IBM, USA). Quantitative variables were expressed as mean, standard deviation, median, interquartile range and range. Qualitative variables were expressed as number and percentage. Chi-square test was utilized to evaluate association between qualitative variables. One-way ANOVA was used to study mean difference among classes of SGNA. P value was regarded significant at levels of 0.05 or less.

Results

The classification of enrolled children with ESRD according to pediatric SGNA is shown in figure 1. Thirty-three children (38.4%) were normal, 39 patients (45.3%) had moderate nutritional deficiency and 14 patients (16.3%) had severe nutritional deficiency.

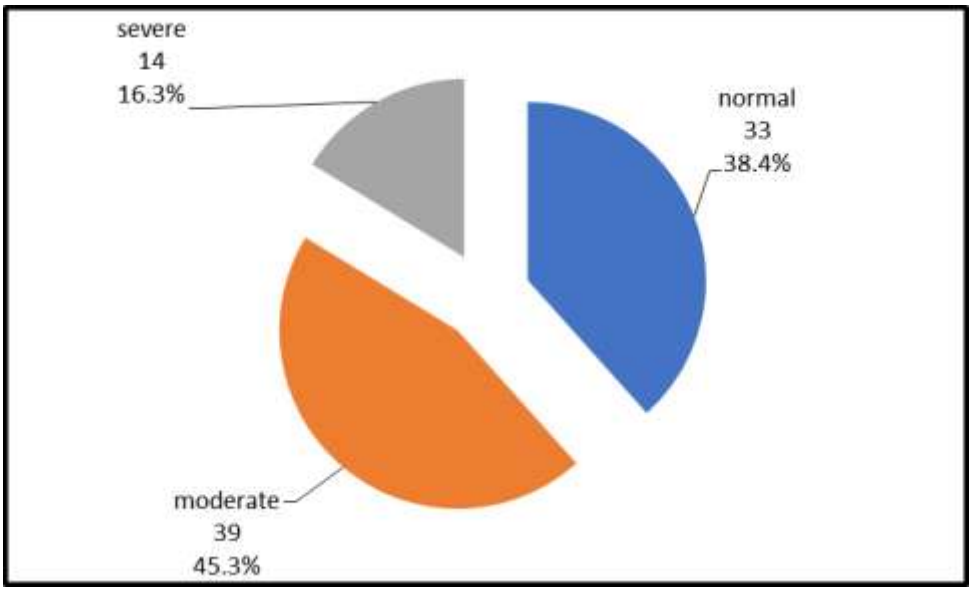

Figure 1. Pie chart showing the frequency distribution of children enrolled in the current study based on the Pediatric Subjective Global Nutrition Assessment (SGNA)

Sociodemographic characteristics of children enrolled in the current study classified based on SGNA are shown in table 1. There was no significant difference in mean age, the frequency of males and females, the place of enrollment, the frequency of residence, the level of education and the care giver status, education and occupation.

Table 1. Sociodemographic characteristics of children enrolled in the current study classified based on Pediatric Subjective Global Nutrition Assessment (SGNA)

Characteristic		SGNA of ESRD children			P value
		Normal *n* = 33	Moderate *n* = 39	Severe *n* = 14	
Age (years)	Mean ±SD	13.06 ±4.82	12.97 ±2.78	14.43 ±2.79	0.426 O
	Range	3-22	7-20	10-20	NS
Gender	Male	18 (54.5 %)	15 (38.5 %)	8 (57.1 %)	0.293 C
	Female	15 (45.5%)	24 (61.5%)	6 (42.9%)	NS
Place	Medical city	3 (9.1%)	5 (12.8%)	3 (21.4%)	0.751 C NS †
	Ibn albaladi	7 (21.2%)	13 (33.3%)	4 (28.6%)	
	Karama	5 (15.2%)	6 (15.4%)	1 (7.1%)	
	Iscan	16 (48.5%)	11 (28.2%)	5 (35.7%)	
	Kadhmia	2 (6.1%)	4 (10.3%)	1 (7.1%)	
Residence	Urban	22 (66.7%)	23 (59.0%)	12 (85.7%)	0.192 C
	Rural	11 (33.3%)	16 (41.0%)	2 (14.3%)	NS
Education	Illiterate	8 (24.2%)	9 (23.1%)	5 (35.7%)	0.344 C NS †
	Read and write	10 (30.3%)	11 (28.2%)	6 (42.9%)	
	Primary	11 (33.3%)	18 (46.2%)	2 (14.3%)	
	Intermediate	4 (12.1%)	1 (2.6%)	1 (7.1%)	
Care giver	Father alive	3 (9.1%)	2 (5.1%)	2 (14.3%)	0.223 C NS †
	Mother alive	1 (3.0%)	1 (2.6%)	2 (14.3%)	
	Other	0 (0.0%)	0 (0.0%)	1 (7.1%)	
	Father/Mother alive	27 (81.8%)	30 (76.9%)	7 (50.0%)	
	Father/other	1 (3.0%)	1 (2.6%)	1 (7.1%)	
	Mother/other	1 (3.0%)	1 (2.6%)	0 (0.0%)	
	Father/Mother/Other	0 (0.0%)	4 (10.3%)	1 (7.1%)	
Education of caregiver	Illiterate	0 (0.0%)	1 (2.6%)	0 (0.0%)	0.944 C NS †
	Read and write	3 (9.1%)	5 (12.8%)	2 (14.3%)	
	Primary	15 (45.5%)	14 (35.9%)	4 (28.6%)	
	Intermediate	7 (21.2%)	8 (20.5%)	3 (21.4%)	
	Secondary	8 (24.2%)	11 (28.2%)	5 (35.7%)	
Occupation	Paid employee	15 (45.5%)	15 (38.5%)	7 (50.0%)	0.670 C NS †
	Private job	12 (36.4%)	18 (46.2%)	5 (35.7%)	
	Unemployed	1 (3.0%)	3 (7.7%)	1 (7.1%)	
	Retired	4 (12.1%)	3 (7.7%)	0 (0.0%)	
	Housewife	1 (3.0%)	0 (0.0%)	1 (7.1%)	

n: number of cases; SD: standard deviation; C: chi-square test; O: One-way ANOVA test; NS: not significant; †: more than 20% of cells have expected count of <5

Medical history and clinical characteristics of children enrolled in the current study classified based on SGNA are shown in table 2. Medical history and family history showed no significant association to SGNA (p>0.05). Uremic symptoms were more significantly associated with severe state of nutritional deficiency (p=0.002). There was no significant difference in mean systolic or diastolic blood pressure among various stages of SGNA (p >0.05).

Dialysis characteristics of children enrolled in the current study classified based on SGNA is shown in table 3. The duration of dialysis was higher in a significant manner in children with severe nutritional deficiency in comparison with children with normal or moderate nutritional deficiency, their mean and interquartile range were 41.00 (76.00) months versus 24.00 (27.00) months and 24.00 (38.00) months, respectively (p=0.001). There was no significant difference in mean frequency of dialysis and mean duration of dialysis session among various stages of SGNA (p>0.05). Access type in form of catheter was more frequently associated with severe nutritional deficiency state in comparison with normal or moderate state, 78.6 % versus 39.4 % and 43.6 %, respectively (p=0.038). Kt/V mean was comparable among various stages of SGNA (p= 0.143).

Table 2. Medical history and clinical characteristics of children enrolled in the current study classified based on Pediatric Subjective Global Nutrition Assessment (SGNA)

Characteristic		SGNA of ESRD children			P value
		Normal *n* = 33	Moderate *n* = 39	Severe *n* = 14	
Medical history	Cystic disease	1 (3.0%)	1 (2.6%)	2 (14.3%)	0.411 C NS †
	Other	29 (87.9%)	34 (87.2%)	10 (71.4%)	
	More than one condition	3 (9.1%)	4 (10.3%)	2 (14.3%)	
Family history of CKD	Positive	4 (12.1%)	7 (17.9%)	4 (28.6%)	0.395 C NS
	Negative	29 (87.9%)	32 (82.1%)	10 (71.4%)	
Uremic symptom	No symptoms	20 (60.6%)	18 (46.2%)	0 (0.0%)	0.002 C * †
	Nausea	7 (21.2%)	6 (15.4%)	2 (14.3%)	
	Poor appetite	0 (0.0%)	1 (2.6%)	1 (7.1%)	
	More than one symptom	6 (18.2%)	14 (35.9%)	11 (78.6%)	
Systolic BP (mmHg)	Mean ±SD	120.85 ±21.00	125.21 ±27.61	131.79 ±30.10	0.407 O NS
	Range	87-160	80-200	90-180	
Diastolic BP (mmHg)	Mean ±SD	80.09 ±17.82	79.79 ±22.31	83.57 ±15.97	0.818 O NS
	Range	55-130	45-150	60-110	

n: number of cases; SD: standard deviation; C: chi-square test; O: One way ANOVA test; NS: not significant; *: significant at p ≤0.05; †: more than 20 % of cells have expected count of <5

Table 3. Dialysis characteristics of children enrolled in the current study classified based on Pediatric Subjective Global Nutrition Assessment (SGNA)

Characteristic		SGNA of ESRD children			P value
		Normal *n* = 33	Moderate *n* = 39	Severe *n* = 14	
Duration of dialysis (months)	Median (IQR)	24.00 (27.00)	24.00 (38.00)	41.00 (76.00)	0.001 K ***
	Range	3-72	3-84	3-192	NS
Frequency HD	Mean ±SD	2.58 ±1.09	2.46 ±0.68	3.00 ±1.36	0.216 O
	Range	1 -7	1 -4	2 -7	NS
Duration per session (hours)	Mean ±SD	3.27 ±0.45	3.15 ±0.37	3.14 ±0.54	0.444 O
	Range	3-4	3-4	2-4	NS
Access type	Fistula	20 (60.6%)	22 (56.4%)	3 (21.4%)	0.038 C *
	Catheter	13 (39.4%)	17 (43.6%)	11 (78.6%)	
Kt/V	Mean ±SD	1.19 ±0.32	1.23 ±0.47	1.48 ±0.70	0.143 O
	Range	0.52 -2.01	0.44 -2.82	0.67 -3.02	NS

n: number of cases; SD: standard deviation; IQR: inter-quartile range; C: chi-square test; O: One way ANOVA test; K: Kruskal Wallis test; NS: not significant; *: significant at p≤0.05; ***: significant at p≤0.001

Biochemical characteristics of children enrolled in the current study classified based on SGNA are shown in table 4. There was no significant difference in mean serum creatinine, calcium, phosphorus, albumin and total protein among various stages of SGNA (p>0.05). Mean serum PTH (parathyroid hormone) was significantly highest in severe state (p=0.05) and mean hemoglobin level was significantly lowest in severe state (p=0.003).

Table 4. Biochemical characteristics of children enrolled in the current study classified based on Pediatric Subjective Global Nutrition Assessment (SGNA)

Characteristic		SGNA of ESRD children			P value
		Normal *n* = 33	Moderate *n* = 39	Severe *n* = 14	
Creatinine (mg/dl)	Mean ±SD	8.5±3.29	7.87±3.05	9.08±4.44	0.482 O
	Range	1.6-15.2	1.98-16.4	5.32-23.55	NS
Calcium (mg/dl)	Mean ±SD	7.92±1.60	7.78±1.77	7.32±1.68	0.539 O
	Range	5.2-11.24	4.5-12.8	3.96-11	NS
Phosphorus (mg/dl)	Mean ±SD	6.89±1.98	6.78±2.76	6.31±2.36	0.754 O
	Range	4.27-13.1	1.58-15.1	3.78-12.15	NS
PTH (pg/ml)	Median (IQR)	498.0 (691.2)	366.2 (580.6)	1405.0 (1301.2)	0.050 K *
	Range	9.3-2200	7.1-2200	136.2-3000	
Albumin (g/dl)	Mean ±SD	4.01±0.49	3.95±0.67	3.83±0.8	0.659 O
	Range	2.5-4.8	2.26-5.3	2.1-4.7	NS
TSP (g/dl)	Mean ±SD	6.35±1.42	6.02±1.05	6.51±1.62	0.387 O
	Range	3.3-9.65	3.3-9.01	4.53-9.65	NS
Hb (g/dl)	Mean ±SD	9.08±1.3	8.65±1.72	7.33±1.61	0.003 O **
	Range	6.6-11.6	5.8-12.4	4.9-11	

n: number of cases; SD: standard deviation; IQR: inter-quartile range; One-way ANOVA test; K: Kruskal Wallis test; NS: not significant; *: significant at p≤0.05; **: significant at p≤0.01, PTH: Parathyroid hormone, TSP: Total serum protein, Hb: Hemoglobin

Nutritional status measurements and growth parameters of children enrolled in the current study classified based on SGNA are shown in table 5. Mean height, mean TSFT (triceps skin fold thickness), mean MAC (mid-arm circumference), mean dry weight and mean BMI showed significant variation with respect to states of SGNA (p<0.05); but there was no significant variation in mean knee height (p= 0.292).

Less than 5th percentile with respect to height for age, weight for age and BMI for age was more significantly associated with severe state of SGNA in comparison with normal state and moderate state (p<0.001). There was no significant difference in mean daily carbohydrate (CHO), protein and fat (p>0.05), but mean total Daily total calories was significantly lowest in severe state of SGNA in comparison to normal and moderate states (p<0.001). The amount of daily protein intake was between 0.4 to 4.6, 0.5 to 3 and 0.6 to 2.6 gram in normal, moderate and severe SGNA states respectively.

Table 5. Nutritional status and growth parameters measurements of children enrolled in the current study classified based on Pediatric Subjective Global Nutrition Assessment (SGNA)

Characteristic		Anthropometrics measurements of children classified based on SGNA			P value
		Normal *n* = 33	Moderate *n* = 39	Severe *n* = 14	
Height (cm)	Mean ±SD	138.21±18.65	130.26±16.12	125.21±14.67	0.034 O *
	Range	93-169	98-158	90-145	
knee height (cm)	Mean ±SD	41.35±7.36	39.58±5.92	38.36±5.61	0.292 O
	Range	24-56	26.5-52	27-48	NS
TSFT (cm)	Mean ±SD	12.42±5.78	10.44±3.97	8.14±4.15	0.018 O *
	Range	3-35	4-27	3-20	
MAC (cm)	Mean ±SD	19.61±5.12	17.14±3.06	16.03±2.04	0.005 O ***
	Range	11-36	12-28	13-20.4	
Dry weight (kg)	Mean ±SD	38.29±15.73	28.41±7.89	23.29±5.52	< 0.001 O ***
	Range	10.8-71	14.8-47	12-32	
BMI (kg/m^2)	Mean ±SD	19.50±4.67	16.79±2.86	14.72±1.76	< 0.001 O ***
	Range	11.25-32.7	12.16-27.8	12.2-18.4	
		Growth parameters of children classified based on SGNA			
Height for age	<5%	14 (42.4%)	35 (89.7%)	14 (100.0%)	< 0.001 C *** †
	5-85%	18 (54.5%)	4 (10.3%)	0 (0.0%)	
	85-95%	1 (3.0%)	0 (0.0%)	0 (0.0%)	
Weight for age	<5%	5 (15.2%)	31 (79.5%)	14 (100.0%)	< 0.001 C *** †
	5-85%	26 (78.8%)	8 (20.5%)	0 (0.0%)	
	85-95%	2 (6.1%)	0 (0.0%)	0 (0.0%)	
BMI for age	<5%	2 (6.1%)	12 (30.8%)	11 (78.6%)	< 0.001 C *** †
	5-85%	26 (78.8%)	25 (64.1%)	3 (21.4%)	
	85-95%	2 (6.1%)	2 (5.1%)	0 (0.0%)	
	>95%	3 (9.1%)	0 (0.0%)	0 (0.0%)	
		Twenty-four hours' dietary intake by children with ESRD classified based on SGNA			
Daily CHO	Mean ±SD	0.57±0.11	0.60±0.12	0.58±0.19	0.556 O
	Range	0.35-0.83	0.245-1	0.2-0.92	NS
Daily protein	Mean ±SD	0.18±0.14	0.14±0.04	0.17±0.05	0.281 O
	Range	0.08-0.919	0.07-0.23	0.07-0.25	NS
Daily fat	Mean ±SD	0.28±0.07	0.32±0.12	0.33±0.16	0.169 O
	Range	0.13-0.45	0.13-0.7	0.06-0.69	NS
Daily total calories	Mean ±SD	1340.0±426.29	1029.74±328.49	850.71±443.7	< 0.001 O ***
	Range	460-2145	300-1805	285-1805	

n: number of cases; SD: standard deviation; O: One way ANOVA test; K: Kruskal Wallis test; C: chi-square test; NS: not significant; ***: significant at p≤0.001; **: significant at p≤0.01; ***: significant at p≤0.001; †: more than 20% of cells have expected count of <5, TSFT: Triceps skin fold thickness, MAC: Mid-arm circumference, BMI: Body mass index, CHO: Carbohydrate

Discussion

In the present study, the use of pediatric SGNA revealed proportions of nutritional status that agree with Iyengar et al. with respect to proportion of moderately malnourished children; however, the proportion of severely malnourished children in current study were less than that reported in Iyengar et al. study. Nevertheless, when all children with malnourishment were taken into consideration, the proportion in this study will be 61.6% and in Iyengar et al. study is 73% indicating that the majority of children with ESRD on dialysis suffer malnutrition [10].

According to the International Pediatric Peritoneal Dialysis Network, among 1001 children receiving chronic peritoneal dialysis worldwide, the prevalence of undernutrition

(based on BMI z scores) was 8.9%, with a larger burden (20%) seen in South Asia [11]. A single reference tool is not yet available, and objective evaluation techniques for the diagnosis of malnutrition in children with CKD may be unreliable in the presence of edema and fluid overload [12,13]. Additionally, BMI as a measurement is unable to distinguish between subcutaneous fat loss and muscle withering. Due to the fact that this is also true for individuals with CKD, a combination of objective and subjective evaluations has been investigated. It is paradoxical that tests like the SGNA have been proven to be more reliable at predicting outcomes for adults receiving dialysis than tests that exclusively use objective measurements [14]. According to reports, children without CKD had a higher malnutrition burden diagnosed by SGNA than by objective anthropometry measures [15,16].

In the present study, we reported that uremic symptoms were more significantly associated with severe state of nutritional deficiency. In addition, we observed no significant difference in mean systolic or diastolic blood pressure among various stages of SGNA. According to Flynn et al. [17], "Thirty-seven percent of children with CKD had either elevated systolic or diastolic blood pressure" indicating that high blood pressure is evenly distributed among those children, thus, in this study it is not surprising to find no significant variation in mean blood pressure among children with ESRD when they were classified based on SGNA because most of those children already has high readings.

In the present study, it was observed that longer duration of dialysis was significantly associated with severe malnutrition state, but, the frequency of sessions and their duration were not associated with nutritional state. However, catheter type of access was associated with severe malnutrition probably because of high rate of infection in association with this kind of access. In a previous study, mean duration of dialysis was longer in children with severe nutritional deficiency state in comparison to children with moderate nutritional deficiency state but the level of significance was (p=0.054) [10] and this is very close to the significant level of (p=0.05). Therefore, we can suggest that longer duration of dialysis will be associated with significantly more deterioration of kidney function and thus worse nutritional status.

In this study, Kt/V mean was comparable among various stages of SGNA. In the study of Cano et al. [18], they found significant correlation between Kt/V and mean protein catabolic rate; however; this correlation is concerned with protein nutritional status and not overall nutritional status so it is difficult to compare our findings to their findings.

There was no significant difference in mean serum creatinine, calcium, phosphorus, albumin and total protein among various stages of SGNA in current study; however, mean serum PTH was significantly highest in severe state and mean hemoglobin level was significantly lowest in severe state. Both, hyperparathyroidism and anemia are well known complications of ESRD in children. Many patients treated with hemodialysis remain anemic despite exogenous erythropoietin therapy, suggesting the anemia experienced by these patients is multifactorial in etiology. Iron deficiency, infection, inflammation, and malnutrition have been implicated in this process. Additionally, secondary hyperparathyroidism has been associated with anemia in adults, but little data exists on this topic in children [19]. Our suggestion is that severe malnourishment contributes to anemia because of lack of essential elements for red blood cells construction and function, thus it is expected to found less hemoglobin level in ESRD children with severe malnutrition state, in addition, a state of hyperthyroidism was linked to anemia and thus higher level of PTH will be associated with anemia and anemia will affect the overall nutrition status of tissue leading to retardation of growth and development.

In this study, mean height, mean TSFT, mean MAC, mean dry weight and mean BMI showed significant variation with respect to states of SGNA and less than 5th percentile with respect to height for age, weight for age and BMI for age was more significantly associated with severe state of SGNA in comparison with

normal state and moderate state. Indeed, these findings were in accordance with the findings of Iyengar et al. [10]. Actually, very few researches have looked at how specific SGNA factors affect how children's nutritional status is classified as a whole. Physical evidence of muscle wasting, gastrointestinal symptoms, and metabolic stress were the three individual SGNA criteria in young children that most significantly predicted SGNA rating in non-CKD children who underwent thoracic or abdominal procedures. The SGNA rating was affected in older children by visible signs of fat wasting, repeated weight loss, gastrointestinal issues, and stunting [20].

In conclusion, malnutrition is an evident problem in pediatric patients on hemodialysis. Several factors contribute to the impairment of nutritional status of these children such as loss of appetite and inadequate intake of calories, severity of their chronic disease status, inflammation and metabolic derangement. Their physical parameters were also greatly affected due to their clinical condition. Using a comprehensive tool such as pediatric SGNAt, was more reflective of their nutritional status than when compared to other parameters such as body mass index or skin fold thickness as these may be affected by fluid retention giving false idea about the patient's condition, and it was significantly correlated with duration of dialysis, type of access, uremic symptoms, total daily caloric intake, anemia and hyperparathyroidism.

The main limitation of this study lies in the lack of new research studies regarding nutritional assessment in CKD patients especially in pediatrics, in Iraq and short duration for data collection period.

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Author contribution

Dr Rashid: Data collection, editing, study design and writing. Both Dr Ali and Dr Azat supervised the study, made the plan and participated in revision of results with interpretation.

Conflict of interest

None.

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References

1. Azzouz JZ, Safdar OY, Awaleh FI, et al. Nutritional assessment and management in paediatric chronic kidney disease. J Nutr Metab. 2021; 2021: 8283471. doi: 10.1155/2021/8283471.
2. Azad Tehrani MM, Sabry YY, Elkersh MM, Elsharkawy, et al. Assessment of nutrition status of children with chronic renal failure undergoing hemodialysis. Tanta Sci Nursing J. 2020; 9(2): 157-83.
3. Haffner D. Strategies for optimizing growth in children with chronic kidney disease. Front Pediatr. 2020; 8: 399. doi: 10.3389/fped.2020.00399.
4. Franke D, Alakan H, Pavičić L, et al. Birth parameters and parental height predict growth outcome in children with chronic kidney disease. Pediatr Nephrol. 2013; 28(12): 2335-41. doi: 10.1007/s00467-013-2604-7.
5. Secker DJ, Jeejeebhoy KN. How to perform Subjective Global Nutritional assessment in children. J Acad Nutr Diet. 2012; 112(3): 424-31.e6. doi: 10.1016/j.jada.2011.08.039.
6. Ghorbani A, Hayati F, Karandish M, eta al. The prevalence of malnutrition in hemodialysis patients. J Renal Inj Prev. 2020; 9(2): e15. doi: 10.34172/jrip.2020.15.
7. Flegal KM, Cole TJ. Construction of LMS parameters for the Centers for Disease Control and Prevention 2000 growth charts. Natl Health Stat Report. 2013; (63): 1-3.
8. GlobalRPH The clinician's ultimate reference. Laboratory values. Available at http://www.globalrph.com/labs.htm.
9. Baloğlu I, Selçuk NY, Evran H, et al. Evaluation of hemodialysis adequacy: Correlation between Kt/Vurea and Other Methods. Turk J Nephrol. 2019; 28(3): 193-6. Doi: 10.5152/turkjnpehrol.2019.3426.
10. Iyengar A, Ashok JMR, Vasudevan A. Subjective Global Nutritional Assessment [SGNA] in Children on chronic dialysis- A prospective observational study. Indian J Nephrol. 2022; 32(4): 334-41. doi: 10.4103/ijn.ijn_340_21.
11. Schaefer F, Benner L, Borzych-Dużałka D, et al. Global variation of nutritional status in children undergoing chronic peritoneal dialysis: A longitudinal study of the International Pediatric Peritoneal Dialysis Network. Sci Rep. 2019; 9(1): 4886. doi: 10.1038/s41598-018-36975-z.

12. Foster BJ, Leonard MB. Nutrition in children with kidney disease: Pitfalls of popular assessment methods. Perit Dial Int. 2005; 25 Suppl 3: S143-6.

13. Nelms CL, Shaw V, Greenbaum LA, et al. Assessment of nutritional status in children with kidney diseases-clinical practice recommendations from the Pediatric Renal Nutrition Taskforce. Pediatr Nephrol. 2021; 36(4): 995-1010. doi: 10.1007/s00467-020-04852-5.

14. Zsom L, Zsom M, Abdul Salim S, et al. Subjective global assessment of nutrition, dialysis quality, and the theory of the scientific method in Nephrology practice. Artif Organs. 2020; 44(10):1 021-30. doi: 10.1111/aor.13762.

15. Minocha P, Sitaraman S, Choudhary A, et al. Subjective global nutritional assessment: A reliable screening tool for nutritional assessment in cerebral palsy children. Indian J Pediatr. 2018; 85(1): 15-9. doi: 10.1007/s12098-017-2501-3.

16. Mahdavi AM, Safaiyan A, Ostadrahimi A. Subjective vs objective nutritional assessment study in children: A cross-sectional study in the northwest of Iran. Nutr Res. 2009; 29(4): 269-74. doi: 10.1016/j.nutres.2009.03.009.

17. Flynn JT, Mitsnefes M, Pierce C, et al. Blood pressure in children with chronic kidney disease: A report from the Chronic Kidney Disease in Children study. Hypertension. 2008; 52(4): 631-7. doi: 10.1161/HYPERTENSIONAHA.108.110635.

18. Cano FJ, Azocar MA, Delucchi MA, et al. Nitrogen balance studies and Kt/V urea in children undergoing chronic peritoneal dialysis. Adv Perit Dial. 2004; 20: 245-50.

19. Smith LB, Fadrowski JJ, Howe CJ, et al. Secondary hyperparathyroidism and anemia in children treated by hemodialysis. Am J Kidney Dis. 2010; 55(2): 326-34. doi: 10.1053/j.ajkd.2009.09.033.

20. Secker DJ, Jeejeebhoy KN. Subjective Global Nutritional assessment for children. Am J Clin Nutr. 2007; 85(4): 1083-9. doi: 10.1093/ajcn/85.4.1083.

Correspondence to Dr. Zina A. Rashid
E-mail: zina.rashid9@yahoo.com
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The Effect of Interferon-Inducible T Cell Alpha Chemoattractant (I-TAC) on Transplanted Mice with Breast Cancer

Hind A. Mohammed[1] *PhD*, **Arwa M. Al-Shuwaikh[2]** *PhD*, **Ahmed M. Al-Shammari[3]** *PhD*

[1]Dept. of Nursing, Al-Farabi University College, Baghdad, Iraq, [2]Dept. of Microbiology, College of Medicine, Al-Nahrain University, Baghdad, Iraq, [3]Experimental Therapy, Iraqi Center for Cancer and Medical Genetics Research, Al-Mustansiriyah University, Baghdad, Iraq

Abstract

Background Interferon-inducible T-cell alpha chemoattractant (I-TAC, CXCL11) is a novel chemokine that used as immunotherapy. CXCL11 develops an effective anti-tumor immune response that relies on the coordinated interactions between immunocompetent cells, suppress angiogenesis and leading to an anti-tumor effect.

Objective To investigate the anti-tumor effect of CXCL11 on breast cancer transplanted in an animal's model.

Methods CXCL11 adoptive immunotherapy inducible, was intratumorally injected in mice breast cancer model one dose weekly for three weeks, each dose concentration 20 ng/100µl. Weight of mouse and size of tumor were recorded every 48 hr until 25 days. Parameters examined on mice transplanted with murine mammary adenocarcinoma (AMN3) cells (to study the therapeutic of CXCL11 in vivo) were included growth inhibition (GI%), relative tumor volume (RTV), relative mice weight (RMW) and survival time in mice.

Results In-vivo CXCL11 treatment showed to induce GI% from day 3 and continued to day 25 with significant increase in mice weight in comparison to non-treated control group during 25 days ($P<0.0001$). Also, the CXCL11 treatment exhibited a significant reduction in RTV and improving survival time in mice.

Conclusion CXCL11 showed an anti-cancer effect on breast cancer AMN3 implanted in mice compared to control. CXCL11 may have immunotherapeutic effect on breast cancer by triggering the activation of immunity against cancer.

Keywords Breast cancer, CXCL11, immunotherapy, AMN3

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List of abbreviations: AMN3 = Murine mammary adenocarcinoma cell line, CCR3 = Cysteine-cysteine chemokine receptor-3, CTLs = Cytotoxic T lymphocytes, CXCL11 = C-X-C motif chemokine ligand 11, CXCR3 = C-X-C motif chemokine receptor 3, GI% = Growth inhibition, IFN-α = Interferon alpha, IFN-β= Interferon beta, IFN-γ = Interferon gamma, IL-2 = Interleukin-2, I-TAC = Interferon-inducible T-cell alpha chemoattractant, NK= Natural killer cells, NKT = Natural killer T, RTV = Reducing relative tumor volume, Th= T helper, TNF-α = Tumor necrosis factor alpha

Introduction

Cancer immunotherapy is considered a new cornerstone in cancer treatment using the patient's immune system to fight cancer [(1)]. The success of immunotherapy can be explained by the enormous complexity of interactions between tumor cells and the immune system [(2-4)]. Migration of the immune cells to specific organs is controlled in part by small proteins called chemokines (i.e.,

chemotactic cytokines) [5,6]. The C-X-C motif (CXC) chemokine have two N-terminal cysteines separated by one amino acid "X". There have been 17 different CXC chemokines described in mammals. CXC chemokines are classified into two groups; with and without Glu-Leu-Arg (ELR) motif [7]. Those with the ELR motif can allow neutrophils to migrate and have an angiogenic effect, whereas those without the ELR motif primarily allow lymphocytic migration and inhibit angiogenesis [8].

C-X-C motif chemokine 11 (CXCL11) is ELR-negative CXC chemokines that attenuate angiogenesis, leading to an anti-tumor effect. However, some reports show that CXCL11 increases tumor proliferation and metastases [8]. CXCL11 is mainly secreted by monocytes, endothelial cells, fibroblasts, and cancer cells in response to interferon gamma (IFN-γ), synergistically enhanced by tumor necrosis factor alpha (TNF-α) [9,10] bind to C-X-C motif chemokine receptor 3 (CXCR3), which is a receptor preferentially expressed on the surface of monocytes, T cells, Natural killer (NK) cells, dendritic cells and cancer cells [11,12].

CXCL11, also known as an interferon-inducible T-cell alpha chemoattractant (I-TAC) or interferon-gamma-inducible protein 9 (IP-9), is induced by IFN-γ and interferon beta (IFN-β), and weakly induced by interferon alpha (IFN-α) [13]. The binding domain of CXCL11 on CXCR3 is located at a different site from that of CXCL9 and CXCL10 [14]. CXCL11 can bind to CXCR7, which is associated with invasiveness and reduces apoptosis of tumor cells [15]. For immune cell migration, each of the CXCR3 ligands is equally effective on activated T helper-1 (Th1) cells, cytotoxic T lymphocytes (CTLs) and NK cells in vivo models of cell recruitment [16,17].

CXCL11 attract Th1 cells and block the migration of Th2 cells in response to cysteine-cysteine chemokine receptor-3 (CCR3) ligands due to their ability to serve as antagonists for CCR3 [18]. On the other hand, NK cell subsets, the anti-tumor effectors that express CXCR3, are also recruited to the site in a CXCR3-dependent manner [17]. For immune cell activation, CXCL11 stimulates immune cells through Th1 polarization and activation. Th1 cells produce IFN-γ, TNF-α, interleukin-2 (IL-2) and enhance anti-tumor immunity by stimulating CTLs, NK cells, natural killer T (NKT) cells and macrophages [19,20]. The IFN-γ-dependent immune activation loop also promotes CXCL11 release. Importantly, NK cells can display immune activity by modulating dendritic cell function and also provide an early source for IFN-γ production [17]. Naturally, immune cells, mainly Th1, CTLs, NK cells and NKT cells, show an anti-tumor effect against cancer cells through paracrine CXCL9, CXCL10, CXCL11, and their receptor (CXCR3) axis in tumor models [21-23].

The research objective was to characterize the in-vivo antitumor effect induced by CXCL11 on breast cancer transplanted mice.

Methods

Animals

Ten female healthy mice, three weeks old, weighed between 15-26 g, obtained from the animal house of Iraqi Center for Cancer and Medical Genetics Research (ICCMGR). All animals were housed in individual cages designed for changing sawdust and feed. The temperature-controlled at 20-30°C with relative humidity ranging from 40-65%, the light:dark period of 12 hr. The adult female mice back were injected with sterile murine mammary adenocarcinoma cell line (AMN3) suspension according to Al-Shammari [24], where was supplied from ICCMGR, the tumor cells were taken from a tumor-bearing mouse used to obtain tumor-cell suspension that transplanted into other mice. The animals were distributed randomly into two groups, one control group and one treated group with CXCL11, each consisting of five mice.

Mouse C-X-C Motif Chemokine 11/I-TAC (CXCL11)

Interferon-inducible T cell alpha - chemoattractant (I-TAC; CXCL11), adoptive

immunotherapy inducible. This chemokine produced by Abbexa, UK. (Catalogue No. abx261542). The origin of chemokine from mouse and it expressed as recombinant from *Escherichia coli*. It was reconstituted in sterile 18 MΩ · cm water to a concentration of 200 µg/ml. After reconstitution, it was stored at below -18°C for long-term storage, and at 4°C for up to 7 days, for immediate use.

Experimental design

Mice were intratumorally injected by CXCL11 one dose weekly for three weeks, each dose concentration 20 ng/100 µl. Via intratumoral injection, with a recorded weight of mouse and size of tumor every 48 hr until 25 days, parameters examined on mice transplanted with AMN3 cells to study the therapeutic effect of CXCL11 in vivo by investigation growth inhibition (GI%), relative tumor volume (RTV), relative mice weight (RMW) and survival time in mice.

Statistical analysis

Graph Pad Prism 7.0 software was analyzed using the unpaired T-test method. P-value of <0.05 was considered to be significant.

Results

Effect of CXCL11 on the GI% of breast cancer in experimental animals

Tumor volume was calculated using vernier to measure tumor length and width, and by using the following equation: tumor volume = (Length)*(Width)²/2. Figure (1) shows an increase in GI% during the period of the experiment. GI% started with 40.67% and continue to increase to reach 70% on day 17, from day 17 until day 25 there was a slight increase to reach approximately 77% on day 25. The highest GI% was reached in the fourth week of experiment as shown in figure (1, A and B).

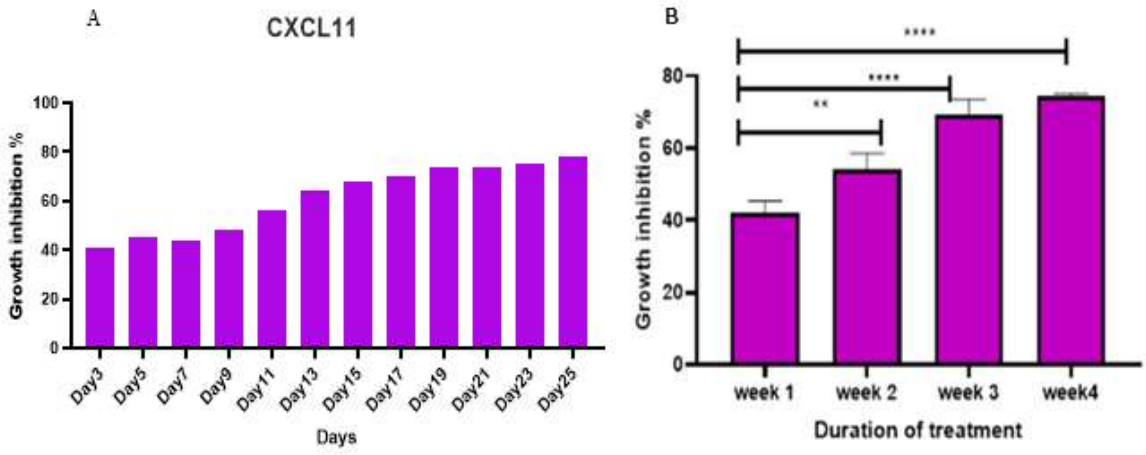

Figure 1. A) The growth inhibition of CXCL11 treatment started on day 3 and continued to day 25. B) Comparison between growth inhibition of CXCL11 treatment group for four weeks. Values represent the (mean±SD), *P <0.05, **P <0.01, ***P <0.001 and ****P <0.0001

Effect of CXCL11 on the weight of experimental animals

Mice weight was used as parameter for toxicity of CXCL11 treatment in this study. Evaluation of the RMW of experimental animals throughout the experimental period after intratumoral injection with CXCL11 and control groups was achieved by using electronic balance and the equation: RMW (day x) = mouse weight in (day x) / mouse weight in (day 0) X 100. RMW value was recorded for each group and CXCL11 treatment appeared to have no cytotoxic effect on mice compared to non-treated

control group during the period of experiment (Figure 2, A and B).

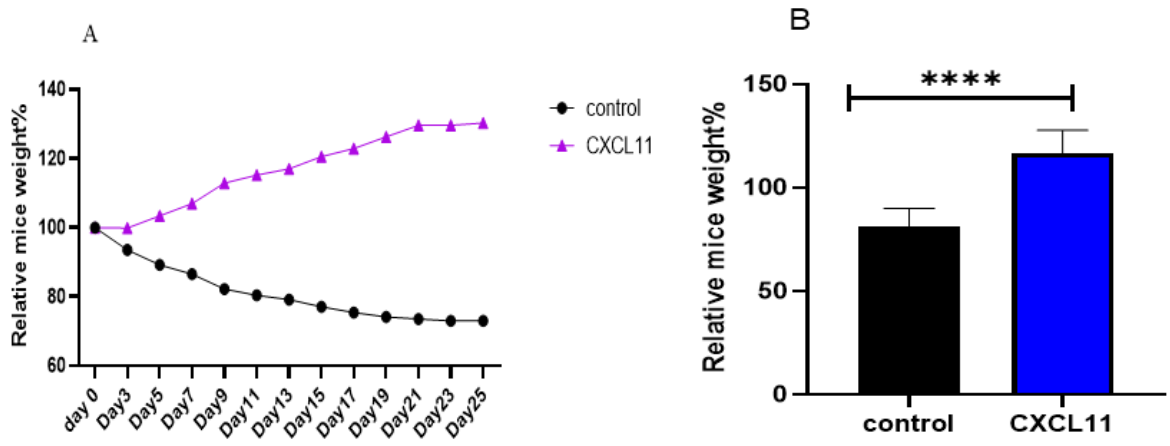

Figure 2. A) The relative mice weight to control and CXCL11 for 25 days. B) Comparison between non treated control and in-vivo CXCL11 treatment group depending on accumulative relative mice weight percentage. Values represent the (mean±SD), *P <0.05, **P <0.01, ***P <0.001 and ****P <0.000

Effect of CXCL11 on the relative tumor volume

To analyze the effects of CXCL11 treatment, RTV for each group from day zero before treatment (considered as 100%) and for each treatment day was calculated by the following equation: RTV (day x) = tumor volume (day x) / tumor volume (day 0) X 100. The result showed a decrease in RTV during experiment compared to control (Figure 3A), and highly significant difference between two groups at the end of the overall experiment (Figure 3B).

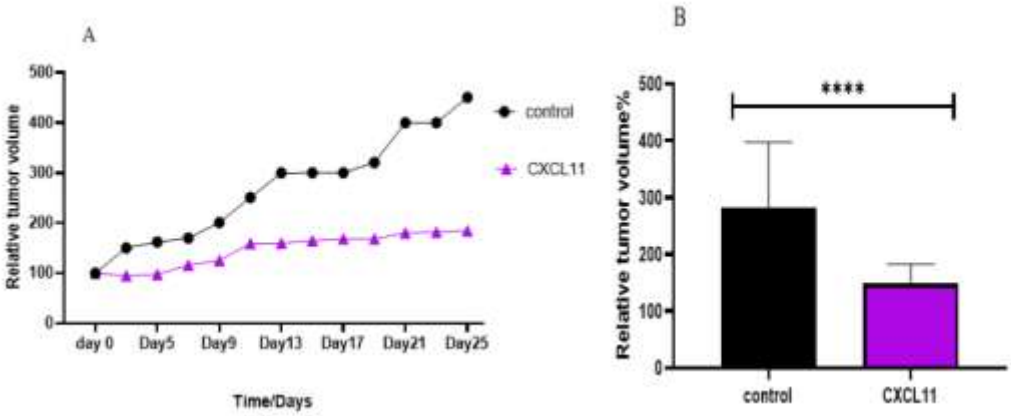

Figure 3. A) The relative tumor volume during treatment period. B): The comparison among groups depending on accumulative relative tumor volume percentage. Values represent the (mean ± SD), *P <0.05, **P <0.01, ***P <0.001 and ****P <0.0001

Survival time in mice model

The survival time between treatment group throughout the experiment explained in Figure (4), which showed a difference in the number of dead mice and prolong survival. In the control group, one mouse was died in the second week, and one was dead at the end of the third week, and three mice remained to live until the end of the experiment. For CXCL11 treated group one mice died at the end of the third week, and one mouse showed complete healing from cancer in the middle of the second week.

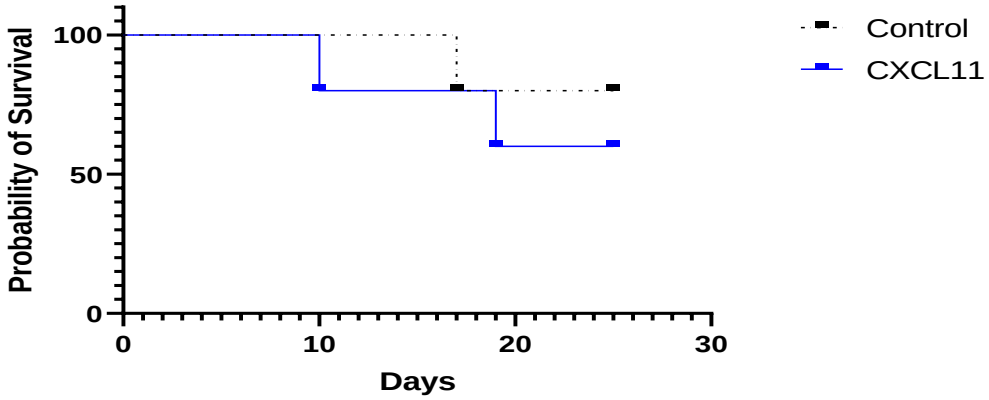

Figure 4. Prolong surviving in mice model

Discussion

In this study, a comparison between the non-treated control group and the CXCL11 treated group showed an anti-tumor effect of CXCL11, and this may be attributed to the presence of CXCL11 in the tumors. A previous study found that high concentration of CXCL11 production in tumor tissue compared to normal adjacent give good prove of healing [25].

The current study also showed that the tumor volume was enlarged in size during the treatment period of the control group. While, the CXCL11 treated group exhibited a steady ratio of tumor volume from day 10 until the end of experiment, when compared depending on accumulative tumor volume, a highly significant difference was shown between the CXCL11 treated group compared to the non-treated control group. This could be explained by the facts that CXCL11 might be able to reset the tumor microenvironment (TME) by modulating CD8+ T cell accumulation, tumor antigen-reactive T cells play a key role in eradicating tumors. In addition, CXCL11 promotes the entry of type-1 effector cells (CTLs, Th1 and NK cells) into inflamed or tumor tissues. CXCL11 is important for the T-cell attraction (effector phase) and the development of adaptive immunity (induction phase). Modulation of TME was achieved via CXCL11-mediated inhibition of suppressor factors, such as decreased transforming growth factor beta (TGF-b), cyclooxygenase-2 (COX2), and chemokine ligand 22 (CCL22). CXCL11 induces an immunotolerizing drives CD4+ T cell polarization into IL-10 high T regulatory (Tr-1) and IL-4high Th2 cells. Also, CXCL11 contributes to inhibiting angiogenesis and tumor progression. CXCL11 suppression tumor growth with subsequently increased survival rates in a therapeutic tumor model [25-31] is in consistent with current result.

Current study showed that there was decrease in mice weight in non-treated control group, while an increase in mice weight was observed in CXCL11 treated group. Also, CXCL11 treated group showed highly significant differences in mice weight in compared to non-treated control group during 25 days, this indicated that CXCL11 treatment has a little cytotoxic effect on mice throughout the entire period of experiment. This is in agreement with another

study that found that mice that received intratumoral injections of CXCL11-armed oncolytic adenoviruses gained steadily weight than mice in the control group and mice that got only oncolytic adenoviruses. The chemokine CXCL11 may cause changes in the types of immune cells within the TME. CXCL11 can increase the chemotaxis of activated T cells and NK cells and potentially suppress M2 macrophage polarization in a murine cancer model. The presence of cytotoxic CD8+ T cells, NK cells, and M1 macrophages within the TME is generally associated with regression of tumors as well as a favorable prognosis [(32)].

In addition to its beneficial effects on reducing tumor volume, CXC11 treatment resulted in a longer life span than the untreated control group. One mouse also showed fully cancer recovered by the middle of the second week after treatment (Figure 4). Another study found that mice given saline as a control developed tumors quickly and died within 30 days, but that a single injection of CXCL11-armed oncolytic adenoviruses considerably slowed tumor growth and increased survival time compared to control [(32)]. In breast cancer, high CXCL11 was determined to be positively correlated with immune response activation, increased antitumour immune cell infiltration, immune checkpoint molecule expression, and enhanced sensitivity to immunotherapy and chemotherapy [(33)].

In conclusion, growth inhibition of cancer and decrease in RTV with no toxicity effect of CXCL11 on mice weight; together these results provide important insights into the fact that CXCL11 could be used as an anti-cancer immunotherapy treatment.

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Author contribution

As part of her PhD thesis, Dr. Mohammed performed the laboratory work and wrote the draft of this study. Dr. Al-Shuwaikh and Prof. Dr. Al-Shammari designed, supervised and co-wrote this manuscript. The final version of this manuscript was read and approved by all authors.

Conflict of interest

The authors reported no potential conflict of interest.

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References

1. Coley WB. The treatment of malignant tumors by repeated inoculations of erysipelas. With a report of ten original cases. 1893. Clin Orthop Relat Res. 1991; (262): 3-11.
2. Grimm EA, Mazumder A, Zhang HZ, et al. Lymphokine-activated killer cell phenomenon. Lysis of natural killer-resistant fresh solid tumor cells by interleukin 2-activated autologous human peripheral blood lymphocytes. J Exp Med. 1982; 155(6): 1823-41. doi: 10.1084/jem.155.6.1823.
3. Mazumder A, Grimm EA, Zhang HZ, et al. Lysis of fresh human solid tumors by autologous lymphocytes activated in vitro with lectins. Cancer Res. 1982; 42(3): 913-8.
4. Kirkwood JM, Ernstoff MS. Interferons in the treatment of human cancer. J Clin Oncol. 1984; 2(4): 336-52. doi: 10.1200/JCO.1984.2.4.336.
5. Zlotnik A, Yoshie O. The chemokine superfamily revisited. Immunity. 2012;3 6(5): 705-16. doi: 10.1016/j.immuni.2012.05.008.
6. Murphy PM, Baggiolini M, Charo IF, et al. International union of pharmacology. XXII. Nomenclature for chemokine receptors. Pharmacol Rev. 2000; 52(1): 145-76.
7. Strieter RM, Polverini PJ, Kunkel SL, et al. The functional role of the ELR motif in CXC chemokine-mediated angiogenesis. J Biol Chem. 1995; 270(45): 27348-57. doi: 10.1074/jbc.270.45.27348.
8. Cambien B, Karimdjee BF, Richard-Fiardo P, et al. Organ-specific inhibition of metastatic colon carcinoma by CXCR3 antagonism. Br J Cancer. 2009; 100(11): 1755-64. doi: 10.1038/sj.bjc.6605078.
9. Ohmori Y, Schreiber RD, Hamilton TA. Synergy between interferon-gamma and tumor necrosis factor-alpha in transcriptional activation is mediated by cooperation between signal transducer and activator of transcription 1 and nuclear factor kappaB. J Biol Chem. 1997; 272(23): 14899-907. doi: 10.1074/jbc.272.23.14899.
10. Ohmori Y, Wyner L, Narumi S, et al. Tumor necrosis factor-alpha induces cell type and tissue-specific expression of chemoattractant cytokines in vivo. Am J Pathol. 1993; 142(3): 861-70.
11. Muehlinghaus G, Cigliano L, Huehn S, et al. Regulation of CXCR3 and CXCR4 expression during terminal differentiation of memory B cells into

plasma cells. Blood. 2005; 105(10): 3965-71. doi: 10.1182/blood-2004-08-2992.
12. Brightling CE, Ammit AJ, Kaur D, et al. The CXCL10/CXCR3 axis mediates human lung mast cell migration to asthmatic airway smooth muscle. Am J Respir Crit Care Med. 2005; 171(10): 1103-8. doi: 10.1164/rccm.200409-1220OC.
13. Rani MR, Foster GR, Leung S, et al. Characterization of beta-R1, a gene that is selectively induced by interferon beta (IFN-beta) compared with IFN-alpha. J Biol Chem. 1996; 271(37): 22878-84. doi: 10.1074/jbc.271.37.22878.
14. Colvin RA, Campanella GS, Sun J, et al. Intracellular domains of CXCR3 that mediate CXCL9, CXCL10, and CXCL11 function. J Biol Chem. 2004; 279(29): 30219-27. doi: 10.1074/jbc.M403595200.
15. Burns JM, Summers BC, Wang Y, et al. A novel chemokine receptor for SDF-1 and I-TAC involved in cell survival, cell adhesion, and tumor development. J Exp Med. 2006; 203(9): 2201-13. doi: 10.1084/jem.20052144.
16. Campanella GS, Medoff BD, Manice LA, et al. Development of a novel chemokine-mediated in vivo T cell recruitment assay. J Immunol Methods. 2008; 331(1-2): 127-39. doi: 10.1016/j.jim.2007.12.002.
17. Martín-Fontecha A, Thomsen LL, Brett S, et al. Induced recruitment of NK cells to lymph nodes provides IFN-gamma for T(H)1 priming. Nat Immunol. 2004; 5(12): 1260-5. doi: 10.1038/ni1138.
18. Xanthou G, Duchesnes CE, Williams TJ, et al. CCR3 functional responses are regulated by both CXCR3 and its ligands CXCL9, CXCL10 and CXCL11. Eur J Immunol. 2003; 33(8): 2241-50. doi: 10.1002/eji.200323787.
19. Schoenborn JR, Wilson CB. Regulation of interferon-gamma during innate and adaptive immune responses. Adv Immunol. 2007; 96: 41-101. doi: 10.1016/S0065-2776(07)96002-2.
20. Mosser DM, Edwards JP. Exploring the full spectrum of macrophage activation. Nat Rev Immunol. 2008; 8(12): 958-69. doi: 10.1038/nri2448.
21. Gorbachev AV, Kobayashi H, Kudo D, et al. CXC chemokine ligand 9/monokine induced by IFN-gamma production by tumor cells is critical for T cell-mediated suppression of cutaneous tumors. J Immunol. 2007; 178(4): 2278-86. doi: 10.4049/jimmunol.178.4.2278.
22. Hensbergen PJ, Wijnands PG, Schreurs MW, et al. The CXCR3 targeting chemokine CXCL11 has potent antitumor activity in vivo involving attraction of CD8+ T lymphocytes but not inhibition of angiogenesis. J Immunother. 2005; 28(4): 343-51. doi: 10.1097/01.cji.0000165355.26795.27.
23. Yang X, Chu Y, Wang Y, et al. Targeted in vivo expression of IFN-gamma-inducible protein 10 induces specific antitumor activity. J Leukoc Biol. 2006; 80(6): 1434-44. doi: 10.1189/jlb.0306212.
24. Al-Shammari AM, Hamad MA, Al-Mudhafar MA, et al. Clinical, molecular and cytopathological characterization of a Newcastle disease virus from an outbreak in Baghdad, Iraq. Vet Med Sci. 2020; 6(3): 477-84. doi: 10.1002/vms3.262.
25. Gao Q, Wang S, Chen X, et al. Cancer-cell-secreted CXCL11 promoted CD8+ T cells infiltration through docetaxel-induced-release of HMGB1 in NSCLC. J Immunother Cancer. 2019; 7(1): 42. doi: 10.1186/s40425-019-0511-6.
26. Kelsoe JR. Arguments for the genetic basis of the bipolar spectrum. J Affect Disord. 2003; 73(1-2): 183-97. doi: 10.1016/s0165-0327(02)00323-3.
27. Hamilton NH, Mahalingam S, Banyer JL, et al. A recombinant vaccinia virus encoding the interferon-inducible T-cell alpha chemoattractant is attenuated in vivo. Scand J Immunol. 2004; 59(3): 246-54. doi: 10.1111/j.0300-9475.2004.01391.x.
28. Berencsi K, Meropol NJ, Hoffman JP, et al. Colon carcinoma cells induce CXCL11-dependent migration of CXCR3-expressing cytotoxic T lymphocytes in organotypic culture. Cancer Immunol Immunother. 2007; 56(3): 359-70. doi: 10.1007/s00262-006-0190-2.
29. Wendel M, Galani IE, Suri-Payer E, et al. Natural killer cell accumulation in tumors is dependent on IFN-gamma and CXCR3 ligands. Cancer Res. 2008; 68(20): 8437-45. doi: 10.1158/0008-5472.CAN-08-1440.
30. Liu Z, Ravindranathan R, Li J, et al. CXCL11-Armed oncolytic poxvirus elicits potent antitumor immunity and shows enhanced therapeutic efficacy. Oncoimmunology. 2015; 5(3): e1091554. doi: 10.1080/2162402X.2015.1091554.
31. Mosmann TR, Coffman RL. TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. Annu Rev Immunol. 1989; 7: 145-73. doi: 10.1146/annurev.iy.07.040189.001045.
32. Wang G, Zhang Z, Zhong K, et al. CXCL11-armed oncolytic adenoviruses enhance CAR-T cell therapeutic efficacy and reprogram tumor microenvironment in glioblastoma. Mol Ther. 2023; 31(1): 134-53. doi: 10.1016/j.ymthe.2022.08.021.
33. Zhang X, Wu J, Hu C, et al. CXCL11 negatively regulated by MED19 favors antitumour immune infiltration in breast cancer. Cytokine. 2023; 162: 156106. doi: 10.1016/j.cyto.2022.156106.

Correspondence to Dr. Arwa M. Al-Shuwaikh
E-mail: arwa_alshwaikh_2004@yahoo.com
arwa.mujahid@nahrainuniv.edu.iq
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Risk Factors of Diabetic Nephropathy Among A Group of Iraqi Children with Type 1 Diabetes Mellitus

Mohammed S. Hammoud[1] *MSc*, **Faris A. F. Alawsi**[2] *FICMS (Ped), FICMS (Ped Neph)*, **Shatha H. Ali**[3] *CAPB*, **Rayah S. Baban**[4] *PhD*

[1]Al-Ramadi Teaching Hospital, Al-Anbar, Iraq, [2]Ibn Albaladi Maternity and Children Hospital, Baghdad, Iraq, [3]Dept. of Pediatrics, College of Medicine, Al-Nahrain University, Baghdad, Iraq, [3]Dept. of Chemistry and Biochemistry, College of Medicine, Al-Nahrain University, Baghdad, Iraq

Abstract

Background Diabetic nephropathy is the major cause of end-stage kidney disease in patients with diabetes mellitus.

Objective To identify risk factors for occurrence of Diabetic nephropathy among group of Iraqi children with type 1 diabetes mellitus.

Methods A cross-sectional study was conducted at Department of Chemistry and Biochemistry, and department of Pediatrics, College of Medicine, Al-Nahrain University from November 2019 to April 2020. Total of 80 children with diabetic nephropathy were divided into two groups: 40 patients without diabetic nephropathy and 40 patients with diabetic nephropathy. Blood and urine samples were taken for biochemical tests.

Results Each of age, duration of diabetes mellitus, fasting blood sugar and HbA1c were significantly associated with diabetic nephropathy. However, duration showed higher predictive value (OR = 18.69, 95% CI = 5.48-63.74, p<0.001). Very low-density lipoprotein (VLDL-c) was the most powerful lipid test as predictor for diabetic nephropathy (OR = 15.07, 95% CI = 3.98-57.1, p<0.001). Albumin-creatinine ratio was the most powerful renal function test predictor for diabetic nephropathy (OR = 107.6, 95% CI = 20.3-569.6, p<0.001). When all variables were entered in multivariate regression model, only 3 (out of 14) remained significantly associated with the development of diabetic nephropathy. Of those, albumin creatinine ratio >25 mg/g was the most powerful independent predictor (OR = 11.78, 95%CI = 7.45-42.82, p = 0.009). In contrast, estimated glomerular filtration rate, was a negative predictor of diabetic nephropathy (OR = 0.26, 95% CI = 2.79-18.93, p = 0.026). HbA1c level >9% was a positive predictor of diabetic nephropathy (OR = 4.15, 95% CI = 3.88-36.91, p value = 0.031).

Conclusion Albumin creatinine ratio and estimated glomerular filtration rate, are the strongest predicting factors for development and progression of diabetic nephropathy in Iraqi children with type 1 diabetes mellitus.

Keywords Diabetes mellitus, diabetic nephropathy, children, risk factors

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List of abbreviations: BMI = Body mass index, DBP = Diastolic blood pressure, eGFR = Estimated glomerular filtration rate, FBS = Fasting blood sugar, HbA1c = Glycated hemoglobin, HDL = High-density lipoprotein, LDL = Low-density lipoprotein, SBP = Systolic blood pressure, TG = Triglycerides, VLDL = Very low-density lipoprotein, uACR = Urinary albumin to creatinine ratio

Introduction

Diabetes mellitus (DM) is metabolic disorder that characterized by chronic hyperglycemia and glycosuria, which

results from insulin deficiency that caused by autoimmune destruction of pancreatic beta cells (T1DM) [1].

T1DM usually occurs in adolescents with a peak begin around puberty [2]. The American Diabetes Association (ADA) in 2020, have issued DM diagnostic criteria [3].

Diabetic nephropathy (DN) is one of the microvascular complications, which represents the major cause of end-stage renal disease in DM patients [4]. DN occurs in 20% to 40% of all diabetic cases [5,6]. ADA (2019) guidelines for screening of chronic kidney disease (CKD) in diabetic patients, and The Kidney Disease: Improving Global Outcomes (KDIGO) recommends annually at least, assessing urinary albumin/creatinine ratio (uACR) and estimated glomerular filtration rate (eGFR) in patients with T1DM with duration ≥5 years, and in all patients with T2DM and hypertension. ACR is considered more sensitive and dependable biomarker to identify the patients who are at a risk for progression to DN [7].

Albuminuria is defined as above than 30 mg/day of albumin urine excretion, and is the initial sign of DN or cardiovascular dysfunction [8,9].

Numerous epidemiological studies show that ethnicity, family history, elevated blood pressure, hyperglycemia, dyslipidemia, obesity, and insulin resistance are the main risk factors of DN and other putative risk factors involve elevated glycosylated hemoglobin level (HbA1c), proteinuria and smoking [10].

This study was conducted to identify risk factors for occurrence and progression of DN among group of Iraqi children with T1DM.

Methods

This cross-sectional study was conducted at the Diabetic Pediatric Clinic in Al-Imamein Al-Kadhimein Medical City, Ibn Al-Balady Children and Maternity Hospital and Al-Ramadi Teaching Hospital for Gynecology and Pediatrics from November 2019 to April 2020.

Diagnosis of DM of all patients was according to ADA (2020) [3].

The study group was divided into two groups:

1. Normoalbuminuria (without DN): N = 40 (22 male and 18 female) included patients with uACR <30 mg/g creatinine.
2. Microalbuminuria (with DN): N = 40 (22 male and 18 female) included patients with uACR = 30-299 mg/g creatinine.

Patient with chronic inflammatory condition (autoimmune disease, systemic Lupus Erythematosus), other renal disease, liver disease, malnourished children and malignancies were excluded.

Ethical approval was taken from parents of children involved in this study. The protocol of study was approved by the Scientific Committee of Department of Pediatrics, College of Medicine, Al-Nahrain University.

Five milliliters of venous blood were obtained from each fasting (8 hour fast) patient. Also freshly voided morning urine samples were collected from each patient.

Blood and urine samples were immediately transferred to Department of Chemistry and Biochemistry, College of Medicine, Al-Nahrain University, for biochemical tests using LINEAR kits (Spain).

Normal reference values of blood chemistries were applied accordingly [11]. Determination of the stage of CKD was done by calculating the eGFR using Schwartz formula [12].

Statistical analysis

All data were analyzed with statistical package for social sciences (SPSS). Continuous variables were express as Mean±standard deviation (SD) and analyzed with independent student t-test. Categorical variables were expressed as number and percentage and analyzed with Chi-square. Univariate and multivariate logistic regressions were used to predict the factors associated with the development DN. Receiver operating characteristic (ROC) curve was used to evaluate the diagnostic value of eGFR and uACR with T1DM. The area under the curve (AUC) provides a useful tool to compare different biomarkers. Whereas an AUC value close to 1 indicates an excellent diagnostic and

predictive marker. Pearson's correlation was used to explore the possible correlation between different quantitative variables in diabetic patients with and without DN. Odds ratio (OR) used to measure association between an exposure and an outcome. A p ≤0.05 was considered statistically significant.

Results

Demographic and clinical characteristics of the patients

The mean±SD of age of diabetic patients with DN significantly was higher than diabetic patients without nephropathy (P <0.001). In contrast, there were no significant differences between the two groups in body mass index (BMI), gender distribution, systolic and diastolic blood pressure P >0.05. However, patients with DN showed significantly higher fasting blood sugar (FBS), HbA1c and duration of than patients without DN as shown in table (1).

Table 1. Demographic and clinical characteristics of the patients

Variables		Without Nephropathy (N=40)	With Nephropathy (N=40)	P value
Age (years)	Mean±SD	11.6±3.04	13.7±2.52	0.001
BMI (kg/m^2)	Mean±SD	19.35±0.85	19.56±1.24	0.380
Gender	Male N (%) Female N (%)	22 (55%) 18 (45%)	22 (55%) 18 (45%)	1.000
Duration (years)	Mean±SD	2.83±1.15	8.35±3.72	<0.001
SBP (mmHg)	Mean±SD	118.5±9.82	123.0±11.47	0.423
DBP (mmHg)	Mean±SD	78.0±7.12	82.75±9.2	0.391
FBS (mg/dl)	Mean±SD	229.9±42.77	278.23±40.03	<0.001
HbA1c (%)	Mean±SD	8.97±0.95	9.76±1.01	0.031

Values are expressed as mean ± standard deviation, BMI: body mass index, SBP: systolic blood pressure, DBP: diastolic blood pressure, FBS: fasting blood sugar, HbA1c: glycated hemoglobin

Lipid profile

The mean±SD of cholesterol, triglycerides (TG), low-density lipoprotein (LDL-c) and very low-density lipoprotein (VLDL-c) in diabetic patients with DN was significantly higher than that of patients without DN (P <0.001). In contrast, patients without DN had significantly higher level of high-density lipoprotein (HDL-c) than those with DN as shown in Table (2).

Table 2. Lipid profile of the patients

Variables		Without Nephropathy (N=40)	With Nephropathy (N=40)	P value
Cholesterol (mg/dl)	Mean±SD	154.9±19.0	174.6±17.56	<0.001
TG (mg/dl)	Mean±SD	117.83±26.95	150.98±28.09	<0.001
LDL-c (mg/dl)	Mean±SD	91.08±16.6	112.09±16.12	<0.001
VLDL-c (mg/dl)	Mean±SD	23.56±5.39	30.1 9± 5.62	0.018
HDL-c (mg/dl)	Mean±SD	38.57±13.3	32.60±13.75	0.044

Values are expressed as mean ± standard deviation, TG: Triglycerides, HDL: High-density lipoprotein, LDL: Low-density lipoprotein, VLDL: Very low-density lipoprotein

Renal functions tests

All parameters included in renal function tests (urea, creatinine, and ACR) were recorded in higher level in patients with DN than those without DN, with highly significant differences. In contrast, eGFR was significantly lower in patients with DN compared to those without DN P <0.001 and significantly lower levels of serum albumin, P <0.021 (Table 3).

Table 3. Renal functions tests of the patients

Variables		Without Nephropathy (N=40)	With Nephropathy (N=40)	P value
B. Urea (mg/dl)	Mean±SD	33.62±3.79	40.92±4.78	<0.001
S. Creatinine (mg/dl)	Mean±SD	0.72±0.14	1.04±0.21	<0.001
S. Albumin (g/dl)	Mean±SD	4.08±0.35	3.35±0.38	0.021
eGFR (ml/min)	Mean±SD	109.22±8.81	84.35±12.28	<0.001
uACR (mg/g)	Mean±SD	18.28±4.32	52.69±39.48	<0.001

Values are expressed as mean ± standard deviation, eGFR: Estimated glomerular filtration rate, uACR: Urinary albumin to creatinine ratio

Predictors of DN

Univariate logistic regression test was used to find out the predication value of each parameter that showed significant variation between patients with and without DN. Through this test, the OR with its corresponding 95% CI were calculated.

Demographic and clinical predictors

Each of age, duration of DM, FBS and HbA1c were significantly associated with DN. However, duration showed higher predictive value followed by FBS, and HbA1c as shown in table (4).

Table 4. Demographic variable as predictors for diabetic nephropathy

Variables		Without Nephropathy (N=40)	With Nephropathy (N=40)	P value	Odds ratio (95%CI)
Age (years)	≤12 N (%)	22 (55%)	11 (27.5%)	0.012	3.22 (1.27-8.19)
	>12 N (%)	18 (45%)	29 (72.5%)		
Duration (years)	≤5 N (%)	36 (90%)	13 (32.5%)	<0.001	18.69 (5.48-63.74)
	>5 N (%)	4 (10%)	27 (67.5%)		
FBS (mg/dl)	≤250 N (%)	30 (75%)	11 (27.5%)	<0.001	7.91 (2.92-21.43)
	>250 N (%)	10 (25%)	29 (72.5%)		
HbA1c (%)	≤9 N (%)	26 (65%)	10 (25%)	<0.001	5.57 (2.12-14.65)
	>9 N (%)	14 (35%)	30 (75%)		

FBS: Fasting blood sugar, HbA1c: Glycated hemoglobin

Lipid profile as predictor for DN

Each of cholesterol, LDL-c and VLDL-c was significantly associated with development of DN, with VLDL-c was the most powerful predictor compared with the other two components. In contrast, HDL-c was inversely associated with the development of DN (OR =

0.04, 95% CI = 0.01-0.15, P <0.001) as shown in table (5).

Table 5. Lipid profile as predictor for diabetic nephropathy

Variables		Without Nephropathy (N=40)	With Nephropathy (N=40)	P value	Odds ratio (95%CI)
Cholesterol (mg/dl)	≤165 N (%) >165 N (%)	26 (65%) 14 (35%)	13 (32.5%) 27 (67.5%)	<0.001	3.86 (1.53-9.75)
TG (mg/dl)	≤150 N (%) >150 N (%)	26 (65%) 14 (35%)	13 (32.5%) 27 (67.5%)	<0.001	3.86 (1.53-9.75)
LDL (mg/dl)	≤100 N (%) >100 N (%)	26 (65%) 14 (35%)	9 (22.5%) 31 (77.5%)	< 0.001	6.4 (2.89-17.15)
VLDL (mg/dl)	≤30 N (%) >30 N (%)	37 (92.5%) 3 (7.5%)	18 (45%) 22 (55%)	< 0.001	15.07 (3.98-57.1)
HDL (mg/dl)	≤35 N (%) >35 N (%)	4 (10%) 36 (90%)	29 (72.5%) 11 (27.5%)	< 0.001	0.04 (0.01-0.15)

TG: Triglycerides, HDL: High-density lipoprotein, LDL: Low-density lipoprotein, VLDL: Very low-density lipoprotein

Renal function tests as predictors for DN

For those parameters which were positively associated with DN (urea, creatinine, and ACR), the ACR was the most powerful predictor for DN. On the other hand, eGFR had a greater prediction value than albumin as a negative predictor for development of DN, Table (6).

Table 6. Renal function tests as Predictors for diabetic nephropathy

Variables		Without Nephropathy (N=40)	With Nephropathy (N=40)	P value	Odds ratio (95%CI)
B. urea (mg/dl)	≤35 N (%) >35 N (%)	28 (70%) 12 (30%)	2 (5%) 38 (95%)	< 0.001	44.33 (9.18-214.06)
S. Creatinine (mg/dl)	≤0.9 N (%) >0.9 N (%)	35 (87.5%) 5 (12.5%)	11 (27.5%) 29 (72.5%)	< 0.001	18.45 (5.85-59.23)
S. Albumin (g/dl)	≤3.5 N (%) >3.5 N (%)	4 (10%) 36 (90%)	27 (67.5%) 13 (32.5%)	< 0.001	0.05 (0.02-0.18)
eGFR (ml/min)	≤95 N (%) >95 N (%)	4 (10%) 36 (90%)	34 (85%) 6 (15%)	< 0.001	0.02 (0.01-0.08)
uACR (mg/g)	≤25 N (%) >25 N (%)	38 (95%) 2 (5%)	6 (15%) 34 (85%)	< 0.001	107.6 (20.3-569.6)

eGFR: Estimated glomerular filtration rate, uACR: Urinary albumin to creatinine ratio

Multivariate regression

When all variables were entered in multivariate regression model, only 3 (out of 14) remained significantly associated with the development of DN. Of those, ACR >25 mg/g was the most powerful independent predictor (OR = 11.78, 95%CI = 7.45-42.82, P = 0.009). In contrast, eGFR, was a negative predictor of DN (OR = 0.26, 95%CI = 2.79-18.93, P = 0.026). HbA1c level >9% was a positive predictor of DN (OR = 4.15, 95% CI = 3.88-36.91) as shown in table (7).

Table 7. Multivariate regression

Variables	P value	Odds ratio (95%CI)
Age (>12 years)	0.112	3.26 (2.71-13.67)
Duration (>5 years)	0.097	2.18 (1.91-17.53)
FBS (>250 mg/dl)	0.107	3.31 (2.24-21.81)
HbA1c (>9%)	0.031	4.15 (3.88-36.91)
Cholesterol (>165 mg/dl)	0.219	4.44 (1.09-38.73)
LDL-c (>100 mg/dl)	0.181	3.92 (2.98-26.51)
VLDL-c (>30 mg/dl)	0.285	1.89 (1.07-13.98)
HDL-c (≤35 mg/dl)	0.107	0.68 (3.65-22.87)
Blood urea (>35 mg/dl)	0.088	4.32 (2.11-17.67)
Serum creatinine (>0.9 mg/dl)	0.071	2.27 (1.16-21.28)
Serum albumin (≤3.5 g/dl)	0.114	0.34 (2.08-24.41)
eGFR (≤95 ml/min)	0.026	0.26 (2.79-18.93)
uACR (>25 mg/g)	0.009	11.78 (7.45-42.82)

FBS: Fasting blood sugar, HbA1c: Glycated hemoglobin, HDL: High-density lipoprotein, LDL: Low-density lipoprotein, VLDL: Very low-density lipoprotein, eGFR: Estimated glomerular filtration rate, uACR: Urinary albumin to creatinine ratio

Diagnostic value

According to the results of multivariate regression, ROC curve was used to assess the diagnostic value of all variables, which showed a significant association with the development of DN.

Glycated hemoglobin (HbA1c):

The AUC was 0.732, 95% CI = 0.622-0.841, P <0.001. The sensitivity and specificity of the test at cut off value of HbA1c = 9.1% were 0.75 and 0.65, respectively (Figure 1).

Urinary albumin/creatinine ratio (uACR)

The area under the curve (AUC) was 0.968, 95% CI = 0.936 - 0.999, P < 0.001. The sensitivity and specificity of the test at cut off value of uACR = 24.5 mg/g were 90% for each (Figure 2).

Estimated glomerular filtration rate

The AUC was 0.938, 95% CI = 0.883-0.993, P <0.001. The sensitivity and specificity of the test at cut off value of eGFR = 97 ml/min were 90% and 85% respectively (Figure 3).

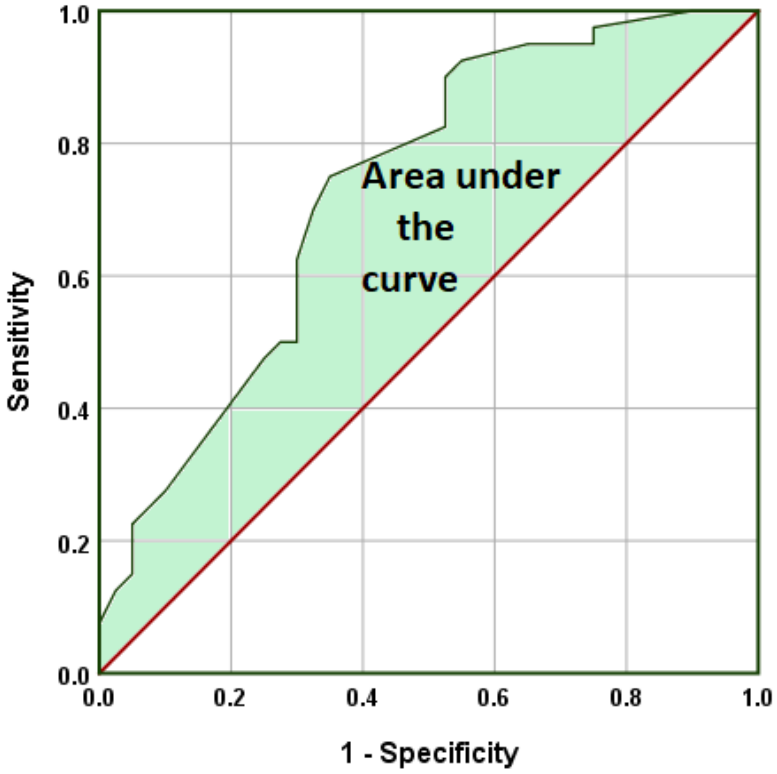

Figure 1. Receiver operating characteristic curve for HbA1c% in the context of detection of nephropathy in patients with T1DM

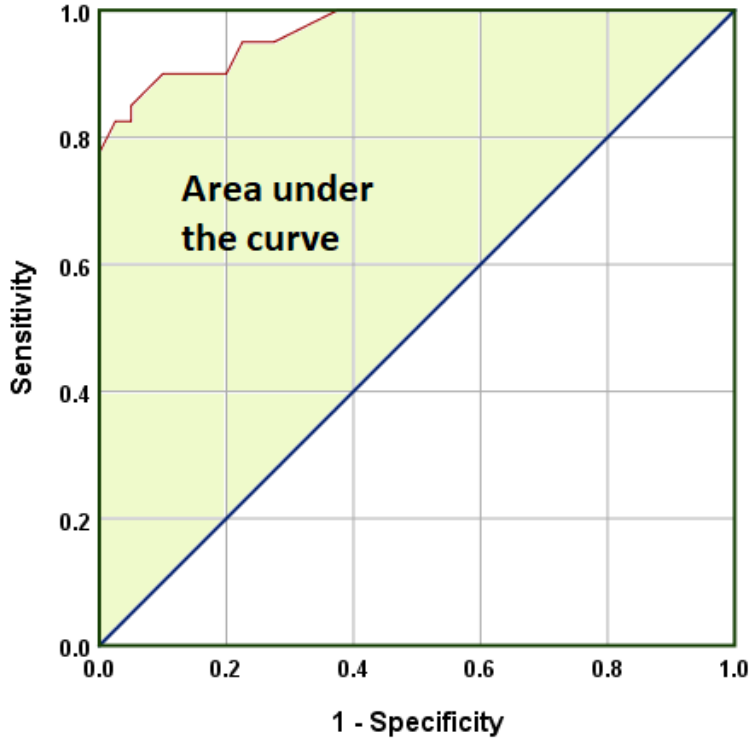

Figure 2. Receiver operating characteristic curve for urinary albumin/creatinine ratio (uACR) in the context of detection of nephropathy in patients with T1DM

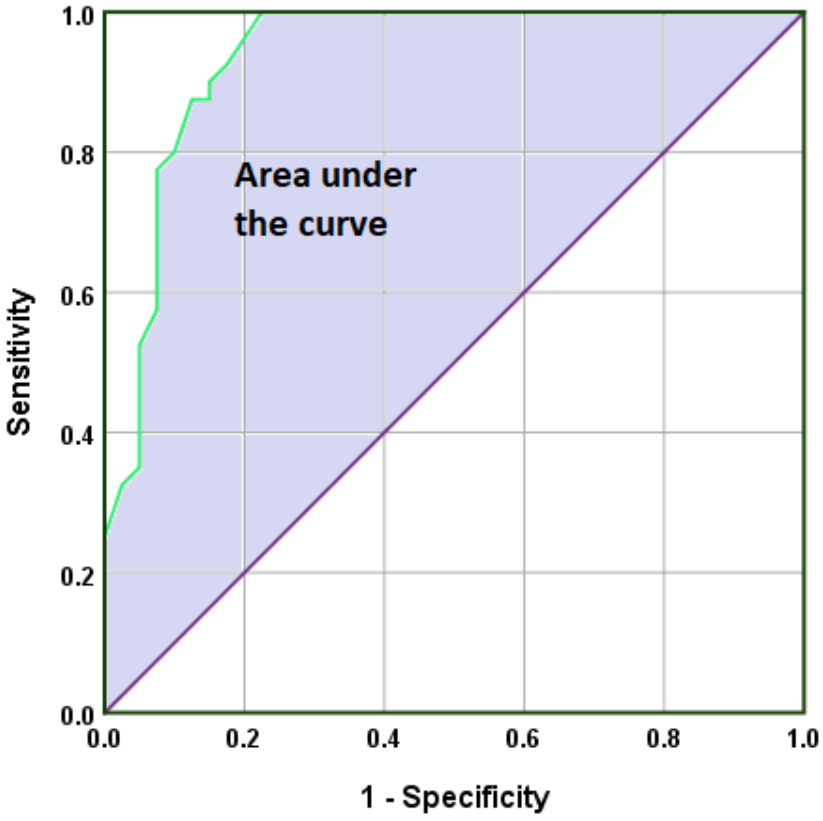

Figure 3. Receiver operating characteristic curve for estimated glomerular filtration rate (eGFR) in the context of detection of nephropathy in patients with T1DM

Discussion

Demographic and clinical characteristics of the patients

Several studies reported that the longer duration of DM and higher HbA1c level were significantly higher in children with Microalbuninuria when compared with normoalbuminuria children [13-19].

Several studies highlighted the effect of older age and longer duration of diabetes as predictor for nephropathy in T1DM patients [13,20,21].

Longer disease duration with longer exposure to hyperglycemia induce advanced glycation end products accumulation and leads to renal endothelial cells dysfunctions, so hyperglycemia is the driving force for the development of DN [17,22].

It has been argued that poor glycemic control in adolescents is directly proportionate to increasing age. This may be correlated to physiologic insulin resistance because of increased sex steroids, growth hormone and reduced free insulin-like growth factor [23].

Compared to other studies, systolic and diastolic blood pressure and BMI showed no statistically significant differences were found in both groups [13,16,24]. In Aljabri et al. (2013) study, there was no significant difference in gender with DN [18]. This result agrees with present study.

Regarding lipid profile, several studies found higher readings of cholesterol, TG, LDL-c and VLDL-c in patients with DN compared to patients without DN. increased cholesterol and LDL-c levels have been proven to be risk factors for DN [16,19, 25,26].

cholesterol is associated with persisted microalbuminuria in adolescents with T1DM [25]. The finding of significant decreased in HDL-c concentration in children with DN compared with normoalbuminuria patients was similar to three studies [19,27,28]. Another study showed that glycemic control significantly influenced

changes in lipid levels except HDL-c. The adverse effect of glycemic control could be due to glycation of lipoproteins, with subsequent reduction of their catabolism and to stimulation of transfer of cholesteryl esters from HDL-c to apolipoprotein B-containing lipoproteins [29]. Abnormal lipid profiles could cause tubule damage via deposition of lipids in renal tubule and may also develop in association with inflammation in vessel wall, therefore patients with hyperlipidemia shows higher ACR [30].

The changes in eGFR typically reflect derangement of renal function due to injury, with increasing the damage of the kidney, leading to decrease in the elimination of the creatinine and urea from the blood [21].

The present study was agreed with Zhang et al. (2019) who found hypoalbuminemia is frequently present in most patients with DN; the lower serum level of albumin was associated with the reduced renal function in patients with DN [31].

Renal function tests and HbA1c as predictors for DN

The current study showed that uACR is the most powerful predictor for DN followed by eGFR and HbA1c. Sueud et al. (2019) reported that the ACR has an excellent diagnostic power and it is a precise biomarker with best specificity, good sensitivity and acceptable accuracy in predicting the severity of nephropathy in patients with diabetes [32].

Two studies reported microalbuminuria was not only as an indicator of DN risk but also as a strong predictor of its progression, and albuminuria remain one of the strongest risk factors for progression of renal disease. ACR improves risk stratification and predicts CKD progression and mortality. Albuminuria and eGFR independently predicted kidney disease progression and the combination of both markers was superior to predict those subjects as highest risk for ESRD development [33,34].

In conclusion, ACR and eGFR are the strongest predicting factors for development and progression of DN in Iraqi children with T1DM but there many associated but inadequately controlled risk factors that need to be followed.

Understanding the relationship among the risk factors for DN in children and adolescents with T1DM helps us to take earlier steps for slowing down CKD.

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Author contribution

All authors have participated sufficiently in the intellectual content, conception and design of this work or the analysis and interpretation of the data, as well as the writing of the manuscript.

Conflict of interest

The authors declare there is no conflict of interest.

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References

1. Marcovecchio ML, Chiarelli F. Diabetic nephropathy in children. In: Avner ED, Harmon WE, Niaudet P, et al. (eds). Pediatric nephrology. 7th ed. New York: Heidelberg Springer; 2016. p. 1545-69.
2. Saberzadeh-Ardestani B, Karamzadeh R, Basiri M, et al. Type 1 diabetes mellitus: Cellular and molecular pathophysiology at a glance. Cell J. 2018; 20(3): 294-301. doi: 10.22074/cellj.2018.5513.
3. American Diabetes Association. 2. Classification and diagnosis of diabetes: Standards of medical care in diabetes-2020. Diabetes Care. 2020; 43(Supplement-1): S14–S31. doi: https://doi.org/10.2337/dc20-S002.
4. Yuan CM, Nee R, Ceckowski KA, et al. Diabetic nephropathy as the cause of end-stage kidney disease reported on the medical evidence form CMS2728 at a single center. Clin Kidney J. 2017; 10(2): 257-62. doi: 10.1093/ckj/sfw112.
5. Gheith O, Farouk N, Nampoory N, et al. Diabetic kidney disease: worldwide difference of prevalence and risk factors. J Nephropharmacol. 2015; 5(1): 49-56.
6. Alicic RZ, Rooney MT, Tuttle KR. Diabetic kidney disease: Challenges, progress, and possibilities. Clin J Am Soc Nephrol. 2017; 12(12): 2032-45. doi: 10.2215/CJN.11491116.
7. de Boer IH, Caramori ML, Chan JCN, et al. Executive summary of the 2020 KDIGO Diabetes Management

in CKD Guideline: evidence-based advances in monitoring and treatment. Kidney Int. 2020; 98(4): 839-48. doi: 10.1016/j.kint.2020.06.024.
8. Hafez MH, El-Mougy FA, Makar SH, et al. Detection of an earlier tubulopathy in diabetic nephropathy among children with normoalbuminuria. Iran J Kidney Dis. 2015; 9(2): 126-31.
9. Haneda M, Utsunomiya K, Koya D, et al. A new classification of diabetic nephropathy 2014: A report from Joint Committee on Diabetic Nephropathy. J Diabetes Investig. 2015; 6(2): 242-6. doi: 10.1111/jdi.12319.
10. Hussain S, Jamali MC, Habib A, et al. Diabetic kidney disease: An overview of prevalence, risk factors, and biomarkers. Clin Epidemiol Global Health. 2021; 9: 2-6. doi: https://doi.org/10.1016/j.cegh.2020.05.016.
11. Sharma J, Vasudevan A. Normal reference values of blood and urine chemistries. In: Phadke K, Goodyer P, Bitzan M (eds). Manual of pediatric nephrology. London, UK: Springer; 2014. p. 533-610.
12. Avner ED, Sreedharan R. Chronic kidney diseases. In: Kliegman RM, Stanton BF, St. Geme JW, et al. (eds). Nelson textbook of pediatrics. 19th ed. Philadelphia: Elsevier; 2011. p. 6558-66.
13. Zabeen B, Nahar J, Islam N, et al. Risk factors associated with microalbuminuria in children and adolescents with diabetes in Bangladesh. Indian J Endocrinol Metab. 2018; 22(1): 85-88. doi: 10.4103/ijem.IJEM_269_17.
14. Chae HW, Shin JI, Kwon AR, et al. Spot urine albumin to creatinine ratio and serum cystatin C are effective for detection of diabetic nephropathy in childhood diabetic patients. J Korean Med Sci. 2012; 27(7): 784-7. doi: 10.3346/jkms.2012.27.7.784.
15. Missambou Mandilou SV, Bouénizabila É, Nganga Ngabou F, et al. Microvascular complications of type 1 diabetes mellitus screening in children, adolescents and young adults: Report from a small center in a limited resource setting, Brazzaville - Congo. Ann Endocrinol Metabol. 2020; 3(1): 43-9. doi: 10.36959/433/565.
16. Kamaleldeen EB, Mohammad HA, Mohamed EF, et al. Microvascular complications in children and adolescents with type 1 diabetes mellitus in Assiut governorate, Egypt. Egyptian Ped Ass Gazette. 2018; 66(4): 85-90. doi: https://doi.org/10.1016/j.epag.2018.10.003.
17. Zeng XF, Lu DX, Li JM, et al. Performance of urinary neutrophil gelatinase-associated lipocalin, clusterin, and cystatin C in predicting diabetic kidney disease and diabetic microalbuminuria: A consecutive cohort study. BMC Nephrol. 2017; 18(1): 233. doi: 10.1186/s12882-017-0620-8.
18. Aljabri KS, Bokhari SA. Glycemic control of patients with type 1 diabetes mellitus in Saudi community. J Diabetes Metabol. 2013, 4(4). 255-7. doi: 10.4172/2155-6156.1000256.
19. Daniels M, DuBose SN, Maahs DM, et al. Factors associated with microalbuminuria in 7,549 children and adolescents with type 1 diabetes in the T1D Exchange clinic registry. Diabetes Care. 2013; 36(9): 2639-45. doi: 10.2337/dc12-2192.
20. Baek JH, Lee WJ, Lee BW, et al. Age at diagnosis and the risk of diabetic nephropathy in young patients with type 1 diabetes mellitus. Diabetes Metab J. 2021; 45(1): 46-54. doi: 10.4093/dmj.2019.0134.
21. Piscitelli P, Viazzi F, Fioretto P, et al. Predictors of chronic kidney disease in type 1 diabetes: a longitudinal study from the AMD Annals initiative. Sci Rep. 2017; 7(1): 3313. doi: 10.1038/s41598-017-03551-w.
22. Al-Eisa AA, Al-Hajri A, Al-Shuaib S, et al. Early-onset microalbuminuria in children with type 1 diabetes in Kuwait. Curr Pediatr Res 2017; 21 (2): 254-9.
23. Kim SH, Park MJ. Effects of growth hormone on glucose metabolism and insulin resistance in human. Ann Pediatr Endocrinol Metab. 2017; 22(3): 145-52. doi: 10.6065/apem.2017.22.3.145.
24. Omar MA, Rezk MM, El-Kafoury AA, et al. Microalbuminuria and glycated hemoglobin in children with type 1 diabetes mellitus. Alexandria J Med. 2015; 51(1): 83-8.
25. Huang CY, Ting WH, Lo FS, et al. Factors associated with diabetic nephropathy in children, adolescents, and adults with type 1 diabetes. J Formos Med Assoc. 2017; 116(12): 924-32. doi: 10.1016/j.jfma.2017.09.015.
26. Vinoth M, Pinto NR, Ferreira A, et al. Lipid profile of diabetic kidney disease patients in rural Goa, India. J Med Dent Sci Res. 2016; 3(4): 25-8.
27. Bulum T, Kolaric B, Duvnjak L. Lower levels of total HDL and HDL3 cholesterol are associated with albuminuria in normoalbuminuric Type 1 diabetic patients. J Endocrinol Invest. 2013; 36(8): 574-8. doi: 10.3275/8850.
28. Dayem SM, Nazif HK, Ei-Kader MA, et al. Study of adiponectin level in diabetic adolescent girls in relation to glycemic control and complication of diabetes. Open Access Maced J Med Sci. 2015; 3(4): 613-8. doi: 10.3889/oamjms.2015.098.
29. Chait A, Subramanian S. Hypertriglyceridemia: Pathophysiology, role of genetics, consequences, and treatment. [Updated 2019 Apr 23]. In: Feingold KR, Anawalt B, Blackman MR, et al., (eds). Endotext [Internet]. South Dartmouth (MA): MDText.com, Inc.; 2000-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK326743/
30. Lee SH, Kim DH, Kim YH, et al. Relationship between dyslipidemia and albuminuria in hypertensive adults: A nationwide population-based study. Medicine (Baltimore). 2016; 95(16): e3224. doi: 10.1097/MD.0000000000003224.
31. Zhang J, Zhang R, Wang Y, et al. The level of serum albumin is associated with renal prognosis in patients with diabetic nephropathy. J Diabetes Res. 2019; 2019: 7825804. doi: 10.1155/2019/7825804.
32. Sueud T, Hadi NR, Abdulameer R, et al. Assessing urinary levels of IL-18, NGAL and albumin creatinine

ratio in patients with diabetic nephropathy. Diabetes Metab Syndr. 2019; 13(1): 564-8. doi: 10.1016/j.dsx.2018.11.022.

33. Uwaezuoke SN. Prevention of diabetic nephropathy in children and adolescents: How effective are the current strategies. Int J Diabetol Vasc Dis Res. 2015; 5: 1-5.

34. Lopez-Giacoman S, Madero M. Biomarkers in chronic kidney disease, from kidney function to kidney damage. World J Nephrol. 2015; 4(1): 57-73. doi: 10.5527/wjn.v4.i1.57.

Correspondence to Dr. Shatha H. Ali
E-mail: shatha6ali@yahoo.com
Shathah666@colmed-alnahrain.edu.iq
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Assessment of Women Knowledge Toward Cesarean Section Complications in Baghdad Teaching Hospital 2022

Fadia M. Abood[1] *HD/FM*, **Aliaa M. H. Alsafi**[2] *FICMS/CM*

[1]Al-Zuwiyah Primary Health Care Center, Al-Rasafa Health Directorate, Baghdad, Iraq, [2]Dept. of Family and Community Medicine, College of Medicine, University of Baghdad, Baghdad, Iraq

Abstract

Background The increasing rate of births through caesarean sections (CS) had become a source of concern in many countries. Maternal beliefs may influence the mode of delivery. Knowledge of pregnant women regarding CS and its complications is an important tool to assess their choice for birth modality.

Objective To assess women knowledge toward complications of CS and to find out the relationship between level of knowledge and their demographic characteristics.

Methods A cross sectional study with analytic elements was conducted in Baghdad Teaching Hospital, Medical City Complex in 2022 on women visited Gynecology and Obstetrics Consultation Outpatient Clinics and Ward for different reasons.

Results The study included 400 women, 67.5% (270) aged <35 years. The majority 78% (312) of study participants were with poor level of knowledge. Most women did not know about (weakness of bowel movement after delivery as a complication of CS), and (placenta progressing and adhesion in the next pregnancy) (260; 56.0%) and (252; 63.0%) respectively.

Conclusion Two third of the patients had low knowledge level regarding complications of CS. knowledge was better in those who were older than 35 years, highly educated and had a job.

Keywords Cesarean section, knowledge, pregnant women, wound infection

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List of abbreviations: CS = Caesarean section, PHCC = Primary health care centers, WHO = World Health Organization

Introduction

The increasing rate of births through caesarean sections (CS) had become a source of concern in many countries. Maternal beliefs may influence the mode of delivery [(1)]. However, many evidence suggests a high risk of poor outcomes (such as psychological or social well-being, maternal, perinatal and neonatal morbidity) associated with unnecessary sections [(2)]. Furthermore, some complications of CS are not immediately seen after delivery, and studies had shown that the hospitalization rate within the first 30 days after birth is two times more likely with a CS than with a normal vaginal delivery [(3)].

As a standard, patient's education, improving pregnant women's knowledge and perception towards CS are essential for a good outcome, patients' decision making, and managing medico-legal conditions. Good clients' knowledge and attitude towards surgery had been shown to have a positive effect on the clients' outcome [(4)]. Women perception about

modes of birth have been found to influence the choice of mode of birth by pregnant women. Therefore, it is essential to study these issues. Knowledge and attitudes of women about modes of delivery are different among them, depending on many factors such as culture, belief, educational levels, socioeconomic status in addition to health care system [5,6]. The main complication of CS is illustrated in the table (1) below [7]:

Table 1. Main complication of cesarean section

Early complications	Late complications
Infections (most common) Endometritis, wound infection, abscess	Cesarean scar defect (most common)
Subfascial hematoma	Abdominal wall endometriosis
Bladder flap hematoma (>4 cm)	Morbidly adherent placenta (Placenta accreta, increta, and percreta)
Uterine dehiscence	Cesarean scar ectopic pregnancy
Uterine rupture	Cesarean scar retained products of conception

Improvement of mothers' knowledge and attitude toward normal vaginal birth is considered as an important strategy to control increasing rate of CS on maternal demand [8]. Maternal perceptions about delivery through CS attributed to delayed presentation of women when needing emergency obstetric care. This increases the risks of complications and affect the achievement of the sustainable development goal target of reducing neonatal mortality to end new-born deaths [9,10].

This study objectives were to assess women knowledge toward complications of CS in Baghdad Teaching Hospital and to find out the relationship between level of knowledge and their demographic characteristics such as (age, education, and multipara-CS).

Methods

Study design, setting and duration

A cross sectional descriptive study with analytic components, was conducted in Baghdad Teaching Hospital, Medical City Complex from the period extended from January - June 2022.

Sample size and sampling technique

A convenient sample of 400 married women at reproductive age was recruited to the study, aged 15-45 years and currently married and accepted to participate in the study.

Tool of the study

A structured questionnaire validated by panel of experts at Department of Family and Community Medicine, College of Medicine, University of Baghdad utilizing information obtained from previous related studies [2,4-6].

The questionnaire consisted of two domains:

First appendix containing the demographic characteristics (studied variables and obstetric characteristics) of women including their age, residency, education, job, parity, and their gestational age of current pregnancy, previous CS.

The second appendix included information about women's knowledge towards CS main complications (bleeding, infection, delayed breast feeding and thromboembolism).

Data collection method

The information was obtained by direct interview with the women using the structured questionnaire at the morning through 4 days/week for two consecutive months (from January to June 2022).

Studied variables

- Age: less than 35 years, and equal or more than 35 years [2]
- Education: illiterate, primary, secondary, college, and higher education [4]
- Early post-operative bleeding: Post procedural hemorrhage of skin and subcutaneous tissue following CS procedure in the first two weeks [4]
- Late post-operative bleeding: Post procedural hemorrhage of skin and subcutaneous tissue following C/S procedure in 15-40 days [11]
- Thromboembolism: A disorder that includes deep vein thrombosis and pulmonary embolism [11]
- Delayed breast feeding: A temporarily delay the large increase in milk production usually seen between 3 to 5 days following birth [11].
- Uterus rupture: It is spontaneous tearing of the uterus that may result in the fetus being expelled into the peritoneal cavity [12,13]

Ethical considerations

A verbal consent was taken from all the women who decided to participate in the study. None of the participants were interviewed in front of any of the relatives, thus; a complete confidentiality was ensured.

Administrative approval

Data collection was started after obtaining the official approval from the Scientific Committee of College of Medicine, University of Baghdad, Iraqi Ministry of Health and Medical City Health Directorate

Scoring

The knowledge part of questions had 3 levels of scores, 0, 1, and 2; 0 for incorrect answer, 1 for uncertain and 2 for correct answer, representing poor, fair and good level of knowledge respectively.

A total score of <50% considered as poor, 50-75% was considered as fair, while >75% was considered as good knowledge.

Calculating and analysis the score of the scale

The score of each participated woman was calculated by application of the following equation:

Overall knowledge score for each participant = (total score (summation of the scores of all items))/(highest possible score)×100

After that, the level of knowledge for each participated woman was divided into 3 levels:

- Poor knowledge: those who achieved <50% score.
- Average knowledge: those who achieved 50-75% score.
- Good knowledge: those who achieved >75% score.

Statistical analysis

Data were introduced into Microsoft excel sheet 2019 and loaded into SPSS (Statistical Package for Social Sciences) version (24). Parametric data are presented as mean and standard deviation. Categorical data presented as numbers and percentages. Chi- square test and Fisher exact test was used. P-value <0.05 was considered significant.

Results

The study sample were 400 women, 67.5% of them of age less than 35, 35.3% of them had primary education, 68.3% were housewives, 55% had 3-5 children and45.7% of them had 1-3 CS. Their sociodemographic features detailed in table (2).

Table 2. Sociodemographic distribution of study sample

Variable		N.	%
Age group	<35	270	67.5
	≥35	130	32.5
Education	Illiterate	100	25.0
	Primary	141	35.3
	Secondary	120	30.0
	College	37	9.3
	Higher education	2	0.5
Occupation	Housewife	273	68.3
	Employer	95	23.8
	Self employed	32	8.0
Residency	Urban	331	82.8
	Rural	69	17.2
Parity	<3	120	30
	3-5	219	55
	>5	61	15
Number of CS	0	178	44.5
	1-3	179	45.7
	>3	43	10.8

N=400

The overall knowledge score with mean of (10±6), minimum score was 0, and maximum achieved score by participants were 28 (which also the highest possible score).

Figure (1) illustrates the distribution of overall level of knowledge among study participants whereas the overwhelming majority with poor level of knowledge corresponding to 78% (312) of study participants.

In table (3), the scale of knowledge of participated women regarding CS complications is illustrated. It shows that most women did not know about weakness of bowel movement after delivery as a complication of CS, and placenta progressing and adhesion in the next pregnancy 260 (56.0%), 252 (63.0%) respectively.

There was a significant association (p <0.001) between age and overall level of knowledge as the highest proportion of good knowledge 63.3% (19) among women ≥35 years, and highest level of poor knowledge 71.2% (222) among women younger than 35 years. A significant association was found between women overall knowledge level and their levels of education, women's occupation and with parity. As all illustrated in table (4).

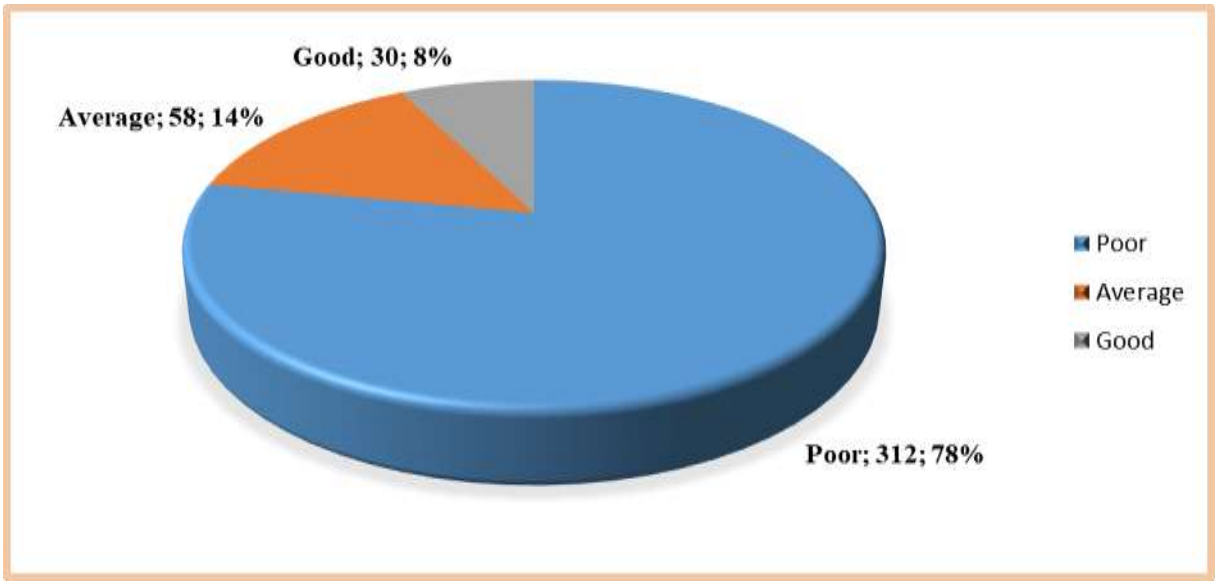

Figure 1. Distribution of overall level of knowledge among study participants (n=400)

Table 3. Women knowledge regarding cesarean section complications

Items	Scale					
	Don't know		Uncertain		know	
	N.	%	N.	%	N.	%
Delayed wound healing and wound infection after the operation	158	39.5	216	54.0	26	6.5
Early post-partum hemorrhage	110	27.5	251	62.7	39	9.8
Late post-partum hemorrhage	232	58.0	120	30.0	48	12.0
Bladder injury	225	56.3	145	36.2	30	7.5
Respiratory tract infection as a result of Anesthesia	111	27.8	244	61.0	45	11.3
Paralytic ileus	260	65.0	114	28.5	26	6.5
Abdominal pannus	167	41.8	174	43.4	59	14.8
Urinary tract infection	143	35.8	219	54.8	38	9.4
Thromboembolism	184	46.0	175	43.7	41	10.3
Endometritis	225	56.2	145	36.3	30	7.5
Placenta progressing and adhesion in the next pregnancy	252	63.0	116	29.0	32	8.0
Uterus rupture	258	64.5	106	26.5	36	9.0
Delayed breast feeding	111	27.2	185	46.3	104	26.0
Cesarean section cost	56	14	170	42.5	174	44.5

N=400

Table 4. Distribution of Overall Knowledge level in relation to Sociodemographic characteristics of study participants

Variable		Total (400)	Overall, Knowledge level						P value
			Poor		Average		Good		
			N.	%	N.	%	N.	%	
Age group	<35	270	222	71.2	37	63.8	11	36.7	<0.001*
	≥35	130	90	28.8	21	36.2	19	63.3	
Education	Illiterate	100	90	28.8	8	13.8	2	6.7	<0.001*
	Primary	141	121	38.8	18	31.0	2	6.7	
	Secondary	120	95	30.4	22	37.9	3	10.0	
	College	37	6	1.9	9	15.5	22	73.3	
	Higher education	2	0	0.0	1	1.7	1	3.3	
Occupation	Housewife	273	240	76.9	29	50.0	4	13.3	<0.001*
	Employer	95	59	18.9	22	37.9	14	46.7	
	Self employed	32	13	4.2	7	12.1	12	40.0	
Residency	Urban	331	255	81.7	47	81.0	29	96.7	0.092*
	Rural	69	57	18.2	11	18.9	1	3.3	
Parity	<3	120	93	29.8	11	19.0	16	53.3	0.008*
	3-5	219	170	54.5	40	69.0	9	30.0	
	>5	61	49	15.7	7	12.1	5	16.7	
Previous Cesarean Section	0	178	143	45.8	20	34.5	15	50.0	0.089**
	1-3	179	131	42.0	35	60.3	13	43.3	
	>3	43	38	12.2	3	5.2	2	6.7	

*Chi-square is significant at p < 0.05, **Fisher-exact test is significant at p < 0.05

Discussion

One of the most frequently performed operations for women is CS, in the past decade it was estimated that one third of all births done by caesarean section in USA [13].

In the current study that included 400 women, more than half of them were aged below 35 years and those with primary education level were the majority that agreed with Saoji et al. [14] and Razzaq et al. [15], Ghasvari et al. [16], which could be related to society nature where a significant number of females get marry and give birth when they are younger in age, in which all are countries that known of poor attention to female education as well as poverty, which could explain the distribution of low proportion of women with high education level between participants. Those mothers with 3-5 parity constituted more than half of participant, which is consistent with Al Sulamy et al. [17], those with 2-3 parity had the highest proportion of participant.

In the current study, pregnant with no previous CS or with previous (2-3) CS were forming the overwhelming majority that agreed with KojoPrah et al. study [18]. In this study, those participants who were younger than 35 years old and women with <3 parities were forming the majority that might explain this high proportion of ≤3 previous CS.

The study demonstrated that nearly two third of participants had a poor knowledge regarding CS complications, which is not different from Razzaq et al. [15] who found a higher acceptable level of knowledge, as well as the finding of Ghasvari et al. [16] who found that only one third of participants had good knowledge of the complications. Low or no knowledge regarding CS were found in 65.1% and this may be related to high proportion of participants had poor education and the low socioeconomic state was also a cause of low awareness in Arabic society, however, the economic status of participants was not mentioned.

By using a scaling list to evaluate knowledge of participants, more than half of the studied sample didn't know about the following complication: “weakness of bowel movement after delivery, placenta progressing and adhesion in the next pregnancy and uterus explosion in the next deliveries” and uncertain regarding “early bleeding after the operation, respiratory tract infection as a result of anaesthesia”.

The finding of the current study agreed with Saleh et al. [19] regarding “placenta progressing and adhesion in the next pregnancy, uterus explosion in the next deliveries and early bleeding after the operation" but differs in the other items regarding participants knowledge.

There was a statistical association between demographic data and overall knowledge level show an increased awareness of CS complication in participants who were older than 35 years, which was different from the studies of Ghasvari et al. [16] and Saleh et al. [19], which showed non statistically significant association between age and level of knowledge. The finding of this study might be explained by what stated in Rydahl et al. study [20] in which, women equal or over 35 years were more likely to be multiparous, and to have comorbid illnesses or pregnancy-related compared to those under 35 years. Those with such risk factors are more to deliver by CS according to their doctor advice, which is mostly decided few months before the date of delivery. CS gives the chance for the patients to know more about the producer, in addition to the information's from their peers or family member which could explained current study findings.

In the current study, low education level had been significantly associated with poor knowledge regarding operation complications, which was agreed with Ghasvari et al. [16] and Razzaq et al. [15] studies, but Saleh et al. [19] who observed no association. The reason behind this finding is that higher education level mothers can easily get access to information regarding the procedure even without need of medical consultation unlike low educated mothers, in addition low educational level had impact on chose delivery method, most of those with good education often chose elective Caesarean section delivery [21,22], this is may be due to patient differences or physician bias, physicians should be aware of this disparity and should attempt to provide unbiased informed consent for all women regardless of their level of education. Furthermore, poorly educated mothers frequently experience the following negative effects: social exclusion, unhealthy lifestyle choices, maltreatment, stress, and depression. Young mothers are also more likely to drop out of school or get a lower level of education [23].

The study also demonstrated that women with <3 parity had been significantly associated with increase knowledge level which disagreed with KojoPrah et al. [18] study that found the increase in parity is associated with increase knowledge about CS. According to Iraqi culture, educated women are preferring fewer pregnancies and small family sizes, moreover, they are searching for their information and adopting their knowledge from reliable sources like health care providers which might explain our finding.

In conclusion, two thirds of the patients had low knowledge level regarding complications of CS, especially complications like “weakness of bowel movement after delivery”, “placenta progressing and adhesion in the next pregnancy”, and “uterus explosion in the next deliveries” while "delay breast feeding" was the most known complication for participants. Knowledge was better in those who were 35 years or older, highly educated and had a job especially women with less than three parities.

The authors recommend put more emphasis on increase women awareness about CS complications by conducting group discussion at primary health care centers (PHCC) for pregnant women attending for ANC, using mass media, booklets, and brochures. Also, guidelines and indications of caesarean section according to World Health Organization (WHO) should be clarified and announced for the patients and need to be followed by obstetricians.

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Author contribution
Dr. Abood: data collection, statistical analysis, writing the manuscript. Dr. Alsafi: study design, literature review, final revision.

Conflict of interest
None.

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References
1. Majlesi M, Montazeri A, Rakhshani F, et al. 'No to unnecessary caesarean sections': Evaluation of a mass-media campaign on women's knowledge, attitude and intention for mode of delivery. PLoS One. 2020; 15(8): e0235688. doi: 10.1371/journal.pone.0235688.
2. Boatin AA, Ngonzi J, Ganyaglo G, et al. Cesarean delivery in low- and middle-income countries: A review of quality-of-care metrics and targets for improvement. Semin Fetal Neonatal Med. 2021; 26(1): 101199. doi: 10.1016/j.siny.2021.101199.
3. Maharlouei N, Rezaianzadeh A, Hesami E, et al. The preference of Iranian women to have normal vaginal or cesarean deliveries. J Res Med Sci. 2013; 18(11): 943-50.
4. Mohammad EM, AL-Ghabban SI, Al-Alak MM. Indications of primary caesarean sections among Iraqi women in Karbala city. Int J Curr Pharmaceut Res. 2019; 11(6): 123-5. doi: doi:10.22159/ijcpr.2019v11i6.36360.
5. Shabila NP. Rates and trends in cesarean sections between 2008 and 2012 in Iraq. BMC Pregnancy Childbirth. 2017; 17(1): 22. doi: 10.1186/s12884-016-1211-6.
6. World Health Organization. Health Cluster Iraq - Annual Report 2019. Available https://reliefweb.int/report/iraq/health-cluster-iraq-annual-report-2019. Accessed at June 2022
7. Ministry of Health. Health Cluster Iraq - Annual Report 2019. Available at: https://reliefweb.int/report/iraq/health-cluster-iraq-annual-report-2019. Accessed at April 26, 2023
8. Saraya YS, Alashkar AH, Ali MA, et al. Indications and rate of first cesarean delivery in central region's maternity and children hospital. Saudi Med J. 2019; 40(12): 1251-5. doi: 10.15537/smj.2019.12.24736.
9. Batieha AM, Al-Daradkah SA, Khader YS, et al. Cesarean section: incidence, causes, associated factors and outcomes: A national prospective study from Jordan. Gynecol Obstet Case Rep. 2017; 3(3): 55. doi:10.21767/2471-8165.1000055.
10. Al-Hammami H, Taleb MJ, Alsharif MN. Prevalence of cesarean section at Al-Tawlid Hospital during the Syrian crisis. J Med Pharmaceut Allied Sci. 2017; 6(2): 947-53. doi:10.22270/jmpas.v6i12.746.
11. Endalew M, Endalew N, Agegnehu A, et al. Knowledge and attitude towards anesthesia for cesarean section and its associated factors among pregnant women attending antenatal care: A cross sectional study. Ann Med Surg (Lond). 2022; 75: 103299. doi: 10.1016/j.amsu.2022.103299.
12. Ahmad I, Afshan G. Knowledge and attitudes of Pakistani women towards anaesthesia techniques for caesarean section. J Pak Med Assoc. 2011; 61(4): 359-62.
13. Ige OA, Oyedepo OO, Adesina KT, et al. Anesthesia for cesarean section in the University of Ilorin Teaching Hospital, Ilorin, Nigeria: A 5-year review. J Med Trop. 2019; 21: 10-3.
14. Saoji A, Nayse J, Kasturwar N, et al. Women's knowledge, perceptions, and potential demand towards caesarean section. Nat J Comm Med. 2011; 2(02): 244-8.
15. Razzaq MS, Jumaah ZN, Atiyah AA. Knowledge of Women Regarding Complications of Caesarean Section in Al-Najaf City Hospitals. Global Sci J. 2021; 9(1): 1595-606.
16. Ghasvari M, Rahmanian V, Rahmanian K. Knowledge of pregnant women in the southwest Iran about complications of cesarean section, 2009. Pars J Med Sci. 2012; 10(2): 37-42. doi: 10.29252/jmj.10.2.37.
17. Al Sulamy AA, Yousuf SA, Thabet HA. Knowledge and attitude of pregnant women toward elective cesarean section in Saudi Arabia. Open Journal of Nursing. 2019; 9(2): 199-208. doi: 10.4236/ojn.2019.92020.
18. KojoPrah J, Kudom A, UwumbornyiLasim O, et al. Knowledge, attitude and perceptions of pregnant women towards caesarean section among antenatal clinic attendants in Cape Coast, Ghana. Texila Int J Public Health. 2017; 5(1): 1-8. doi: 10.21522/TIJPH.2013.05.01.Art007.
19. Saleh BB, Wardia WI. Assessment of mothers' knowledge toward cesarean section complications at the hospitals in Baghdad city hospitals. Medico-legal Update. 2020; 20(4): 1997-2005. doi: https://doi.org/10.37506/mlu.v20i4.2137.
20. Rydahl E, Declercq E, Juhl M, et al. Cesarean section on a rise-Does advanced maternal age explain the increase? A population register-based study. PLoS One. 2019; 14(1): e0210655. doi: 10.1371/journal.pone.0210655.
21. Tollånes MC, Thompson JM, Daltveit AK, et al. Cesarean section and maternal education; secular trends in Norway, 1967-2004. Acta Obstet Gynecol Scand. 2007; 86(7): 840-8. doi: 10.1080/00016340701417422.

22. Ardic C. Role of mother education level in delivery method preference. Family Pract Palliative Care. 2018; 3(1): 28-32. doi: https://doi.org/10.22391/fppc.382429.
23. Zimbeck M, Mohangoo A, Zeitlin J, et al. The European perinatal health report: Delivering comparable data for examining differences in maternal and infant health. Eur J Obst Gynecol Reprod Biol. 2009; 146(2): 149-51.

Correspondence to Dr. Fadia M. Abood
E-mail: fadiamunir3@gmail.com
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Detection of Human Bocavirus in Nephrotic Syndrome Children with Acute Upper Respiratory Tract Infections

Husam H. Lazim[1] *PhD*, **Shatha H. Ali[2]** *CABP*, **Asmaa B. Salim[3]** *PhD*

[1]Dept. of Basic Sciences, College of Dentistry, Ibn Sina University of Medical and Pharmaceutical Sciences, Baghdad, Iraq, [2]Dept. of Pediatrics, College of Medicine, Al-Nahrain University, Baghdad, Iraq [3]Dept. of Microbiology, College of Medicine, Al-Nahrain University, Baghdad, Iraq

Abstract

Background Nephrotic syndrome (NS) is a frequent chronic illness marked by changes in permselectivity at the glomerular capillary wall, as a result, it is unable to limit protein loss through the urine. Bacterial and viral infections are more common in patients with NS. Human Bocavirus (HBoV) is an emerging pathogen suspected to cause respiratory and GIT infections in children.

Objective To investigate the frequency of HBoV in children with NS who have acute upper respiratory tract infections, and compare it with normal controls.

Methods A case-control study carried out on 120 nasal swabs from children divided into three groups; 40 children each group (nephrotic syndrome and immunocompetent children with acute upper respiratory tract infections, and apparently children in good health without respiratory infections as control group). Viral DNA extracted from these samples and HBoV detected using real-time polymerase chain reaction.

Results HBoV was detected in 30 (75%) of patients with NS, and 18 (45%) in normal children with acute upper respiratory infections, while it was 3 (7.5%) in apparently healthy control group. The mean cycle threshold (CT) of HBoV in the three groups were 18.99 in nephrotic patients, 20.21 in normal children with acute upper respiratory infections and 24.33 in control group

Conclusion HBoV is relatively common among nephrotic children with acute upper respiratory infection, with a lowest CT (high viral load) as compared to the apparently normal children with acute upper respiratory infections and children in good health.

Keywords Nephrotic syndrome, Human Bocavirus, acute upper respiratory infections, children

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List of abbreviations: CCL = Chemokine (C-C motif) ligand, CT = Cycle threshold, HBov = Human bocavirus, IC = Internal control, IFN = interferon, IL = Interleukin, NS = Nephrotic syndrome, PCR = Polymerase chain reaction, SD = Standard deviation, TGF = Transforming growth factor

Introduction

Nephrotic syndrome (NS) is a prevalent chronic illness characterized by permselectivity changes at the glomerular capillary wall, as a result, it is unable to limit protein loss through urine. Nephrotic range proteinuria is defined as proteinuria exceeding 1000 mg/m² per day or spot (random) urinary protein-to creatinine ratio exceeding 2 mg/mg. The proteinuria in childhood NS is relatively discerning, mostly composed of albumin [(1)]. Patients with NS, bacterial and viral infections are more common in them. Urinary losses of immunoglobulins, complement, and properdin cause an increase

in infection susceptibility. Altered T-cell mechanisms, the usage of immunosuppressive therapy for a long time, and the presence of edema also contribute to infections [2].

Because of the immunodeficiency status in patients with NS, respiratory infections are the most common complications among them [3].

Human Bocavirus (HBoV) was first described by Allander and colleagues in 2005 [4]. The HBoV virions are icosahedral, non-enveloped and small, roughly 18-26 nm in diameter and their linear single-stranded DNA genome is 5543 bp in length [5]. HBoV has been linked to the upper and lower respiratory tract in a number of studies. Cough, fever, rhinorrhea, asthma exacerbation, bronchiolitis, severe wheezing, and pneumonia are the most commonly described clinical manifestations of HBoV infection in this regard [4,6-8].

The objective of this study was to investigate the frequency of HBoV in children with NS who have acute upper respiratory tract infections, and compare it with normal controls.

Methods

A case-control study carried out on 40 children to each (NS children with acute upper respiratory infection, normal children with acute upper respiratory apparently healthy children without respiratory infections as control group) aged 3-18 years admitted to Al-Imamein Al-Kadhimein Medical City, Central Teaching Hospital of Pediatric, Children Welfare Teaching Hospital at (Baghdad Medical City), and Al-Karama Teaching Hospital – Baghdad. Nasal swabs were collected during winter and autumn of 2020. This study was approved by the Institutional Review Board (IRB) of the College of Medicine, Al-Nahrain University (approval no. 327 in 24/12/2019). Nasal swabs were placed in 1 ml virus transport media (VTM) tube (Heinz Herenz, Germany), which provided with the swab and stored at (-20C°) and the DNA extraction was done by using Viral Gene-spin™ Viral DNA/RNA Extraction Kit (Intron, Cat No 17151, Korea). Each sample was analyzed for the presence of HBoV by using ARVI-screen-FRT polymerase chain reaction (PCR) kit (R-V57-100-F) (AmpliSens, Russia). This kit is an in vitro nucleic acid amplification test for multiplex detection and identification of specific nucleic acid fragments of pathogens that cause acute respiratory viral infections. In this study, only the reagents of HBoV were used. The PCR mix (total volume 25 µl) composed of 10 µl of PCR-mix-1-FL-F, 5 µl of PCR-mix-2-FRT, Polymerase (TaqF) (0.5) µl, 3 µl were added of internal control, from this mixture, 15 µl is taken and 10 µl of the sample or positive control was added to it. PCR procedures were carried out on a real-time PCR detection system (Sa-cycler-96, Italy). Analysis of the PCR data was performed with computer software provided by the instruments company (Sa-cycler-96, Italy). Cycling conditions for the real time-PCR procedures were 1 cycle (95°C, 15 min) followed by 10 cycles (95°C, 10 sec) (54°C, 25 sec) (72°C, 72 sec) and then 35 cycles (95°C, 10 sec) (54°C, 25 sec) (72°C, 72 sec). The results were based on the number of positive cases for HBoV in each group and make a comparison between the mean of cycle threshold (CT) values in the three groups, and find out which group has the lowest CT value (higher viral load).

Statistical analysis

The data were processed using statistical package for social sciences (SPSS) version 16.0.0, and Microsoft Excel 2010. The data of the current study were scrutinized carefully in terms of being parametric or non-parametric using normality tests. Analysis of variance (ANOVA) test was used to measure the difference in means between groups.

Results

The age of NS ranged from 3-16 years; their mean age was 9.15 years. The respiratory presentations, sex and age of NS patients whom positive for HBoV were summarized in table (1).

The positive cases with HBoV were 30 (75%) out of 40 in nephrotic patients with acute upper respiratory infection, 18 (45%) in normal children with acute upper respiratory infection

and 3 (7.5%) in apparently healthy control group. The mean of CT values of HBoV in three groups were 18.99 in nephrotic patients, 20.21 in normal children and 24.33 in control group as shown in table (2).

Table 1. Age, sex and respiratory presentations of nephrotic syndrome patients whom +ve for HBoV

Parameter		NS, URTI +ve HBo N (%)	Normal, URTI +ve HBov N (%)	Normal +ve HBov N (%)
Sex	Male	15 (50.0)	12 (66.66)	2 (66.66)
	Female	15 (50.0)	6 (33.33)	1 (33.33)
p. value		1.030	0.222	0.977
Age (year)	3-5	4 (13.33)	7 (38.88)	2 (66.66)
	5-10	16 (53.33)	6 (33.33)	1 (33.33)
	10-20	10 (33.33)	5 (27.77)	0 (0)
p. value		0.07	0.221	0.977
Respiratory presentations	Common cold	25 (83.33)	10 (55.55)	---
	Pharyngitis	10 (33.33)	8 (44.44)	---
p. value		0.004	0.746	---

NS: Nephrotic syndrome, URTI: Upper respiratory tract infection

Table 2. The mean of cycle threshold values of HBoV in the three groups

Group	HBov +ve N (%)	Cycle threshold mean±SD	P value
Nephrotic syndrome with URTI	30 (75.0)	18.99±2.27	
Normal with URTI	18 (45.0)	20.21±1.75	0.222
Normal	3 (7.5)	24.33±1.31	

URTIs: Upper respiratory tract infections, SD = standard deviation

Discussion

There were several Iraqi studies, the results of which showed the presence of HBoV among children in Iraqi society [9-11]. In this study, 3/4 of NS patients have HBoV infection, also HBoV CT was lowest among patients with NS. There were more than one study linking between viral respiratory infection and exacerbation of the condition of patients with NS [12-14]. As for the direct relationship between HBoV and NS, to the best of our understanding, there is no study so far that explains the details of the mechanism of infection with this virus in patients with NS, but there is a study that showed the relationship between one of the important respiratory viruses, which is the respiratory syncytial virus (RSV) and NS. Rats infected with RSV developed proteinuria, according to the study, this was followed by a significant amount of podocyte destruction, and very modest alterations in renal tubular epithelia cells and mesangial cells. The connection between rat immune responses to RSV and nephrotic infection was explored in this study. Following RSV infection, blood levels of the cytokines (interleukins) IL-6, IL-17, and transforming growth factor (TGF)- were raised, and serum levels of IL-6 and IL-17 were higher following RSV re-infection than following RSV main infection. These findings suggested that abnormal adaptive immune responses to viral infection may exacerbate nephrotic damage.

RSV infection causes the production of cytokines such as type I (interferon) IFN and IFN-, IL-6, IL-8, IL-10, IL-13, and IL-17, as well as chemokines such as Chemokine (C-C motif) ligand (CCL3, CCL2, and CCL5) [15]. According to Turner et al. (2010), cytokines such as IL-17, IL-6, and IL-21 can bind to receptors on mesangial cells and renal tubular epithelial cells, causing chemokine production and neutrophil and monocyte recruitment to the kidney. These processes have the potential to cause pathological injury of kidney [16]. Respiratory tract infection a risk factor for the onset and relapse in NS patients [17-19]. Perhaps the HBoV performs the same mechanism when infecting patients with NS, as it is one of the respiratory viruses or there was a co-infection with other viruses.

There was a noticeable difference in the number of positive cases in the three groups, which the highest number and lowest CT (high viral load) in nephrotic group children and the expected reasons for this in the presence of a weakened immune system, exposure to HBoV during immunosuppression can lead to persistent infection and prolonged viral shedding [20].

In conclusion, HBoV had a wide distribution and lowest CT (high viral load) among nephrotic children with acute upper respiratory infection in compared to the normal children with acute upper respiratory infection and healthy children.

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Author contribution

The authors, altogether, conceived and planned the study. The experiment was done by Dr. Lazim under supervision of Dr. Ali and Dr. Salim.

Conflict of interest

There is no conflict of interest.

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References

1. Bagga A, Mantan M. Nephrotic syndrome in children. Indian J Med Res. 2005;122(1):13-28.
2. Mantan M, Singh S. Infection associated relapses in children with nephrotic syndrome: A short-term outcome study. Saudi J Kidney Dis Transpl. 2019; 30(6): 1245-53. doi: 10.4103/1319-2442.275468.
3. Wei CC, Yu IW, Lin HW, et al. Occurrence of infection among children with nephrotic syndrome during hospitalizations. Nephrology (Carlton). 2012; 17(8): 681-8. doi: 10.1111/j.1440-1797.2012.01650.x.
4. Allander T, Tammi MT, Eriksson M, et al. Cloning of a human parvovirus by molecular screening of respiratory tract samples. Proc Natl Acad Sci U S A. 2005; 102(36): 12891-6. doi: 10.1073/pnas.0504666102.
5. Gurda BL, Parent KN, Bladek H, et al. Human bocavirus capsid structure: insights into the structural repertoire of the parvoviridae. J Virol. 2010; 84(12): 5880-9. doi: 10.1128/JVI.02719-09.
6. Bastien N, Brandt K, Dust K, et al. Human Bocavirus infection, Canada. Emerg Infect Dis. 2006; 12(5): 848-50. doi: 10.3201/eid1205.051424.
7. Jartti T, Hedman K, Jartti L, et al. Human bocavirus-the first 5 years. Rev Med Virol. 2012; 22(1): 46-64. doi: 10.1002/rmv.720.
8. Kesebir D, Vazquez M, Weibel C, et al. Human bocavirus infection in young children in the United States: molecular epidemiological profile and clinical characteristics of a newly emerging respiratory virus. J Infect Dis. 2006; 194(9): 1276-82. doi: 10.1086/508213.
9. Rasheed ZS, Al-Shuwaikh AMA, Issa KR. Multiplex RT-PCR based detection of Human Bocavirus and other respiratory viruses in infants and young children with lower respiratory tract infection. Iraqi JMS. 2019; 17(1): 74-82. doi: 10.22578/IJMS.17.1.11.
10. Yaseen ZT, Khudair MK, Naseef AS, et al. Detection of Human Bocavirus in Children Suffering from respiratory tract infection in Diyala province. Diyala J Med. 2020; 18(2): 121-9.
11. Al-Mayah QS, Hussein AA, Hasan DA. Detection of Human Bocavirus infection in children with lower respiratory. Rafidain J Sci. 2018; 27(4): 64-74. doi: 10.33899/rjs.2018.159335
12. Xiong X-L, Yu C-Y, Wang M. Etiologic features of respiratory tract infections seen in children with primary nephrotic syndrome: A cross-sectional retrospective study. Iran J Pediatr. 2020; 30(6): e99276. doi: 10.5812/ijp.99276.
13. Pardede SO, Rafli A, Odilo J, et al. Respiratory tract infection and diarrhea as risk factors for relapsing nephrotic syndrome. Asian J Pediatr Nephrol. 2018; 1(1): 38. doi: 10.4103/AJPN.AJPN_1_18.
14. Jung SJ, Park SE, Lee JH. A case of severe influenza infection in a child with nephrotic syndrome on steroid therapy. J Korean Soc Pediat Nephrol. 2014; 18(1): 47–50. doi: DOI:10.3339/jkspn.2014.18.1.47.

15. Zhai S, Zhong L, Zhang L, et al. Re-infection with respiratory syncytial virus aggravates renal injury. Int J Clin Exp Pathol. 2016; 9(3): 4149-56.
16. Turner JE, Paust HJ, Steinmetz OM, et al. The Th17 immune response in renal inflammation. Kidney Int. 2010; 77(12): 1070-5. doi: 10.1038/ki.2010.102.
17. Han H, Wang S, Liang Y, et al. Respiratory tract infection: A risk factor for the onset and relapse of adult-onset minimal change disease in Southern China. Biomed Res Int. 2018; 2018: 1657208. doi: 10.1155/2018/1657208.
18. Harambat J, Allard L, Godron-Dubrasquet A. Relapse rate of nephrotic syndrome in the time of COVID-19. Pediatr Nephrol. 2021; 36(1): 211-12. doi: 10.1007/s00467-020-04814-x.
19. Ali SH, twfeek ZA, Nariman Fahmi Ahmed Azat NFA, et al. Triggering factors for relapses in steroid sensitive nephrotic syndrome. Int J Curr Microbiol App Sci. 2016; 5(8): 842–51. doi: http://dx.doi.org/10.20546/ijcmas.2016.508.095.
20. Schenk T, Maier B, Hufnagel M, et al. Persistence of human bocavirus DNA in immunocompromised children. Pediatr Infect Dis J. 2011; 30(1): 82-4. doi: 10.1097/INF.0b013e3181f12fcf.

Correspondence to Husam H. Lazim
E-mail: hussam_lazim@yahoo.com
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Hyperthyroidism in Patients with Chronic Lymphocytic Leukemia

Waleed H. Saeed *PhD*

Dept. of Nursing, Akre Technical Institute, Duhok Polytechnic University, Duhok, Iraq

Abstract

Background Chronic lymphocytic leukemia (CLL) is a hematological malignancy characterized by the accumulation of immunologically mature cluster of differentiation (CD) 5+ B-lymphocytes in the blood, bone marrow and lymphatic tissues. Hyperthyroidism can occasionally be caused by non-thyroid cancers infiltrating the thyroid and cause hyperthyroidism.

Objective To assess the thyroid function of patients with CLL.

Methods Patients and participant controls are subjected to prevalence tests of thyroid function as thyroid stimulating hormone (TSH), thyroxine (T4) and triiodothyronine (T3). Serum samples were analyzed by specialized kits of immunodiagnostic mini VIDAS Industry system (BioMérieux, France).

Results Out of 37 males and 17 females CLL patients and 60 volunteer controls (36 males and 24 females), only two male patients at their sixth decade were diagnosed with hyperthyroidism, these patients were classified as Stage (A) to the Binet staging system. The test results were showed as follow: TSH; <0.05 miu/ml (Normal range 0.25-5.0 miu/ml); T4; 172.11, 182.34 nmol/l (Normal range 60-120 nmol/) and T3 3.4, 3.6 nmol/l (Normal range 0.9-2.3 nmol/l) for both patients respectively. All controls subject's thyroid function test results were normal.

Conclusion Accurate assessment of thyroid function and determination the origin of hyperthyroidism to identify any thyroid gland infiltration by chronic lymphocytic malignant cells in CLL patients using medical advanced diagnostic techniques can help treating hyperthyroidism.

Keywords Chronic lymphocytic leukemia (CLL), hyperthyroidism, malignancy, metastasis, infiltration, thyroid gland

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List of abbreviations: CD markers = Cluster of differentiation markers, CLL = Chronic lymphocytic leukemia, SLL = Small lymphocytic lymphoma, T3 = Triiodothyronine, T4 = Thyroxine, TSH = Thyroid stimulating hormone

Introduction

Chronic lymphocytic leukemia (CLL) is a clonal disorder characterized by the accumulation of morphologically mature cluster of differentiation (CD) 5+ B-lymphocytes in the peripheral blood, bone marrow and lymphatic tissues [(1)]. In patients with CLL as a result of defective apoptosis, B lymphoid cells build up and invade the spleen, lymph nodes, bone marrow, and, in extremely rare circumstances, other organs [(2)].

A heterogeneous category of malignant lymphocyte neoplasms known as lymphoma diseases accounts for about 2% of all thyroid gland malignancies [(3)]. The thyroid gland infiltration in patients with CLL is a part of systemic disease involvement [(4)].

Rare cases of CLL/SLL (chronic lymphocytic leukemia/small lymphocytic lymphoma) manifesting as thyroid abnormalities have been documented [(5)]. Instances of CLL affecting the thyroid have been reported to be extremely

rare, accounting for only 3-4% of all thyroid lymphoproliferative neoplasms [6].

Clinical diagnosis of thyroid cancer with metastatic illness is uncommon. But there have been a rising number of clinical cases with metastases to the thyroid gland, as a result of the development of new diagnostic methods [7].

Hyperthyroidism can occasionally be caused by non-thyroid cancers infiltrating the thyroid gland. It is possible for hematological cancers to invade the thyroid gland and this process may be combined with euthyroidism, hypothyroidism, or hyperthyroidism [8].

The aim of the study is the assessment of thyroid gland function in patients with CLL.

Methods

At the Hematology Clinics at the Nanakali and Azadi Hospitals in the cities of Erbil and Duhok in Iraqi Kurdistan region, a total of 54 patients with CLL were enrolled and 60 normal individuals with no history of hematologic malignancies were included as controls. The World Health Organization (WHO) diagnostic criteria were used to diagnose these patients. Absolute lymphocytosis, characteristic morphology, and immunophenotyping by immunohistochemistry and flow cytometry are some of these criteria [9]. Equal to or more than 5×10^9/l small monoclonal cells in peripheral blood are needed for diagnosis of CLL. These patients were identified as having CLL according to the WHO criteria, and flow cytometry showed clonality as indicated by kappa or lambda light chain restriction. CLL cells are frequently positive for CD5, CD23, CD19, and CD20 [9].

All patients were divided into staging categories (stage A, B, and C) in accordance with the International Working Party categorization created by Binet and his colleagues [10].

All patients were initially informed about the study and written consent was obtained. Three milliliters of peripheral venous blood were drawn from patients and control subjects clot activator gel tubes, and a centrifugation technique was utilized to obtain clear serum.

Thyroid function tests were performed using mini VIDAS system (BioMérieux, France).

For statistical analysis, the obtained data were analyzed using (statistical package for social sciences (SPSS) 24 program system. Frequency and its percentage value were used for description of the data.

Results

As shown in table (1), out of the 54 CLL patients and 60 healthy individuals with no history of malignancy (including CLL disease) were subjected to thyroid function tests during this study. The age of hospitalized CLL patients as revealed in table (2) were ranged from 44 to 82 years old (mean 61.31), of which 37 patients (68.5%) were males and the remaining 17 patients (31.5%) were females, with a male to female ratio (M:F) 2.18:1. For control participants, their ages ranged from 40 to 90 years (mean 57.53) with 36 (60%) males and 24 (40%) females. CLL cases were seen most frequently in males and according to decades of age among (61-70) year old patients with coincidence of hyperthyroidism disorder within same age's decade.

The Binet staging system was used to categorize CLL cases [10]. Stage A was more common than the other stages, as illustrated in table (3). Stage A was represented by 29 (53.7%) patients of the cases, Stage B by 15 (27.8%) patients, and Stage C, which was the least prevalent, by 10 (18.5%) patients.

Thyroid stimulating hormone (TSH), thyroxine (T4), and triiodothyronine (T3) tests were performed on all patients to evaluate the thyroid gland function, 2 patients age 63 and 67 years old men revealed thyroid dysfunction as hyperthyroidism and represented 3.7% of patients with CLL with TSH, <0.05 miu/ml (Normal range 0.25-5.0 miu/ml); T4, 172.11, 182.34 nmol/l (Normal range 60-120 nmol/l) and T3, 3.4, and 3.6 nmol/l (Normal range 0.9-2.3 nmol/l) respectively as illustrated in table (4). Noteworthy, both patients were within Stage (A) of CLL disease.

Table 1. Thyroid function Status of CLL patients according to gender

Patient gender	Thyroid function Status		Total	
	Euthyroidism	Hyperthyroidism	No.	%
Male	35	2	37	68.5
Female	17	0	17	31.5
Total	52	2	54	100
Controls gender				
Male	36	0	36	60
Female	24	0	24	40
Total	60	0	60	100

Table 2. Thyroid function Status of CLL patients according to age group

Age group (yr)	Thyroid function Status		Total	
	Euthyroidism	Hyperthyroidism	No.	%
40-50	6	0	6	11.11
51-60	16	0	16	29.63
61-70	21	2	23	42.60
71-80	8	0	8	14.81
81-90	1	0	1	1.85
Total	52	2	54	100

Table 3. Thyroid function Status of CLL patients according to disease stage

Age group (yr)	Thyroid function Status		Total	
	Euthyroidism	Hyperthyroidism	No.	%
A	27	2	29	53.7
B	15	0	15	27.8
C	10	0	10	18.5
Total	52	2	54	100

Table 4. Frequency and percentage of CLL patients related to thyroid function

Thyroid function	N	%
Euthyroidism	52	96.3
Hyperthyroidism	2	3.7
Total	54	100

Discussion

Previous researches have shown that leukemia and lymphoma, particularly CLL malignant cells infiltrate the thyroid gland [8]. The thyroid disorders can be developed by metastatic disease in the sixth or seventh decades of life in older people [11,12]. Due to their plentiful blood supply, endocrine glands generally can serve as targets for metastases from a variety of non-endocrine tumors [13]. The majority of individuals with metastases to the thyroid gland are euthyroid, although some patients may also have hypothyroidism or hyperthyroidism [8].

In the current study, thyroid gland function tests were performed on all CLL patients to investigate any abnormalities of its function, such as hypothyroidism or hyperthyroidism. Only two patients were 63- and 67-years old men revealed thyroid dysfunction as hyperthyroidism identified throughout this study, making up just 3.7% of all patients, while the participant volunteers control revealed non-hyperthyroidism status (euthyroidism). However, according to another study, the prevalence of CLL affecting the thyroid is remarkably low, accounting for only 3-4% of all thyroid lymphoproliferative neoplasms [6]. Obviously, these patients were located within CLL disease stage (A) according to Binet staging system and was reported by another study as having the same staging by Andrysiak-Mamos and his colleagues [14].

In conclusion, infiltration of malignant cells like CLL cells into the thyroid gland may induce tissue damage and hyperthyroidism. Therefore, a pathologist's correct determination of the origin of hyperthyroidism utilizing a variety of diagnostic procedures (for instance, cytological evaluation by fine needle aspiration biopsy) may allow a therapist to make the best decision for managing hyperthyroidism.

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Conflict of interest

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References

1. Gaidano G, Foà R, Dalla-Favera R. Molecular pathogenesis of chronic lymphocytic leukemia. J Clin Invest. 2012; 122(10): 3432-8. https://doi.org/10.1172/JCI64101
2. Derringer GA, Thompson LD, Frommelt RA, et al. Malignant lymphoma of the thyroid gland: a clinicopathologic study of 108 cases. Am J Surg Pathol. 2000; 24(5): 623-39. doi: 10.1097/00000478-200005000-00001.
3. Thieblemont C, Mayer A, Dumontet C, et al. Primary thyroid lymphoma is a heterogeneous disease. J Clin Endocrinol Metab. 2002; 87(1): 105-11. doi: 10.1210/jcem.87.1.8156.
4. Reid-Nicholson M, Moreira A, Ramalingam P. Cytologic features of mixed papillary carcinoma and chronic lymphocytic leukemia/small lymphocytic lymphoma of the thyroid gland. Diagn Cytopathol. 2008; 36(11): 813-7. doi: 10.1002/dc.20894.
5. Shin J, Chute D, Milas M, et al. A rare case of chronic lymphocytic leukemia/small lymphocytic lymphoma presenting in the thyroid gland. Thyroid. 2010; 20(9): 1019-23. doi: 10.1089/thy.2010.0089.
6. Gonçalves A, Vale S, Nobre E, et al. Thyroid nodule: first manifestation of chronic lymphocytic leukaemia. Arch Endocrinol Metab. 2015; 59(2): 190-4. doi: 10.1590/2359-3997000000034.
7. Nixon IJ, Coca-Pelaz A, Kaleva AI, et al. Metastasis to the thyroid gland: A critical review. Ann Surg Oncol. 2017; 24(6): 1533-9. doi: 10.1245/s10434-016-5683-4.
8. Jonklaas J. Infiltration of the thyroid gland by non-thyroid malignancy: A literature review reveals this to be an unusual cause of hyperthyroidism. J Clin Transl Endocrinol. 2020; 20: 100221. doi: 10.1016/j.jcte.2020.100221.
9. Rawstron AC, Kreuzer KA, Soosapilla A, et al. Reproducible diagnosis of chronic lymphocytic leukemia by flow cytometry: An European Research Initiative on CLL (ERIC) & European Society for Clinical Cell Analysis (ESCCA) Harmonisation project. Cytometry B Clin Cytom. 2018; 94(1): 121-8. doi: 10.1002/cyto.b.21595.
10. Binet JL, Auquier A, Dighiero G, et al. A new prognostic classification of chronic lymphocytic leukemia derived from a multivariate survival analysis. Cancer. 1981; 48(1): 198-206. doi:

10.1002/1097-0142(19810701)48:1<198::aid-cncr2820480131>3.0.co;2-v.

11. Autelitano F, Santeusanio G, Mauriello A, et al. Patologia latente della tiroide: studio epidemiologico e statistico di tiroidi prelevate in corso di 507 autopsie consecutive [Latent pathology of the thyroid: an epidemiological and statistical study of thyroids sampled during 507 consecutive autopsies]. Ann Ital Chir. 1992; 63(6): 761-81.
12. Mistelou A, Papadatos SS, Kousi C, et al. Thyroid gland as a target of secondary malignancies - an autopsy study and review data. Folia Med (Plovdiv). 2019; 61(2): 277-88. doi: 10.2478/folmed-2018-0077.
13. Angelousi A, Alexandraki KI, Kyriakopoulos G, et al. Neoplastic metastases to the endocrine glands. Endocr Relat Cancer. 2020; 27(1): R1-R20. doi: 10.1530/ERC-19-0263.
14. Andrysiak-Mamos E, Becht R, Sowińska-Przepiera E, et al. Case report: rare case of infiltration of small lymphocytic B-cell lymphoma in the thyroid gland of female patient with B-cell chronic lymphocytic leukemia (CLL-B/SLL-B). Thyroid Res. 2013; 6(1): 1. doi: 10.1186/1756-6614-6-1.

Correspondence to Dr. Waleed H. Saeed
E-mail: waleed.Saeed@dpu.edu.krd
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Myrtus Communis Linn and its Potential Health Effects: A Review

Hend A. Abass[1] *PhD*, **Bayader A. Mahmeed**[1] *MSc*, **Zahraa S. Omran**[2] *PhD*

[1]Dept. of Chemistry and Biochemistry, College of Medicine, Al-Nahrain University, Baghdad, Iraq, [2]Dept. of Biochemistry, College of Medicine, Karbala University, Karbala, Iraq

Abstract

The communal Myrtus of Linn (Myrtus communis L.) can be defined as one of the most potent medicinal plants in the world is (family: Myrtaceae). The Mediterranean and Middle Eastern regions are home to the common myrtle, Myrtus communis L. The fruit of the myrtle has a distinct flavor and comes in either black or white. It is frequently used to treat a variety of ailments, including gastric ulcers, diarrhea, dysentery, vomiting, rheumatism, bleeding, deep sinusitis, leucorrhoea, and cosmetic issues including hair loss. Wine and cuisine are flavored with the blades, berries, and branches. Due to its high vitamin content, mature fruits were once utilized as a food integrator. It has been demonstrated in numerous studies that the myrtle plant's various parts contain a variety of bioactive substances. The plant's fruit and leaves both contain phenolic chemicals and anthocyanin, as well as quercetin, catechin, and myricetin. Numerous biologically active compounds are produced by the plant, including tannins, flavonoids, coumarins, strong oil, essential oils, fibers, carbohydrates, citric acid, malic acid, and antioxidants. The bioactive chemicals found in various myrtle plant sections are regarded to have beneficial benefits on health. According to earlier research, the plant has antioxidant, antibacterial, antidiabetic, anti-inflammatory, anti-ulcerative, anti-diarrheal, analgesic, and hair-growing properties. More human researches are required because it has been shown that the majority of these investigations are conducted on animals.

Keywords Myrtus communis Linn; antioxidant activity, antidiabetic and anti-inflammatory activity, antiulcerative and antidiarrheal activity; analgesic effect and effect on hair growth

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List of abbreviations: DPPH = 1, 1-diphenyl-2-picrylhydrazyl, ALT = Alanine transaminase, ALP = Alkaline phosphatase, AST = Aspartate aminotransferase, ATP = Adenosine triphosphate, GERD = Gastroesophageal reflux disease, GIS = Gastrointestinal system, H_2O_2 = Hydrogen peroxide, Myrtus communis Linn = Myrtus communis L, t-BOOH = Tert-butyl hydroperoxide

Introduction

Myrtus communis Linn (Myrtus communis L.), also referred to as myrtle, is a genus of flowering plants in the family Myrtaceae that Linnaeus first described in 1753. Is a blooming shrub that thrives in the Middle East and the Mediterranean region [1,2]. It can be found in the wild in nations including Turkey, Iraq, Iran, and Syria as well as in Africa, Europe, and Asia [3]. Communist mysticism Linn is a perennially green, fragrant shrub that is grown in gardens and in the wild in Iraq. The plant has tiny green leaves and is between 1.8 and 2.4 meters long. The myrtle fruit has a distinct flavor, can be either black or white, and is covered in a waxy covering as shown in figure (1). Myrtle has apparently been consumed or used as a spice since the dawn of time to treat a number of conditions, including urethritis, conjunctivitis, lung, and skin issues, as well as bleeding, headaches, palpitations, and gastric ulcers [4,5-8]. Essential oils made from various plant parts have long been used in research and industry,

including aromatherapy, phytotherapy, cosmetics, medicine, and the food business [9]. Many different biological processes are carried out by plant parts and essential oils [10].

Figure 1. Fruit and branches of Myrtus communis [11]

Myrtle's nutritional profile

The same chemicals are present in varying degrees in extracts made from different plant sections. The myrtle's leaves include myricetin, catechin, and quercetin [12]. Myrtle fruit is primarily composed of phenolic acids and anthocyanins, two beneficial substances [1,13]. The dark blue fruit of the myrtle has substantial antioxidant activity while the white fruit of the myrtle mostly contains unsaturated fatty acids including myrtenyl acetate, linoleic acid, and oleic acid [5]. The myrtle berries had 11.21 kcal/g, 4.17%, 17.41%, 2.37%, 8.64%, and 76.11 mg/100 g of calories, protein, fiber, fat, sugar, tannin, and essential oil, respectively [14]. The predominant fatty acids in myrtle berries are 72.1% oleic acid and 15.7% palmitic acid, making up 74.1% of the unsaturated fats and 25.7% of the saturated fats. Various polyphenolic chemicals can be found in myrtle. Linalool (8.3%), 1,8-cineol (24.6%), limonene (14.8%), and -pinene (31.8%) are all present in the leaf essential oil [15]. Its berries include ellagic acid (54.64%), gallic acid (12.70%), quercetin (3.72%), and quercetin 3-O-rhamnoside (3.71%) among other polyphenols [16]. The myrtle plant's phytochemical richness has led to speculation that it has advantageous health effects [13]. Figure (2) depicts the possible health benefits of the myrtle and its components based on data from in vitro and in animal studies.

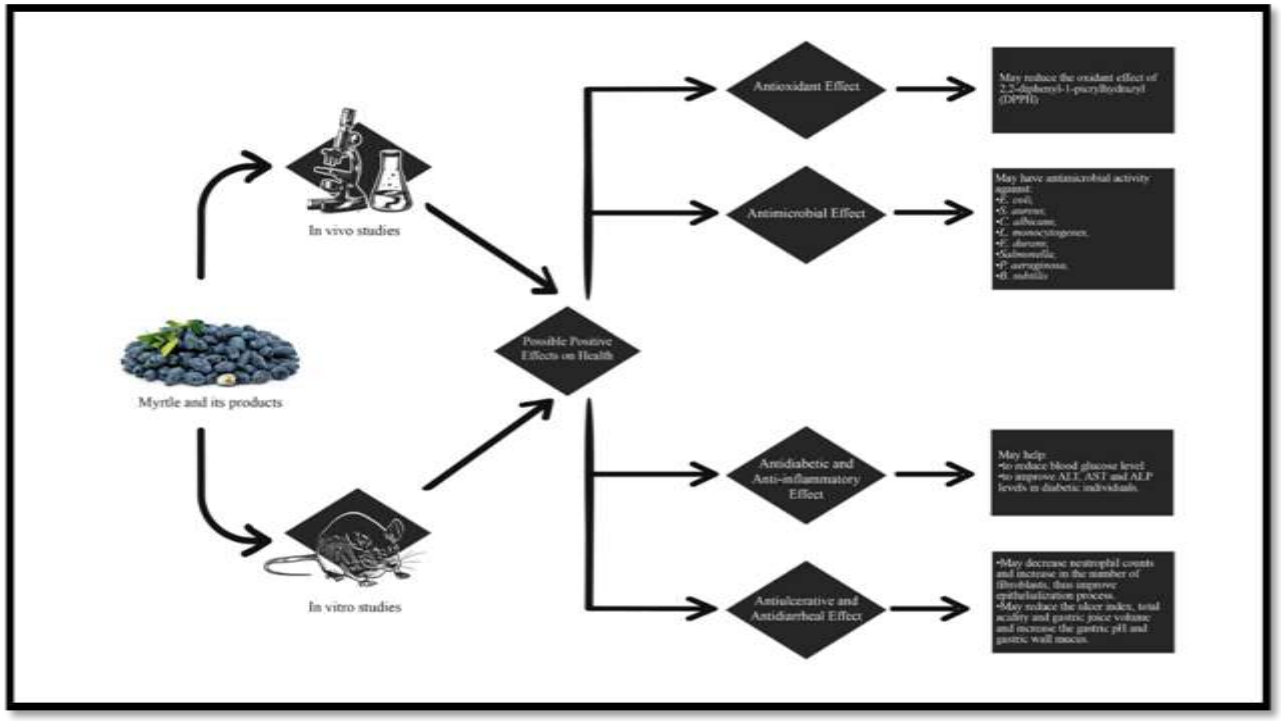

Figure 2. According to data from in vitro and in vivo research, myrtle and its compounds may have good effects on health [17]

Antioxidant activity of myrtle

In order to produce energy, the cell consumes oxygen, and the creation of adenosine triphosphate (ATP) results in the generation of free radicals. Usually, reactive oxygen and nitrogen species are present in these byproducts [18]. These molecules generate oxidative stress when they are present in excessive quantities, which can lead to a variety of chronic diseases as inflammation, diabetes, and atherosclerosis [19]. Anthocyanins are the C15 phenolic glycosides that give plants their color. It has been established that anthocyanins are good for conditions caused by oxidative stress [20]. Studies have demonstrated that myrtle plant essential oils have considerable antioxidant activity [21]. The plant's essential oil lessens the oxidizing effects of 1, 1-diphenyl-2-picrylhydrazyl (DPPH) and the mutagen effects of t-BOOH. Myrtle leaves were used to make the myricetin-3-o-rhamnocide and myricetin-3-o-galactoside, have been shown to have xanthine oxidase activities that prevent lipid peroxidation and DPPH's oxidative effects while blocking the carcinogenic effects of aflatoxin B1, nifuroxazide, and H_2O_2. Aflatoxin B1 and nifuroxazide's genotoxic properties were suppressed by extracts of the myrtle plant in methanol and ethyl acetate [22,23]. A study that compared the antioxidant capacities of white and dark blue myrtle liquors discovered that white liquor contains a higher level of gallic acid and its derivatives [24].

Antidiabetic and anti-inflammatory activity of myrtle

An irregularity in the release of insulin characterizes the metabolic disorder diabetes or hyperglycemia brought on by inadequate insulin. Diabetes-related chronic hyperglycemia can damage or destroy various organs, including the eyes, kidneys, nerves, heart, and blood vessels [25]. Using diabetic rats as test subjects, the myrtle's aqueous extract was found to have anti-diabetic and antioxidant properties. The serum glucose, aspartate aminotransferase (AST), alanine transaminase

(ALT), and alkaline phosphatase (ALP) levels of diabetic mice that ingested 1000 mg/kg of myrtle aqueous extract for 14 days were significantly lower than those of the control group. It was found that aqueous myrtle extract significantly reduced malondialdehyde levels, raised glutathione levels, and superoxide dismutase activity in diabetic rats as compared to the control group [26]. According to a study done on mice, myrtle may have an anti-inflammatory effect on disorders associated with inflammation and reduce edema [27].

Antiulcerative and antidiarrheal activity of myrtle

The mouth is the starting point of the gastrointestinal system (GIS), a long organ that extends for about 10 meters and passes through the chest, belly, and pelvic areas before coming to an end in the anus. The main role of GIS is to convert food nutrients into forms that body cells can use for particular tasks [28]. Anywhere on the GIS mucosa, ulcers can appear as lesions of mucosal tissue that show a slow disintegration of tissue or as a bare wound on the skin [29]. In an animal investigation, it was discovered that the powder made from myrtle berries significantly influences the healing of oral lesions [30]. Discomfort in the intestines brought on by a bacterial or viral infection, drug reaction, food allergy, or systemic sickness are typical signs of diarrhea [31]. An 80% methanol extract of myrtle leaves showed antidiarrheal properties in mice [32]. One of the main chronic gastrointestinal disorders, gastroesophageal reflux disease (GERD), can produce symptoms like chest pain, indigestion, dysphagia, chronic cough, and epigastric pain [33]. Spasm or reduced lower oesophageal relaxation are symptoms of the illness, impairs food absorption in the stomach and causes the contents of the stomach to move toward the oesophagus [34]. In a double-blind, randomized, controlled study, it was found that myrtle syrup reduced disease-induced symptoms in those with gastroesophageal reflux [35].

Analgesic effect of myrtle

Arabic traditional medicine has employed Myrtus communis L. aerial parts as an analgesic [36]. We conducted hot plate and writhing tests to evaluate this activity. In the hot plate test, the aerial portions' aqueous and ethanolic extracts both exhibited notable antinociceptive action, which was reduced by naloxone. The hot plate test is primarily intended to assess central antinociceptive activity, therefore this effect may be mediated by the central opioid receptors or brought on by an increase in endogenous opiopeptide synthesis. The extracts also demonstrated antinociceptive action in a writhing test against acetic acid, which was unaffected by naloxone. These results lead to the hypothesis that the extract's peripheral impact is not mediated by opioid receptors. Hence, it was suggested that there may be additional mechanisms of action, such as reduction of prostaglandin release or suppression of cyclooxygenase [37].

Effect on hair growth of myrtle

In French and Persian traditional medicine, Myrtus communis L. essential oil has been employed as a hair tonic. The effectiveness of using a combination of 100% essential plant oils along with mild electromagnetic pulses to treat androgenetic alopecia was evaluated. Along with other plant essential oils, Myrtus communis L. was present in the solution. By promoting nutritional intake of the hair papilla cells due to the stimulation of the microcirculation and by controlling the activity of sebaceous glands, using the oils alone avoided hair loss and occasionally produced light hair growth. However, the cells were triggered when electromagnetic pulses were used in conjunction with a complementary approach. As a result, the described treatment not only stopped hair loss but also encouraged hair growth. Results revealed that there was an increase in the proliferation index, which was

seen in the immunohistochemical analysis, in addition to an increase in hair density and total hair ratio. Additionally, the expression became more pronounced of the cell proliferation marker Ki67 [38].

Conclusion

As a result of the phytochemical composition, myrtle extracts have antioxidant, antiulcerative, antibacterial, antidiabetic, antiinflammatory, analgesic, and effect on hair development properties. Myrtle eating may therefore be beneficial to health. Due to the fact that the researchers have only found impacts at the cellular level or in animals, their effects on the human body are not fully known. Hence, significant human experimentation is needed.

References

1. Asgarpanah J, Ariamanesh A. Phytochemistry and pharmacological properties of Myrtus communis L. Indian J Trad Knowl. 2015; 1(1): 82-7.
2. Aleksic V, Knezevic P. Antimicrobial and antioxidative activity of extracts and essential oils of Myrtus communis L. Microbiol Res. 2014; 169(4): 240-54. doi: 10.1016/j.micres.2013.10.003.
3. Barboni T, Cannac M, Massi L, et al. Variability of polyphenol compounds in Myrtus communis L. (Myrtaceae) berries from Corsica. Molecules. 2010; 15(11): 7849-60. doi: 10.3390/molecules15117849.
4. Söke P, Elmaci Y. [Processing of candies from black and white myrtle (Myrtus communis L.)]. Akademik Gıda. 2015; 13(1): 35-41.
5. Messaoud C, Béjaoui A, Boussaid M. Fruit color, chemical and genetic diversity and structure of Myrtus communis L. var. italica Mill. morph populations. Biochem Syst Eco. 2011; 39(4-6): 570-80. doi: https://doi.org/10.1016/j.bse.2011.08.008
6. Akin M, Aktumsek A, Nostro A. Antibacterial activity and composition of the essential oils of Eucalyptus camaldulensis Dehn. and Myrtus communis L. growing in Northern Cyprus. Afr J Biotechnol. 2010; 9(4): 531-5.
7. Mahmoudvand H, Ezzatkhah F, Sharififar F, et al. Antileishmanial and cytotoxic effects of essential oil and methanolic extract of Myrtus communis L. Korean J Parasitol. 2015; 53(1): 21-7. doi: 10.3347/kjp.2015.53.1.21.
8. Aksay S. Total phenolic content and antioxidant properties of various extracts of myrtle (Myrtus communis L.) berries. Cukurova Tarim Gida Bil Der. 2016; 31(2): 43-50.
9. Dönmez IE, Salman H. Volatile compounds of myrtle (Myrtus communis L.) leaves and berries. Turk J Forestry. 2017; 18(4): 328-32.
10. Ben Hsouna A, Hamdi N, Miladi R, et al. Myrtus communis essential oil: chemical composition and antimicrobial activities against food spoilage pathogens. Chem Biodivers. 2014; 11(4): 571-80. doi: 10.1002/cbdv.201300153.
11. Gorjian H, Khaligh NG. Myrtle: a versatile medicinal plant. Nutrire. 2023; 48, 10. doi: https://doi.org/10.1186/s41110-023-00194-y.
12. Alipour G, Dashti S, Hosseinzadeh H. Review of pharmacological effects of Myrtus communis L. and its active constituents. Phytother Res. 2014; 28(8): 1125-36. doi: 10.1002/ptr.5122.
13. Sumbul S, Ahmad MA, Asif M, et al. Myrtus communis L. A review. IJNPR. 2011; 2(4): 395-402.
14. Aydin C, Ozcan MM. Determination of nutritional and physical properties of myrtle (Myrtus communis L.) fruits growing wild in Turkey. J Food Eng. 2007; 79(2): 453-458.
15. Ghasemi E, Raofie F, Najafi NM. Application of response surface methodology and central composite design for the optimisation of supercritical fluid extraction of essential oils from Myrtus communis L. leaves. 2011; Food Chem 126(3): 1449-53.
16. Correddu F, Maldini M, Addis R, et al. Myrtus communis Liquor Byproduct as a Source of Bioactive Compounds. Foods. 2019; 8(7): 237. doi: 10.3390/foods8070237.
17. Bagcilar S, Gezer C. Myrtle (Myrtus communis L.) and potential health effects. EMU J Pharmaceut Sci. 2020; 3(3), 205-214.
18. Lobo V, Patil A, Phatak A, et al. Free radicals, antioxidants and functional foods: Impact on human health. Pharmacogn Rev. 2010; 4(8): 118-26. doi: 10.4103/0973-7847.70902.
19. Percário S, da Silva Barbosa A, Varela ELP, et al. Oxidative stress in Parkinson's disease: Potential benefits of antioxidant supplementation. Oxid Med Cell Longev. 2020; 2020: 2360872. doi: 10.1155/2020/2360872.
20. Skrovankova S, Sumczynski D, Mlcek J, et al. Bioactive compounds and antioxidant activity in different types of berries. Int J Mol Sci. 2015; 16(10): 24673-706. doi: 10.3390/ijms161024673.
21. Dahmoune F, Nayak B, Moussi K, et al. Optimization of microwave-assisted extraction of polyphenols from Myrtus communis L. leaves. Food Chem. 2015; 166: 585-95. doi: 10.1016/j.foodchem.2014.06.066.
22. Mimica-Dukić N, Bugarin D, Grbović S, et al. Essential oil of Myrtus communis L. as a potential antioxidant and antimutagenic agents. Molecules. 2010; 15(4): 2759-70. doi: 10.3390/molecules15042759.
23. Hayder N, Bouhlel I, Skandrani I, et al. In vitro antioxidant and antigenotoxic potentials of myricetin-3-o-galactoside and myricetin-3-o-rhamnoside from Myrtus communis: modulation of

expression of genes involved in cell defense system using cDNA microarray. Toxicol In Vitro. 2008; 22(3): 567-81. doi: 10.1016/j.tiv.2007.11.015.
24. Serreli G, Jerković I, Gil KA, et al. Phenolic compounds, volatiles and antioxidant capacity of white myrtle berry liqueurs. Plant Foods Hum Nutr. 2017; 72(2): 205-10. doi: 10.1007/s11130-017-0611-8.
25. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care 2014; 37(Supplement_1): S81–S90. doi: https://doi.org/10.2337/dc14-S081.
26. Demir GM, Gulaboglu M, Aggul AG, et al. Antioxidant and antidiabetic activity of aqueous extract of Myrtus communis L. Berries on streptozotocin-induced diabetic rats. Int J Pharm Biol Sci. 2016; 11(5): 183-90. doi: 10.9790/3008-1105011116.
27. Touaibia M. Composition and anti-inflammatory effect of the common myrtle (Myrtus communis L.) essential oil growing wild in Algeria. Phytothérapie. 2018; 16(51): S143-8. doi: https://doi.org/10.3166/phyto-2019-0142
28. McErlean L. The digestive system. In: Peate I, Nair M, (eds). Fundamentals of anatomy and physiology: for nursing and healthcare students. 2nd ed. Chichester, England: Wiley Blackwell; 2016. p. 412-4.
29. Kahn MA, Hall JM. The American Dental Association practical guide to soft tissue oral disease. 1st ed. Chichester, England: Wiley Blackwell; 2014. p. 5-6.
30. Hashemipour MA, Lotfi S, Torabi M, et al. Evaluation of the effects of three plant species (Myrtus Communis L., Camellia Sinensis L., Zataria Multiflora Boiss.) on the healing process of intraoral ulcers in rats. J Dent (Shiraz). 2017; 18(2): 127-35.
31. World Health Organization. Diarrhoea. 2020. URL: https://www.who.int/health-topics/diarrhoea#tab=tab_1. Retrieved 26 November 2020.
32. Sisay M, Engidawork E, Shibeshi W. Evaluation of the antidiarrheal activity of the leaf extracts of Myrtus communis Linn (Myrtaceae) in mice model. BMC Complement Altern Med. 2017; 17(1): 103. doi: 10.1186/s12906-017-1625-3.
33. Fock KM, Poh CH. Gastroesophageal reflux disease. J Gastroenterol. 2010; 45(8): 808-15. doi: 10.1007/s00535-010-0274-9.
34. Boeckxstaens GE, Annese V, des Varannes, et al. Pneumatic dilation versus laparoscopic Heller's myotomy for idiopathic achalasia. N Engl J Med. 2011; 364(19): 1807-16. doi: 10.1056/NEJMoa1010502.
35. Salehi M, Azizkhani M, Mobli M, et al. The effect of Myrtus communis L. syrup in reducing the recurrence of gastroesophageal reflux disease: A double-blind randomized controlled trial. Iran Red Crescent Med J. 2017; 19(7): e55657. doi:10.5812/ircmj.55657
36. Twaij H, El-Jalil H. Evaluation of narcotic (opioid like), analgesic activities of medicinal plants. Eur J Sci Res. 2009; 33: 179-82.
37. Hosseinzadeh H, Khoshdel M, Ghorbani M. Antinociceptive, anti-inflammatory effects and acute toxicity of aqueous and ethanolic extracts of Myrtus communis L. Aerial parts in mice. J Acupunct Meridian Stud. 2011; 4(4): 242-7. doi: 10.1016/j.jams.2011.09.015.
38. Bureau JP, Ginouves P, Guilbaud J, et al. Essential oils and low-intensity electromagnetic pulses in the treatment of androgen-dependent alopecia. Adv Ther. 2003; 20(4): 220-9. doi: 10.1007/BF02850093.

Correspondence to Hend A. Abass
E-mail: Hind.abbas@nahrainuniv.edu.iq
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Association between Single-Nucleotide Polymorphism (rs2072493) and Serum Level of TLR-5 and Interleukin-6 and Interleukin-12 Response to *Toxoplasma gondii* in Women with Miscarriage and Pregnant Women

Adel N. Al-Baldawy[1] *PhD*, **Huda D. Al-Marsomy[2]** *PhD*, **Inas I. Khaleel[3]** *CABOG*

[1]Higher Health Institute, Balad, Salah Al-Dien, Iraq, [2]Dept. of Microbiology, College of Medicine, Al-Nahrain University, Baghdad, Iraq, [3]Dept. of Gynecology, Balad General Hospital, Salah Al-Dien, Iraq

Abstract

Background *Toxoplasma gondii* (*T. gondii*) is well-known to cause congenital diseases, or ocular diseases, and miscarriage in pregnancy. Earlier infections are threatening in pregnant women. The activity of Toll-like receptors (TLR) in defense against *T. gondii* infections was observed such as TLR-5 molecule, and some interleukins (like IL-6, and IL-12) that may lead to abortion.

Objective To study association between TLR-5 gene polymorphisms (rs2072493) with the susceptibility to toxoplasmosis in a sample of women with miscarriage and to study the association of TLR-5 polymorphisms (rs2072493) with the serum level of TLR-5, IL-6 and IL-12.

Methods This is a case-control study was conducted on 200 women, of which, 50 pregnant women seropositive (IgG, IgM) for *T. gondii* (Group1), 50 pregnant women seronegative (IgG, IgM) for *T. gondii* (Group2) as a control group, 50 women with miscarriage seropositive (IgG, IgM) for *T. gondii* (Group3), and 50 women with miscarriage seronegative (IgG, IgM) for *T. gondii* (Group4) as a control group. They were recruited from Balad General Hospital, Salah al-Din and private laboratory in the period from January 2021 to December 2021. The serum level of TLR-5, IL-6 and IL-12 were measured. The selected single-nucleotide polymorphism (SNPs) (rs2072493) in TLR-5 were detected by using real-time polymerase chain reaction with specific primers.

Results The frequency of the heterozygous genotype (TC) for SNPs TLR-5 gene (rs2072493) was significantly higher in Group1 than Group2 (P=0.004). At allelic level, the frequency of mutant allele (allele C) was significantly higher in Group1 than in Group2 (p=0.009), and in Group3 than in Group4 (P=0.025). There was significant increase in serum level of TLR-5 in Group1 and in Group3. There was significant increase in serum level of IL-6 and IL-12 in Group1.

Conclusion There is significant association of toxoplasmosis with mutant allele (allele C) of the SNP rs2072493, which may be considered as a risk factor for toxoplasmosis and stimulate miscarriage in pregnant women. TLR-5 level is high in both groups.

Keywords TLR-5, polymorphism, Toxoplasma gondii, IL-6, IL-12

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List of abbreviations: ELISA = Enzyme linked immunosorbent assay, IL = Interleukin, IFN = Interferon, IgM = Immunoglobulin, IgG = Immunoglobulin γ, NF-κB = Nuclear factor kappa, SNPs = Single-nucleotide polymorphisms, Th2 = T helper, TLRs = Toll-like receptors

Introduction

Toxoplasmosis infection is rarely symptomatic in immune - competent individuals, but in immunocompromised

host may result in a severe disease or even fatal damage [1]. Toxoplasmosis is caused by *Toxoplasma gondii* (*T. gondii*), which is an obligate intracellular parasite, and considered the most public global parasite [2]. It is one of the most common infections during pregnancy that are passed from mother to child. As well as is the main factor in perinatal morbidity and mortality [3].

Profilin, a pathogen-associated molecular pattern (PAMP) produced by *T. gondii*, is recognized by receptors on dendritic cells and macrophages, activating the cells and causing the release of proinflammatory cytokines like interleukin (IL)-6 and IL-12 [2].

It has been demonstrated that activating Toll-like receptor (TLR)-11 by profilin causes powerful production of protein from mouse dendritic cells, but the human TLR-11 gene contains many stop codons, which lead to transcription of it does not produce a functional protein [4].

The TLR phylogenetic tree has an ancient cluster that includes both human TLR-5 and mouse TLR-11; as a result, human TLR-5 may have preserved mouse TLR-11's biological function and mediated *T. gondii* profilin identification [5,6]. The ectodomain of human TLR-5 contains shared binding sites for flagellin and profiling [6].

TLR-5 is encoded by a gene that has six exons and is located on the long arm of human chromosome 1 (hCh1q). There are now nine reported possible polymorphisms in the gene's promoter and coding regions [7], the functional TLR gene polymorphisms (rs2072493) are within exon region [8].

This research aimed to:

1. Study the association between TLR-5 gene polymorphisms and the susceptibility to toxoplasmosis in a sample of women with miscarriage.
2. Study the association of TLR-5 polymorphisms with the serum level of TLR-5 and cytokines (IL-6 and IL-12).

Methods

The study was approved by the Institutional Review Board (IRB) in the College of Medicine, Al-Nahrain University. The present Case-control study was conducted on 200 women, of which 50 pregnant women seropositive (IgG, IgM) for *T. gondii* (Group1), 50 pregnant women seronegative (IgG, IgM) for *T. gondii* (Group2) as a study group, 50 women with miscarriage seropositive (IgG, IgM) for *T. gondii* (Group3), and 50 women with miscarriage seronegative (IgG, IgM) for *T. gondii* (Group4) as a study group; which were collected in the period from January 2021 to December 2021.

All women participated in the study were recruited from Obstetrics and Gynecology Department of Balad General Hospital in Balad city, Salah al-Din province/Iraq, and all women participating in the study examined by a consultant specialist of gynecologist and obstetrician.

Five ml of whole venous blood was taken from each patient, and patients' information was taken from the data recorded. For the purpose of extracting DNA, two ml of ethylene diamine tetraacetic acid (EDTA) was collected, and both the EDTA tube and the extracted DNA were preserved at -20 °C until use. The remaining three ml of blood were collected in a plain gel tube for serum separation and kept at -20 °C until needed. All women participating in the study were investigated for the presence of anti-*T. gondii* antibodies by rapid chromatographic immune technique, and confirmed by enzyme linked immunosorbent assay (ELISA) technique. To confirm the diagnosis of toxoplasmosis, ELISA was used to detect IgM and IgG levels in all samples, whether they were positive or negative for anti-*T. gondii* Abs. The serum level of TLR-5 and cytokines (IL-6, IL-12) were measured by ELISA technique. Real-time polymerase chain reaction (RT-PCR) was used with particular primers to detect the selected Single-nucleotide polymorphisms (SNPs) (rs2072493) in TLR-5.

Statistical analysis

The program (Graph Pad Prism version 7) was utilized, and the one-way ANOVA (by Tukey's multiple comparisons test) was used to compare the observed parameters and SNP numbers between subdivided groups. Mean Standard Error was used to represent the results. Using the Chi-square test, nominal variables were given as frequency and percentage (%) and compared between study groups.

The MedCalc tool was used to calculate the odds ratios (OR), 95 % of confidence intervals (CI), and p values used to express the results of nominal regression. Significance of differences was determined at ($p < 0.05$). To determine the correlation between markers, correlation coefficients were computed. Mega Stat (Version v 10.12) for Excel 2010 was used to calculate the descriptive statistics and correlation coefficients (9).

Results

Age groups and study groups

The mean age of study groups were 30.36± 0.43 years, the minimum age was 18 years and the maximum age was 43 years (Table 1).

Table 1. Age groups distribution among study groups

Groups	Age groups (years) <20 N (%)	20-30 N (%)	31-40 N (%)	>40 N (%)	Total N (%)
Group1	2 (4)	25 (50)	21 (42)	2 (4)	50 (100)
Group2	3 (6)	26 (52)	21 (42)	0 (0.0)	50 (100)
Group3	0 (0.0)	24 (48)	23 (46)	3 (6)	50 (100)
Group4	0 (0.0)	21 (42)	27 (54)	2 (4)	50 (100)

Anti-*T. gondii* antibodies

According to cassette screening test, 32 (64%) women with miscarriage were positive for both anti-*T. gondii* IgM and IgG antibodies, 5 (10%) of them were positive for anti-*T. gondii* IgM antibodies only, and 13(26%) of them were positive for anti-*T. gondii* IgG antibodies only, while 21 (42%) pregnant women were positive for both anti-*T. gondii* IgM and IgG antibodies, 7 (14%) of them were positive for anti-*T. gondii* IgM antibodies only, and 22 of them (44%) were positive for anti-*T. gondii* IgG antibodies only.

Positive for anti-*T. gondii* IgM antibodies acute cases were excluded, because its percentage is low and no statistically significant and only chronic cases were taken, therefore, the fifty women with miscarriage with (IgM, IgG) seropositive for *T. gondii* and fifty pregnant women with (IgM, IgG) seropositive for *T. gondii* by cassette screening test were positive for anti-*T. gondii* (IgM, IgG) antibodies (100%) by ELISA, while the controls groups were negative result (0%) have for that antibodies as shown in table (2).

TLR-5 gene polymorphism rs2072493

Genetic polymorphism of TLR-5 gene (rs2072493) was observed with three genotypes (TT, TC and CC) (Figure 1). Compared to Group2, which had a homozygous genotype (TT) frequency of 48(96%), Group1 had a homozygous genotype (TT) with a frequency of 30(60%), revealed a high significant difference (P=0.0004) (OR=0.06, CI=0.01 to 0.28), and the frequency of the heterozygous genotype (TC) was higher in group1 20 (40%) than Group2 2 (4%) with high significant difference (P = 0.0004) (OR = 16, CI = 3.48 to 73.4), but homozygous genotype CC was not recorded any frequency 0(0%) in both groups (Table 3).

Table 2. Anti-*T. gondii* antibodies

Groups	Cassette test IgM, IgG N (%)	Cassette test IgM N (%)	Cassette test IgG N (%)	ELISA IgM, IgG N (%)
Group1	21 (42.0)	7 (14.0)	22 (44.0)	50 (100)
Group2	0 (0.0)	0 (0.0)	0 (00.)	0 (0.0)
Group3	32 (64.0)	5 (10.0)	13 (26.0)	50 (100)
Group4	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Total		100		100

P value = 0.6441

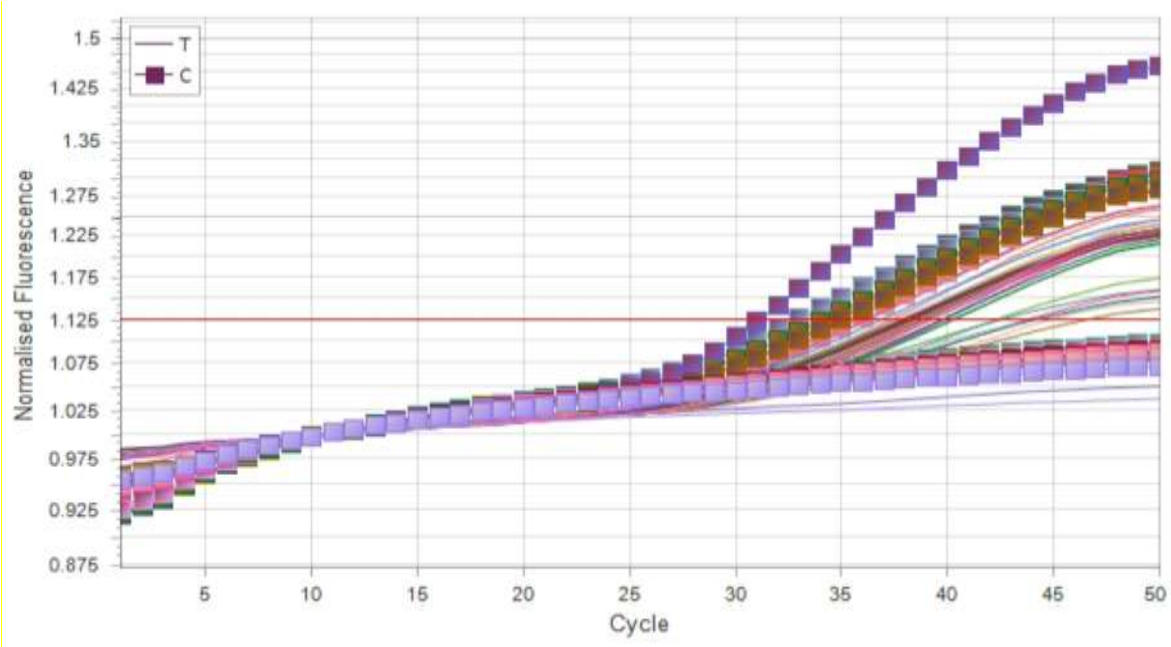

Figure 1. RT-PCR Result for Genetic Polymorphism of TLR-5 gene (rs2072493)

Table 3. Genotypes and alleles of TLR-5 gene polymorphism rs2072493 in group1 and group2

	rs2072493	Group1 (n =50)	Group2 (n =50)	OR (95 % CI)	P value
Genotypes	TT	30 (60%)	48 (96%)	0.06 (0.01 to 0.28)	0.0004
	TC	20 (40%)	2 (4%)	16.00 (3.48 to 73.41)	
	CC	0 (0.0%)	0 (0.0%)	--	
Alleles	T	80 (80%)	98 (98%)	0.08 (0.01 to 0.35)	0.0009
	C	20 (20%)	2 (2%)	12.25 (2.77 to 53.99)	

At allelic level, the frequency of normal allele (allele T) was in group1 and group2 (80% versus 98%) with high significant difference (P = 0.0009) also (OR = 0.08, CI = 0.01 to 0.35), while in mutant allele (allele C) was in group1 and group2 (20% versus 2%) with high significant difference (p=0.0009) also (OR = 12.25, CI = 0. 2.77 to 53.99), as shown in table (3).

Also, the frequency of the homozygous genotype (TT) frequency 41 (82%) was not significant P = 0.07 (OR = 0.29, CI = 0.07 to 1.14) in group3 compared to Group4 47 (94%), and the heterozygous genotype (TC) was higher in group3 6 (12%) than group4 3(6%) with no significant difference P = 0.30 (OR = 2.13, CI = 0.50 to 9.06), while the homozygous genotype CC frequency was no significant difference P = 0.18 (OR = 7.44, CI = 0.37 to 147.93) in Group3 3 (6%) compared to Group4 0 (0%), the frequency of normal allele (allele T) at allelic level was in group3 and group4 (88% versus 97%) with significant difference P = 0.025 also (OR = 0.22, CI = 0.06 to 0.83), while in mutant allele (allele C) was in Group3 and Group4 (12% versus 3%) with significant difference P = 0.025 also (OR = 4.40, CI = 1.20 to 16.14), as shown in table (4).

Table 4. Genotypes and alleles of TLR-5 Gene Polymorphism rs2072493 in group3 and group4

	rs2072493	Group3 (n =50)	Group4 (n =50)	OR (95 % CI)	*P-value*
Genotypes	TT	41 (82%)	47 (94%)	0.29 (0.07 to 1.14)	0.07
	TC	6 (12%)	3 (6%)	2.13 (0.50 to 9.06)	0.30
	CC	3 (6%)	0 (0.0%)	7.44 (0.37 to 147.93)	0.18
Alleles	T	88(88%)	97 (97%)	0.22 (0.06 to 0.83)	0.025
	C	12 (12%)	3 (3%)	4.40 (1.20 to 16.14)	

The serum soluble level of TLR-5, IL-6 and IL-12

Table 5 shows the results of TLR-5, IL-6 and Il-12, they were significantly higher in group1 (mean = 48.43, 151.1 and 27.71) than group2 (mean = 14.72, 151.1 and 27.71) respectively, also, they were significantly higher in group3 (mean = 28.30, 138 and 30.91) than group4 (mean =10.92, 91.89 and 24.63) respectively.

Table 5. The serum soluble level of TLR-5, IL-6 and Il-12

Groups	TLR-5 (ng/l) Mean±SE	IL-6 (ng/l) Mean±SE	IL-12 (ng/l) Mean±SE
Group1	48.43±3.7	151.1±17.42	27.71±2.22
Group2	14.72±2.2	83.45±7.5	15.77±2.28
Group3	28.30±1.93	138±13.36	30.91±2.62
Group4	10.92±1.86	91.89±9.74	24.63±3.75
P value	<0.0001	0.0002	0.0013

P value: one-way ANOVA

Correlations among TLR-5, IL-6 and IL-12 and rs2072493 in group1

As shown in table (6), the results of the present study revealed a non-significant negative correlation (P >0.05) among TLR-5, IL-6 and rs2072493, and a non-significant positive correlation (P>0.05) between IL-12 and rs2072493; also, a non-significant positive correlation (P>0.05) among TLR-5, IL-6 and IL-12, while showed a significant positive correlation (P<0.05) between IL-6 and IL-12.

Table 6. Correlations among TLR-5, IL-6 and IL-12 and rs2072493 in group1

		rs2072493	TLR-5	IL-12	IL-6
rs2072493	r	1.000	-0.255	0.076	-0.025
	P value	.	0.074	0.598	0.861
TLR-5	r		1.000	0.070	0.142
	P value		.	0.627	0.325
IL-12	r			1.000	0.831**
	P value			.	0.000

** = Spearman's rho Correlation is significant at the 0.05 level (2-tailed)

Correlations among TLR-5, IL-6 and IL-12 and rs2072493 in group2

As shown in table (7) the results of the present study revealed a non-significant positive correlation and difference (P>0.05) among TLR-5, IL-6, IL-12 and rs2072493, and a non-significant negative correlation (P>0.05) between TLR-5 and IL-12, also, a significant positive correlation (P<0.05) between TLR-5 and IL-6, moreover, a non-significant positive correlation and no significant difference (P>0.05) between IL-6 and IL-12.

Correlations among TLR-5, IL-6 and IL-12 and rs2072493 in Group3

As shown in table (8), the results of the present study revealed a non-significant negative correlation (P>0.05) among TLR-5, IL-6, IL-12 and rs2072493, and a significant positive (P<0.05) among TLR-5, IL-6 and IL-12; also, a significant positive correlation (P<0.05) between IL-6 and IL-12.

Table 7. Correlations among TLR-5, IL-6 and IL-12 and rs2072493 in group2

		rs2072493	TLR-5	IL-12	IL-6
rs2072493	r	1.000	0.202	0.014	0.057
	P value	.	0.160	0.922	0.696
TLR-5	r		1.000	-0.032	0.745**
	P value		.	0.828	0.001
IL-12	r			1.000	0.120
	P value			.	0.405

** = Spearman's rho Correlation is significant at the 0.05 level (2-tailed)

Table 8. Correlations among TLR-5, IL-6 and IL-12 and rs2072493 in group3

		rs2072493	TLR-5	IL-12	IL-6
rs2072493	r	1.000	-0.240	-0.179	-0.247
	P value		0.094	0.214	0.084
TLR-5	r		1.000	0.373**	0.554**
	P value		.	0.008	0.000
IL-12	r			1.000	0.741**
	P value			.	0.000

** = Spearman's rho Correlation is significant at the 0.05 level (2-tailed)

Correlations among TLR-5, IL-6 and IL-12 and rs2072493 in group4

As shown in table (9), the results of the present study revealed a non-significant positive correlation (P>0.05) among TLR-5, IL-6 and rs2072493, and a non-significant negative correlation (P>0.05) between IL-12 and rs2072493; and a significant positive correlation (P<0.05) between TLR-5 and IL-6, also, a non-significant negative correlation (P>0.05) between TLR-5 and IL-12; in addition, a non-significant positive correlation (P>0.05) between IL-6 and IL-12.

Table 9. Correlations among TLR-5, IL-6 and IL-12 and rs2072493 in group4

		rs2072493	TLR-5	IL-12	IL-6
rs2072493	r	1.000	0.096	-0.044	0.108
	P value	.	0.506	0.763	0.455
TLR-5	r		1.000	-0.085	0.722**
	P value		.	0.559	0.001
IL-12	r			1.000	0.013
	P value			.	0.927

** = Spearman's rho Correlation is significant at the 0.05 level (2-tailed)

Discussion

Maternal acute toxoplasmosis or congenital toxoplasmosis during pregnancy are two of the main factors that raise the risk of abortion. Toxoplasmosis has a significant frequency worldwide, according to serological evidence [10-12]. Innate immunity has a major role in toxoplasmosis and recurrent miscarriages, including TLR-5 and some cytokines, since both mouse and human TLR-5 appear to be the earliest evolutionary relatives of mouse TLR-11, human TLR-5 may have preserved mouse TLR-11's biological function and mediated the identification of *T. gondii* profilin. The ectodomain of human TLR-5 contains shared binding sites for flagellin and profilin [5].

TLR-5 might play an important role in the pathogenesis of unexplained recurrent spontaneous abortion since TLR-5 signaling could result in inflammatory cytokine production (such as IL-6 and IL-12) [13], especially when there is no reason for the miscarriages, so *T. gondii* infection could be considered a potential risk factor for abortion. Host genetics play a key role in determining disease susceptibility, clinical symptoms, treatment response, and disease outcome in addition to the pathogen's virulence characteristics.

Age groups and study groups

The current study showed the distribution of toxoplasmosis among age groups was converged. These findings agreed with Ahmed et al. in 2019, who reported that the distribution among these ages was converged, may be because the women participating in this study were in the reproductive stage [14].

Anti-*T. gondii* antibodies

The current study revealed that the anti-*T. gondii* IgM positivity was too low among study groups, 7(14%) in group1, and 5 (10%) in group3, these results are disagreed with Turkey et al. in 2019, who proved that 33 (66%) of aborted women were positive for IgM antibodies [15], this disagreement might because that the current study has been conducted on women with chronic toxoplasmosis only.

TLR-5 gene polymorphism

Throughout the human genome, a huge number of single nucleotide polymorphisms (SNPs) have been discovered. SNPs are becoming more important and useful in the search for the causes of human diseases and features, as well as in drug development and the research of human treatment response and may affect the innate immune response, by changing the amplitude and quality of intracellular signaling cascades, which has consequences for infection susceptibility, and disease results. This is backed up by a growing body of evidence [16].

Furthermore, there was a significant association between the heterozygous genotype (TC) of TLR-5 gene polymorphism rs2072493 and susceptibility to toxoplasmosis in group1. This suggests that carrier of (TC) genotype of this polymorphism are higher risk of having the disease, compared with (TT) genotype carrier, causes according to odd ratio by 16 under 95% CI (3.48 to 73.4). While it showed no significant difference between the heterozygous genotype (TC) of TLR-5 gene polymorphism rs2072493 and susceptibility to *T. gondii* in Group 2.

There are very a few studies that have researched into this topic (TLR-5 gene polymorphism with toxoplasmosis) globally. The detected allele frequency for (rs2072493) polymorphism in the current study were 40% in group1, these results disagreed with some researches, who reported that allele frequencies of 15% in Caucasians [17], Chinese were 26% [18], and north Indians were 12 % [19], may be because these researches used a population large sample, as well the genetic heterogeneity among different ethnicities, variety can be attributed to the differences in the minor allele incidences, and SNP rs2072493 was associated with various infectious such as colorectal cancer, graves' disease, and chronic hepatitis B virus (HBV) infection [20- 22].

The serum soluble level of TLR-5

In the present study, the serum soluble level of TLR-5 was significantly increased in group1 ($P< 0.05$), this results were agreed with Dabagh-Gorjani et al. in 2014, who indicated that the expressions of TLR-5 was significantly increased in both maternal part (the maternal and fetal parts of the placenta), although it used the expression for TLR-5, the results were agree, TLR-5 ligation activates the production of proinflammatory cytokines via the nuclear factor kappa (NF-κB) pathway, regardless of the trigger factors or ligands, and chronic inflammation is thought to be a primary contributor to the progression of abortion [23].

According to Salazar Gonzalez et al. in 2014, this result is in accordance with the theory that human TLR-5 is involved in innate recognition and initiation of cytokine production by *T. gondii* – derived profiling [6].

The serum level of IL-6

In the current study, IL-6 serum level showed a significant increase ($P<0.05$) more in the serum of group1, this finding was agreed with Zhang et al. in 2017, and Tyagi and Alharthi in 2020, who indicated that plasma concentrations of IL-6, IL-10, and IL-18 are higher in women with successful pregnancies than in women with recurrent pregnancy loss, and IL-6 was decreased in pregnant women with a history of

recurrent spontaneous miscarriage patients [24,25].

IL-6 plays a role in trophoblast proliferation, differentiation, and invasion, as well as follicle development and embryonic implantation, the IL-6 protein is also involved in the first spiral artery remodeling process, which necessitates the production of vascular smooth muscle cells and morphological changes. IL-6 levels that are lower inhibit trophoblast invasion and spiral artery remodeling [26]. Therefore, in this regard, the current result is consistent with numerous earlier investigations [27,28].

The general rule is that T helper2 (Th2) cytokines, such as IL-6, are thought to encourage a normal pregnancy, IL-6 functions as a messenger for notifying the body to the occurrence of an unexpected event. In an infected lesion, IL-6 is produced and transmits a warning signal throughout the body. Increased or decreased levels of this cytokine in serum or gestational tissues appear to have negative consequences for pregnancy. Excessive IL-6 may limit the development of cluster of differentiation (CD4+) T regulator cells, which are essential for pregnancy tolerance, according to one theory [29].

The serum level of IL-12

In the current study, the serum level of IL-12 showed a significant increase ($P<0.05$) more in the serum of group1, this result was disagreed with Tyagi and Alharthi in 2020, who reported that Th1 activity (including IL-12) was higher in pregnant women with a history of recurrent spontaneous abortion irrespective of whether continuing their pregnancy or aborting in contrast to healthy pregnant, maybe this disagree because that might be a persistent imbalance of Th1/Th2 in pregnant women [25].

The generation of interferon-gamma (IFN-γ) by interleukin-12 (IL-12) causes Th1 cells to differentiate. Researchers have discovered that Th1-type immunity may be harmful during pregnancy and that an increased Th1-type cytokine response may result in pregnancy loss because Th1-dependent processes are likely involved in allograft rejection [24], also, according to a study by Rezende-Oliveira et al. in 2012, who suggested that immunomodulation, which was observed during pregnancy, was involved in *T. gondii* evading the immune response [30].

IL-12 level in the blood were shown to be higher in failing pregnancies than in normal pregnancies. Important regulatory role was played by cytokines during pregnancy; an excessive decrease or increase of these cytokines may result in spontaneous abortion, implantation failure, or preeclampsia in patients with a history of miscarriage [31].

Therefore, in this regard, the current result is consistent with numerous earlier investigations [25,30,31]. This concordance, however, does not fit the IL-12 results in group2, may be because different in study group.

According to our knowledge, there is not any previous studies included study the correlations among TLR-5, IL-6 and IL-12 and SNPs rs2072493 in pregnant women. To the best of our knowledge, this study is the first to suggest.

In conclusions, firstly, there is a significant correlation between the heterozygous genotype (TC) of TLR-5 gene polymorphism rs2072493 and susceptibility to toxoplasmosis in pregnant women seropositive group, but there is no significant difference in women with miscarriage seropositive. Secondly, there is a significant increase serum soluble level of TLR-5 in both group women with miscarriage seropositive and of pregnant women seropositive. Thirdly, there are a significant increase serum level of IL-6 and IL-12 in pregnant women seropositive, but there are no significant in women with miscarriage seropositive. Lastly, both women with miscarriage seropositive and pregnant women seropositive with rs2072493 in the current study shows a negative correlation between TLR-5 and IL-6, but IL-12 was negatively correlation in women with miscarriage seropositive, and positive correlation in pregnant women seropositive.

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Author contribution
Dr. Al-Baldawy: did the laboratory work, wrote the article, and statistical analysis. Dr. Al-Marsomy: designed, supervised and co-wrote this article. Dr. Khaleel: diagnosed and follow up patients, and cooperated in samples collection.

Conflict of interest
The authors declare that they have no conflict of interest.

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References
1. Elmore SA, Jones JL, Conrad PA, et al. *Toxoplasma gondii*: epidemiology, feline clinical aspects, and prevention. Trends Parasitol. 2010; 26(4): 190-6. doi: 10.1016/j.pt.2010.01.009.
2. Tenter AM, Heckeroth AR, Weiss LM. *Toxoplasma gondii*: from animals to humans. Int J Parasitol. 2000; 30(12-13): 1217-58. doi: 10.1016/s0020-7519(00)00124-7.
3. Wujcicka W, Wilczyński J, Nowakowska D. SNPs in toll-like receptor (TLR) genes as new genetic alterations associated with congenital toxoplasmosis? Eur J Clin Microbiol Infect Dis. 2013; 32(4): 503-11. doi: 10.1007/s10096-012-1763-y.
4. Yarovinsky F, Andersen JF, King LR, et al. Structural determinants of the anti-HIV activity of a CCR5 antagonist derived from Toxoplasma gondii. J Biol Chem. 2004; 279(51): 53635-42. doi: 10.1074/jbc.M410550200.
5. Takeda K, Kaisho T, Akira S. Toll-like receptors. Annu Rev Immunol. 2003; 21: 335-76. doi: 10.1146/annurev.immunol.21.120601.141126.
6. Salazar Gonzalez RM, Shehata H, O'Connell MJ, et al. Toxoplasma gondii- derived profilin triggers human toll-like receptor 5-dependent cytokine production. J Innate Immun. 2014; 6(5): 685-94. doi: 10.1159/000362367.
7. Sameer AS, Nissar S. Toll-Like Receptors (TLRs): Structure, functions, signaling, and role of their polymorphisms in colorectal cancer susceptibility. Biomed Res Int. 2021; 2021: 1157023. doi: 10.1155/2021/1157023.
8. Seok H, Lee BC, Kim YO, et al. Association between Exonic SNPs of TLR5 and Benign Prostate Hyperplasia in Korean Population. Cooperative Res Prog Agricult Sci Technol Devel. 2013; 21(4): 331-7.
9. Motulsky H, Christopoulos A. Fitting models to biological data using linear and nonlinear regression: A practical guide to curve fitting. San Diego: GraphPad Software Inc., 2003.
10. Kheirandish F, Ezatpour B, Fallahi SH, et al. Toxoplasma serology status and risk of miscarriage, a case-control study among women with a history of spontaneous abortion. Int J Fertil Steril. 2019; 13(3): 184-9. doi: 10.22074/ijfs.2019.5740.
11. Robert-Gangneux F, Dardé ML. Epidemiology of and diagnostic strategies for toxoplasmosis. Clin Microbiol Rev. 2012; 25(2) :264-96. doi: 10.1128/CMR.05013-11.
12. Almushait MA, Dajem SM, Elsherbiny NM, et al. Seroprevalence and risk factors of Toxoplasma gondii infection among pregnant women in south western, Saudi Arabia. J Parasit Dis. 2014; 38(1): 4-10. doi: 10.1007/s12639-012-0195-z.
13. Morse CB, Sammel MD, Shaunik A, et al. Performance of human chorionic gonadotropin curves in women at risk for ectopic pregnancy: exceptions to the rules. Fertil Steril. 2012; 97(1): 101-6.e2. doi: 10.1016/j.fertnstert.2011.10.037.
14. Ahmed BA, Nasralla BA, Muhsen SS. Alteration of genetic sequence of Toxoplasma gondii by using the bands missing in ppGalNAc strains as a treatment. J Pharmaceut Sci Res. 2019; 11(2): 527-30.
15. Turkey SA, Hathal HD, Abbas AA. Association of toll –like receptor 4 gene polymorphisms with serum level of toll –like receptor 4 and interferon gamma in aborted women with acute toxoplasmosis from baghdad. A PhD Thesis. College of Medicine, Al-Nahrain University, 2019. p. 41.
16. Robert F, Pelletier J. Exploring the Impact of Single-Nucleotide Polymorphisms on Translation. Front Genet. 2018; 9: 507. doi: 10.3389/fgene.2018.00507.
17. Hawn TR, Verbon A, Lettinga KD, et al. A common dominant TLR5 stop codon polymorphism abolishes flagellin signaling and is associated with susceptibility to legionnaires' disease. J Exp Med. 2003; 198(10): 1563-72. doi: 10.1084/jem.20031220.
18. Zeng HM, Pan KF, Zhang Y, et al. Genetic variants of toll-like receptor 2 and 5, helicobacter pylori infection, and risk of gastric cancer and its precursors in a Chinese population. Cancer Epidemiol Biomarkers Prev. 2011; 20(12): 2594-602. doi: 10.1158/1055-9965.EPI-11-0702.
19. Meena NK, Ahuja V, Meena K, et al. Association of TLR5 gene polymorphisms in ulcerative colitis patients of north India and their role in cytokine homeostasis. PLoS One. 2015; 10(3): e0120697. doi: 10.1371/journal.pone.0120697.
20. Klimosch SN, Försti A, Eckert J, et al. Functional TLR5 genetic variants affect human colorectal cancer survival. Cancer Res. 2013; 73(24): 7232-42. doi: 10.1158/0008-5472.CAN-13-1746.
21. Xiao W, Liu Z, Lin J, et al. Association of TLR4 and TLR5 gene polymorphisms with Graves' disease in

Chinese Cantonese population. Hum Immunol. 2014; 75(7): 609-13. doi: 10.1016/j.humimm.2014.05.001.
22. Cao L, Zhang T, Zhu J, et al. Polymorphism of TLR5 rs5744174 is associated with disease progression in Chinese patients with chronic HBV infection. APMIS. 2017; 125(8): 708-16. doi: 10.1111/apm.12707.
23. Dabagh-Gorjani F, Anvari F, Zolghadri J, et al. Differences in the expression of TLRs and inflammatory cytokines in pre-eclamptic compared with healthy pregnant women. Iran J Immunol. 2014; 11(4): 233-45.
24. Zhang M, Xu J, Bao X, et al. Association between genetic polymorphisms in interleukin genes and recurrent pregnancy loss - A systematic review and meta-analysis. PLoS One. 2017; 12(1): e0169891. doi: 10.1371/journal.pone.0169891.
25. Tyagi P, Alharthi NS. Evaluation of pro-inflammatory cytokine level in cases of idiopathic recurrent spontaneous miscarriage in Saudi Arabia. Biomed Biotechnol Res J (BBRJ).2020; 4(3): 225-31. doi: 10.4103/bbrj.bbrj_71_20.
26. Pitman H, Innes BA, Robson SC, et al. Altered expression of interleukin-6, interleukin-8 and their receptors in decidua of women with sporadic miscarriage. Hum Reprod. 2013; 28(8): 2075-86. doi: 10.1093/humrep/det233.
27. Pernas L, Ramirez R, Holmes TH, et al. Immune profiling of pregnant Toxoplasma-infected US and Colombia patients reveals surprising impacts of infection on peripheral blood cytokines. J Infect Dis. 2014; 210(6): 923-31. doi: 10.1093/infdis/jiu189.
28. El-Hashimi WK, Khalil HI, Al-Mayah QS, et al. The role of cytokines, TNF-α, IL-6 and pregnancy associated hormones in Toxoplasma gondii induced abortion. Eur J Experiment Biol. 2014, 4(6): 98-104.
29. Prins JR, Gomez-Lopez N, Robertson SA. Interleukin-6 in pregnancy and gestational disorders. J Reprod Immunol. 2012; 95(1-2): 1-14. doi: 10.1016/j.jri.2012.05.004.
30. Rezende-Oliveira K, Silva NM, Mineo JR, et al. Cytokines and chemokines production by mononuclear cells from parturient women after stimulation with live *Toxoplasma gondii*. Placenta. 2012; 33(9): 682-7. doi: 10.1016/j.placenta.2012.05.013.
31. Comba C, Bastu E, Dural O, et al. Role of inflammatory mediators in patients with recurrent pregnancy loss. Fertil Steril. 2015; 104(6): 1467-74.e1. doi: 10.1016/j.fertnstert.2015.08.011.

Correspondence to Adel N. Al-Baldawy
E-mail: adel.albaldawy@yahoo.com
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Stress Peptic Ulcers in a Sample of Iraqi Patients

Taqi S. Atiyah *FICMS*

Dept. of Surgery, College of Medicine, Al-Nahrain University, Baghdad, Iraq

Abstract

Background Psychological stress may cause stress peptic ulcers, regardless of *Helicopecter pylori* (*H. pylori*) infection or use of non-steroidal anti-inflammatory drugs (NSAID). Studies of sociodemographic characteristics and peptic ulcers identified various risk factors, such as low salary, household member crowding, unemployment, marital strain, and psychological and physical stress.

Objective To determine the clinical picture of stress peptic ulcer in a sample of Iraqi patients.

Methods A cross-sectional study was performed in Al-Imamein Al-Khadimein Medical City, Iraq to determine the clinical features of stress peptic ulcer in a sample of Iraqi patients. Sample collection was done in a period of two years, from April 2021, to April 2023. At first, the process was explained to the patients and informed consent taken from all enrolled individuals. The diagnosis of stress peptic ulcer was done by gastroduodenoscopy, or by laparotomy for acute abdomen (perforated stress ulcer). All selected patients were tested for the presence of active *H. pylori* infection by stool antigen test (and it was negative). The patients were divided into two groups; Group A patients were presented as an (emergency cases) with complications of stress peptic ulcer either perforation or upper gastrointestinal bleeding stress ulcer; and Group B patients were presented with dyspepsia (as a cold cases), proved by gastroduodenoscopy to be due to stress peptic ulcer.

Results A total of 86 patients with stress ulcer, 37 (43.02%) males, and 49 (56.97%) females, their age ranged from 13-76 years (mean age 36.16±76 years). Group A were 31 (36.04%) patients, their presentations were either acute abdomen proved to be due to perforated stress ulcer by laparotomy in 8 (9.30%) patients, or hematemesis in 23 (26.74%) patients. Group B were 55 (63.59%) patients. The incidence of stress ulcer was 0.02% and the incidence of operations for perforated stress ulcer was 0.01%. The mortality rate was one (1.16%) old female patient with perforated stress ulcer.

Conclusion Stress ulcer can bleed and perforate, so there should be awareness about management of pain, stress and anxiety in all age groups. There should be good and effective postoperative analgesia, reassurance and empathy for patients and advice for protections against stress ulcer when needed.

Keywords Stress ulcer, bleeding, perforation

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List of abbreviations: DU = Duodenal ulcers, GIT = Gastrointestinal tract, *H. pylori* = *Helicopter pylori*, ICU = Intensive care unit, NSAID = none steroidal anti-inflammatory drugs, OGD = Oeophagogastroduodenoscopy, PTT = Partial thromboplastin time

Introduction

Understanding the etiology, investigation and treatment of peptic ulcer disease has changed markedly in recent years. Stress peptic ulceration commonly occurs in patients with major injury or illness, who have undergone major surgery or who have major comorbidity. Many such patients are found in intensive care units. There is no doubt that it is far better to prevent this condition than to try to treat it once it occurs. Endoscopic means of treating stress ulceration may be ineffective

and operation may be required. The principles of management are the same as for the chronic ulcer. Pain and psychological stress can cause peptic ulcers in patients with different age groups. Secondary peptic ulcer disease develops as a result of the acute stress of a severe systemic illness such as head trauma or overwhelming sepsis [1].

The prevalence of *Helicopecter pylori* (*H. pylori*) shows large geographical variations reaching up to 50% of the population in some developing countries [2].

The discovery of *H. pylori* switched the understanding of the etiology of peptic ulcer disease from that of an acid driven disease to an infectious disease [3]. Peptic ulcer disease has changed profoundly in the last decades in Western countries in both children and adults. Indeed, the prevalence of *H. pylori*-positive ulcers has declined, and a new disease has emerged: *H. pylori*-negative gastric or duodenal ulcers (DU) [4].

It is interested to ask whether ALEXANDER THE GREAT who died at age of 32 years, with acute abdominal pain may be due to stress peptic ulcer (the stress life of his great empire) [5]. Some of those patients with stress ulcer were presented with either dyspepsia, acute abdomen (proved to be due to perforated peptic ulcer), or with hematemesis due to severe pain (any sever pain whether it is renal colic or postoperative pain with inadequate analgesia). There are well known causes of peptic ulcer like *H. pylori* infection, non-steroid anti-inflammatory drugs (NSAID) and smoking, but still there are patients with peptic ulcer due to other causes [6]. It was mentioned in a study that 5-20% of patients with peptic ulcer are considered as idiopathic [7].

Researches and literatures advice more study about the pathogenesis of peptic ulcer disease [8]. It was mentioned in literatures that stress, depression and anxiety impair healing of peptic ulcer may be due to its effect on blood flow and gastric secretions [9]. A study on 233,093 Swedish males shows that decreased stress resilience significantly increased the risk of peptic ulcers [10].

Stress may promote peptic ulcer through increased acid load, effects of hypothalamic-pituitary-adrenal axis activation on healing, altered blood flow, or cytokine-mediated impairment of mucosal defenses. Some studies showed that behavioral mediators such as smoking, alcohol consumption and poor sleep may be behind the mechanism of stress peptic ulcer. Although one study showed that there is no synergy between stress and *H. pylori* and effect modification by socioeconomic status, other studies show the ability of stress to affect the course of *H. pylori* infection [11].

There are evidences to support the observations of the association between the effect of *H. pylori* infection on peptic ulcer development and socioeconomic status, age and tobacco smoking [12].

The observed 0.2% per-year rate of new ulcers during follow-up underestimates the true incidence rate, both because ulcers diagnosed as outpatients among subjects lost to follow-up were not included and because asymptomatic ulcers were unlikely to have been detected. Other studies not believe in stress as a cause of peptic ulcer [13]. Although some studies observe a decrease in the incidence of stress ulcer and its complications of bleeding and perforation in the United States as well as elsewhere, but still, it continues to cause substantial morbidity and mortality in the United States as well as elsewhere [14].

The aim of the study was to determine the clinical features of stress peptic ulcer and its complications in a sample of Iraqi patients.

Methods

A cross-sectional study was performed Al-Imamein Al-Khadimein Medical City, Baghdad, Iraq to determine the clinical features of stress peptic ulcer in a sample of Iraqi patients. Sample collection was done in a period of two years, from April 2021, to April 2023. At first, the process was explained to the patients and informed consent taken from all enrolled individuals. The study was approved by the

Institution Review Board in the College of Medicine Al-Nahrain University. The total number of patients attending the hospital in two years with acute abdomen was 621; operations done for 617 of them, and the other four patients were treated conservatively (their acute abdomen was due to non-surgical causes). The study population included 86 patients with stress peptic ulcer, aged 13–76-year-old who attended the hospital and were diagnosed to have stress peptic ulcer by endoscopic findings, or by laparotomy for acute abdomen (perforated stress ulcer). All selected patients were tested for the presence of active *H. pylori* infection by stool antigen test (and it was negative).

The patients were divided into two groups; Group A patients were presented as an (emergency cases) with complications of stress peptic ulcer either perforation or upper gastrointestinal bleeding stress ulcer; and Group B patients were presented with dyspepsia (as a cold cases), proved by gastroduodenoscopy to be due to stress peptic ulcer.

Group A patients were managed by resuscitation and emergency laparotomy for perforated peptic ulcer; while the patients with bleeding stress peptic ulcer were managed conservatively by resuscitation, gastroduodenoscopy and medical management. Group B patients were managed by conservative treatment for stress ulcer. All the patients had no risk factors of peptic ulcer other than stress (they were not smokers, negative biopsy for *H. pylori*, no take of NSAID, and no other risk factors of peptic ulcer other than stress).

The diagnosis was made with either patients attending the hospital with dyspepsia, perforated stress ulcer, or upper gastrointestinal tract (GIT) bleeding stress ulcer; or the patients were already admitted to the hospital and underwent operations for other diseases not related to the GIT, like nephrectomy for renal cell carcinoma, hernia operations, ureteric stricture and lower ureteric stone, and other diseases) and then they develop stress ulcer 1-2 postoperative day may be due to pain and inadequate postoperative analgesia, or psychological stress especially in patients with carcinoma.

Inclusion criteria

Patients presented with stress ulcer with no previous history of dyspepsia or peptic ulcer.

Exclusion criteria

1) Patients with history of dyspepsia or peptic ulcer.
2) Patients who had risk factors for peptic ulcer other than stress (like *H. pylori*, smokers, alcoholic, and NSAID or steroid users).

Statistical analysis

Analysis of data was done using the available statistical package of SPSS-27 (statistical packages for social sciences- version 27). Data were presented in simple measures of frequency, percentage, mean, standard deviation, and range (minimum-maximum values) accordingly whether they were categorical or continuous.

Results

The total number of patients with stress ulcer was 86 patients, 37 males (43.02%), and 49 females (56.97%), their age ranged from 13-76 years, their mean age was 36.16±76 years. Their biopsies were negative for *H. pylori* and they had no history of other risk factors for peptic ulcer.

The incidence of stress ulcer during two years in this study was 0.02% of patients attending the hospital and the incidence of operations for perforated stress ulcer was 0.01%. The mortality was 1 (1.16%), and three (4.65%) patients need blood transfusion for upper GIT bleeding.

Group A (complicated stress ulcer; perforation and bleeding) were 31 (36.04%) patients; and Group B were 55 (63.59%) patients whom complain of dyspepsia (in the course of management for other diseases) and proved to be due to stress ulcer by gastroduodenoscopy and were treated accordingly.

In group A, there were 8 (9.30%) patients presented with acute abdomen proved to be

due to perforated stress ulcer by laparotomy, and 23 (26.74%) patients were presented with hematemesis proved to be due to stress ulcer by gastroduodenoscopy. Table 1 shows the distribution of study groups according to gender.

Table 1. Distribution of study groups according to gender

Group A (Complicated stress ulcer) (31)				Group B (Dyspepsia) (55)		Total
Bleeding stress ulcer (23)		Perforated stress ulcer (8)				
Male	Female	Male	Female	Male	Female	
7 (8.13%)	16 (18.60%)	4 (4.65%)	4 (4.65%)	26 (30.23%)	29 (33.72%)	86

In group A, 8 (9.304%) patients were developed perforated stress ulcer; 6(6.97%) patients of them were (out-patients) presented to the emergency department with acute abdomen proved to be due to perforated stress ulcer by laparotomy (they were unemployed poor patients with a lot of social and psychological problems and stress), while the other 2 (2.32%) patients were in-patients (they were admitted to the hospital and developed perforated peptic ulcer in the course of elective operations for other diseases). One of those in-patients 1 (1.16%) was a male with lower ureteric stricture and impacted lower ureteric stone and prepared for removal of the ureteric stone and implantation of the ureter, he was developed acute abdomen the night before the operation in the ward due to severe pain and inadequate analgesia for ureteric colic; laparotomy was done for him as an emergency condition (there was purulent peritonitis due to perforated peptic ulcer; cleaning of the peritoneal cavity and repair of the perforated stress ulcer with omental patch was done, then closure of the peritoneal cavity and removal of the lower ureteric stone with re-implantation of the ureter into the bladder), the patient improved and discharged well. The other in-patient 1 (1.16%) was an old female patient and developed acute abdomen in the second postoperative day (postoperative radical nephrectomy for renal cell carcinoma) and proved to be due to perforated stress ulcer by laparotomy but unfortunately the patient died second day due to fluid and electrolyte imbalance. So, the mortality rate was1 (1.16%) patient. Table 2 shows the distribution of patients presented with perforated stress ulcer in Group A.

Table 2. Distribution of patients presented with perforated stress ulcer in Group A

	Out-patients	In-patients		Total
Perforated stress ulcer	6 (6.97%)	Pre-operative (lower ureteric stricture and impacted lower ureteric stone)	1 (1.16%)	
		postoperative radical nephrectomy for renal cell carcinoma	1 (1.16%)	
Total	6 (6.97%)		2 (2.32%)	8 (9.304%)

In group A, there were 23 (26.74%) patients were presented with upper GIT bleeding, which proved to be due to stress ulcer by gastroduodenoscopy (with negative biopsy for *H. pylori* and there were no other risk factors for peptic ulcer like smoking, alcohol, or NSAID). There were 16 (18.60%) patients presented to the emergency room and out-patients with hematemesis due to stress (they were unemployed poor patients with a lot of social and psychological problems and stress). Three of them 3 (3.48%) were in-patients underwent operations for hernia repair and presented with hematemesis; two of them were young males developed hematemesis in the day of operation due to severe pain and inadequate analgesia, and the third patient was an old man who develops hematemesis during hernia repair under local anesthesia. Another three 3 (3.48%) patients were developed hematemesis proved to be due to stress ulcer by gastroduodenoscopy in the course of management of severe abdominal sepsis (in the course of operation not involving GIT). One female patient 1(1.16%) with mastectomy for breast carcinoma developed hematemesis proved to be due to stress ulcer by gastroduodenoscopy (due to severe pain, apprehension, and psychological stress due to cancer and loss her breast). Table 3 shows the distribution of patients presented with hematemesis due to stress ulcer in Group A.

Table 3. Distribution of patients presented with hematemesis due to stress ulcer in Group A

	Out-patients	In-patients		Total
Hematemesis	16 (18.6%)	Postoperative (hernia repair)	3 (3.48%)	
		Severe abdominal sepsis	3 (3.48%)	
		Postoperative (mastectomy)	1 (1.16%)	
Total	16 (18.6%)		7 (8.23%)	23 (26.74%)

Three (4.65%) patients with bleeding stress ulcer need blood transfusion. One (1.16%) old female patient who developed perforated stress ulcer second postoperative day after radical nephrectomy for renal cell carcinoma was developed peritonitis but unfortunately, she died second postoperative day (laparotomy for perforated peptic stress ulcer) due to sepsis and fluid and electrolyte disturbances. Table 4 shows the rate of blood transfusion and mortality in patients with complicated stress ulcer.

Table 4. Rate of blood transfusion and mortality in patients with complicated stress ulcer

Complicated stress ulcer	N (%)
Need for blood transfusion (patients with upper GIT bleeding)	3 (4.65)
Mortality (patients with perforated stress ulcer)	1 (1.16)

Discussion

Peptic ulcer disease has changed profoundly in the last decades in Western countries in both children and adults. Indeed, the prevalence of *H. pylori*-positive ulcers has declined, and a new disease has emerged: *H. pylori*-negative gastric or DU [4]. *H. pylori*-negative ulcers, due to unknown causes, are more frequent in

younger children, do not have a gender preference, and tend to have a higher recurrence rate, particularly in Chinese children [15]. In the past two decades, primary peptic ulcer disease has been more widely recognized as a diagnosis worthy of consideration in the pediatric age group. In the present study, a child presented with perforated stress ulcer; she was a 13 years old female child who was presented with acute right lower abdominal pain for one-day duration. On examination, there was tenderness and rebound tenderness in the right iliac fossa. She was considered as a case of acute appendicitis, and explored through a right grid iron incision; but surprisingly there was a fluid coming from upper abdomen through the right paracolic gutter, and so a second incision was made (upper midline incision) and the findings was a perforated stress ulcer in the first part of the duodenum anteriorly with generalized peritonitis. Cleaning of the peritoneal cavity and wash with normal saline and repair of the perforated duodenal ulcer with omental patch using vicryl sutures and anti-ulcer treatment was given. Her biopsy was negative for *H. pylori.* The patient had stress about her examination in her school. Peptic ulcers in children can be classified into primary and secondary ulcers. Secondary peptic ulcer disease develops as a result of the acute stress of a severe systemic illness such as head trauma or overwhelming sepsis. Excluding those secondary peptic ulcers, primary peptic ulcers are even less commonly seen in pediatric practice [16]. In Goggin, Ireland, founded that the age range was 9.8-14.25 years and that was near to the results of the current study [17]. Single-center series from different parts of the world showed that primary peptic ulcer disease was diagnosed in only 1.8-3.6% of the total number of upper endoscopies performed to investigate GIT symptoms in children [18]. *H. pylori* infection is considered to be the most important cause of primary DU in children, and eradication of the bacteria is effective in preventing ulcer relapse [19].

The second patient in this study was a 32 years old male patient who was admitted to the hospital as a case of impacted lower ureteric stone and ureteric stricture proved by contrast study (intravenous urography) with recurrent attacks of ureteric colic and was prepared for surgery next morning. At midnight suddenly he develops another type of sudden severe generalized abdominal pain, tenderness and rigidity. Exploration shows generalized peritonitis due to big perforated stress ulcer in the first part of the duodenum with generalized peritonitis. Surgical treatment of perforated stress peptic ulcer was done. Then closure of the peritoneal layer, exposure of the ureter, removal of the stone and surgical repair of the ureteric stricture was done (to treat and get rid of the main cause of his disease and primary pathology). Post-operative anti- peptic ulcer was given and the patient was improved. The cause of stress ulcer was severe recurrent attacks of pain due to ureteric colic with inadequate analgesia.

The third patient was 75 years old female patient underwent left radical nephrectomy for right renal cell carcinoma through a left paramedian incision. The patient was developed sudden sever generalized abdominal pain with generalized abdominal tenderness and rigidity in the first postoperative day. After resuscitation she was explored by laparotomy and a generalized peritonitis was found due to big perforated stress ulcer in the first part of the duodenum. Surgical treatment of perforated stress peptic ulcer was done. But unfortunately, the patient was died second postoperative day due to fluid and electrolyte imbalance and septicemia. Her stress ulcer was due to postoperative pain and stress.

The fourth patient was a 56 years old man was presented with sudden generalized abdominal pain with generalized tenderness and rigidity. Exploration showed generalized peritonitis due to perforated stress ulcer in the first part of the duodenum (the stress was due to socioeconomic state and poverty because he was unemployed). Surgical treatment of perforated stress peptic ulcer was done. The patient was improved and discharged well. The Fifth patient was a 20 years old young old

man was undergoing repair of inguinal hernia at morning and he was ready for discharge next morning, but he developed sudden attack of fresh bloody vomiting (hematemesis) proved to be due to stress peptic ulcer by gastroduodenoscopy next morning. After resuscitation and antiulcer treatment, the patient was discharged well.

The Sixth patient was a 72 years old man with obstructed inguinal hernia and unfit for general or spinal anesthesia and the surgical repair of his hernia was done under local anesthesia, he developed hematemesis during surgical repair of the hernia due to pain and stress. After resuscitation and proper management gastroduodenoscopy prove the presence of stress ulcer (the biopsy was negative for *H. pylori*) and there was no history of other causes and risk factors of peptic ulcer.

The seventh patient was a 37 years old poor man who had was unemployed and had a lot of social and economic problems presented with acute abdomen, which proved to be due to perforated big stress ulcer about one centimeter in diameter, he was develop postoperative leak from the site of repair of the ulcer for few days and pelvic abscess which was drained and treated accordingly then he improved and discharged well.

Many other patients were developed postoperative hematemesis proved to be due to stress ulcer by gastroduodenoscopy (their surgeries were not related to gastrointestinal canal, but their operations were mastectomy for breast carcinoma, and different types of hernias (without opening the bowel).

So, after those patients, there was a lot of concern about the condition with good and enough postoperative analgesia and reassurances with early alarm for any dyspepsia. Many patients whom complain of (even mild dyspepsia) were underwent gastroduodenoscopy, a lot of them had stress ulcer proved by gastroduodenoscopy, and was treated appropriately without complications of neither bleeding nor perforation.

Literatures and scientific researches prove the effect of stress in creation of peptic ulcer and its complications. Levenstein et al. concluded that psychological stress increased the incidence of peptic ulcers, regardless of H. pylori infection or NSAID use [6].

Other literatures found that participants with the highest self-perceived stress level had a 2.2-fold higher risk of peptic ulcer treatment in 33 months of follow-up compared to participants with the lowest level of stress. The cumulated incidence of treatment was approximately 1.2% for those with the highest stress levels and 0.4% for those with the lowest levels of stress [20,21].

In a sample of 233,093 Swedish males, decreased stress resilience significantly increased the risk of peptic ulcers [13]. Melinder et al. found that low stress resilience in adolescent males increased the risk of peptic ulcers in adulthood compared with high stress resilience [10]. Ruigómez et al. reported increased odds of peptic ulcers in a nested case control study among patients who had been diagnosed with stress before their peptic ulcer diagnosis [8], and Levenstein et al. found an increased risk in another Danish sample using a stress index preceding 12 years of follow-up [6].

Other studies found that stress should be considered a determinant of peptic ulcer disease. Numerous studies of sociodemographic characteristics and peptic ulcers identified various risk factors, such as low salary, household member crowding, unemployment, marital strain, psychological and physical stress, and previous peptic ulcers. These findings were supported by several previous studies. Anda et al. found an increased risk of peptic ulcers in individuals with self-perceived stress during the month preceding baseline. The study further found evidence of a graded relationship between levels of self-perceived stress and the risk of a peptic ulcer [22]. Wachirawat et al. also found evidence of higher increased odds of a peptic ulcer in patients with high self-perceived stress levels [23]. Some studies found that there are association between *H. pylori* infection and stress. Stress affected *H. pylori*-related ulcers at least as much as those related to neither non-steroidal anti-inflammatory drugs nor *H. pylori*. These results support a multicausal model of

peptic ulcer etiology with intertwined biological and psychosocial components. Clinicians treating ulcer patients should investigate potential psychological stress among other risk factors. Rosenstock et al. found that individuals in a Danish sample with *H. pylori* infection had a significantly lower odds ratio for reporting mental stress than those with no infection [(14)]. On the other hand, some studies show that not all the patients with *H. pylori* or NSAID drug developed ulcers, and in 16-31% of ulcers neither can be implicated so there are other factors must be implicated [(24)] and still the role of psychological stress considered as one of the risk factors for peptic ulcer and should be taken into account [(25, 26)]. Examining life stress at baseline among a defined Danish population cohort in relation to medically confirmed ulcers during the next 11-12 years; had suggested associations with psychological factors and there is a complementary effect of psychological, social, behavioral, and bacteriologic factors in development of stress ulcer.

A vast literature links peptic ulcer to stress and many other researchers showed that ulcer diagnoses after societal stressors such as wartime bombing or earthquake [(27-29)] and worsening of prognosis by psychological factors [(30-32)].

Other cross-sectional studies were unconvincing, because the results were inaccurate due to the distressing effects of disease [(33)]. Previously histamine-2 blockers, and proton pump inhibitors were available only by prescription and patients with dyspepsia were investigated for peptic ulcer using oeseophagogastrodeuodenoscopy (OGD), so, the incidence of ulcer was accurately evaluated, but nowadays dyspepsia is commonly self-treated [(34)], so, many ulcers remain undiagnosed [(35)]. The incidence of uncomplicated ulcer was decreased and although the incidence of complicated stress ulcer (bleeding and perforation) is also decreased, but they carry high morbidity and mortality in the United States and worldwide. The stress index used had the merit of taking into account objective life stressors such as unemployment as well as the subjective distress. A high perceived stress-level was associated with an increased risk of peptic ulcers. The group with the highest stress level had a 2.2-fold increased risk of having a peptic ulcer compared to the individuals with the lowest stress level. Cohen's perceived stress scale has been validated as a measure of stress with consistent results for decades [(36)]. There is a suggestion to give stress ulcer prophylaxis therapy when indicated; if the patient has one of the following four major risk factors: (coagulopathy {platelet count of 1.5, partial thromboplastin time (PTT) of >2 times the control}; mechanical ventilation longer than 24 hours; recent GIT ulcers/bleeding within 12 months of admission; traumatic brain injury, traumatic spinal cord injury, or thermal injury {>35 percent of the body surface area}. Stress ulcer prophylaxis therapy also given if the patient has two or more of the following minor risk factors: (sepsis, shock, intensive care u nit (ICU) >1-week, occult bleeding within 6 days, high dose corticosteroids {250 mg hydrocortisone, 50 mg methylprednisone}, hepatic failure, renal failure, organ transplantation, administration of non-steroidal anti-inflammatory agent, or injury severity score >15). The use of intragastric enteral nutrition may have additive cytoprotective effects when used in conjunction with histamine 2 receptor blockers, but whether administration of intragastric enteral nutrition alone provides adequate protection is controversial. Patients with stress-related mucosal damage have much higher mortality rates than those without (57% vs. 24%) that's why the advice for stress ulcer prophylaxis therapy [(37)].

In conclusion, stress ulcer can bleed and perforate, so there should be awareness about management of pain, stress and anxiety in all age groups. There should be good and effective postoperative analgesia, reassurance and empathy for patients and advice for protections against stress ulcer when needed.

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Conflict of interest
The author declares that have no conflict of interest.

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References
1. Tawfeeq WF, Ibraheem MF, Kadhem ZG. Clinico-epidemiological study of peptic ulcer disease among children in three tertiary health care centers in Baghdad. Iraqi J Med Sci. 2013; 11(4): 381-7.
2. Eissa AA, Mirza SS. The relevance of Helicobacter pylori Infection to iron deficiency anemia in Duhok City. Iraqi J Med Sci. 2021; 19(1): 33-8. doi: 10.22578/IJMS.19.1.5
3. Sullivan PB. Peptic ulcer disease in children. Pediat Child Health J. 2010; 20(10): 462-4. doi: https://doi.org/10.1016/j.paed.2010.04.002.
4. Arents NL, Thijs JC, van Zwet AA, et al. Does the declining prevalence of Helicobacter pylori unmask patients with idiopathic peptic ulcer disease? Trends over an 8 year period. Eur J Gastroenterol Hepatol. 2004; 16(8): 779-83. doi: 10.1097/01.meg.0000108367.19243.73.
5. Levenstein S. Stress and peptic ulcer: life beyond Helicobacter. BMJ. 1998; 316(7130): 538-41. doi: 10.1136/bmj.316.7130.538.
6. Levenstein S, Rosenstock S, Jacobsen RK, et al. Psychological stress increases risk for peptic ulcer, regardless of Helicobacter pylori infection or use of nonsteroidal anti-inflammatory drugs. Clin Gastroenterol Hepatol. 2015; 13(3): 498-506.e1. doi: 10.1016/j.cgh.2014.07.052.
7. Chung CS, Chiang TH, Lee YC. A systematic approach for the diagnosis and treatment of idiopathic peptic ulcers. Korean J Intern Med. 2015; 30(5): 559-70. doi: 10.3904/kjim.2015.30.5.559.
8. Ruigómez A, Johansson S, Nagy P, et al. Risk of uncomplicated peptic ulcer disease in a cohort of new users of low-dose acetylsalicylic acid for secondary prevention of cardiovascular events. BMC Gastroenterol. 2014; 14: 205. doi: 10.1186/s12876-014-0205-y.
9. Holtmann G, Armstrong D, Pöppel E, et al. Influence of stress on the healing and relapse of duodenal ulcers. A prospective, multicenter trial of 2109 patients with recurrent duodenal ulceration treated with ranitidine. RUDER Study Group. Scand J Gastroenterol. 1992; 27(11): 917-23. doi: 10.3109/00365529209000163.
10. Melinder C, Udumyan R, Hiyoshi A, et al. Decreased stress resilience in young men significantly increases the risk of subsequent peptic ulcer disease - a prospective study of 233 093 men in Sweden. Aliment Pharmacol Ther. 2015; 41(10): 1005-15. doi: 10.1111/apt.13168.
11. Lazzarino AI, Hamer M, Stamatakis E, et al. The combined association of psychological distress and socioeconomic status with all-cause mortality: a national cohort study. JAMA Intern Med. 2013; 173(1): 22-7. doi: 10.1001/2013.jamainternmed.951.
12. Kanno T, Iijima K, Abe Y, et al. Peptic ulcers after the Great East Japan earthquake and tsunami: possible existence of psychosocial stress ulcers in humans. J Gastroenterol. 2013; 48(4): 483-90. doi: 10.1007/s00535-012-0681-1.
13. Song EM, Jung HK, Jung JM. The association between reflux esophagitis and psychosocial stress. Dig Dis Sci. 2013; 58(2): 471-7. doi: 10.1007/s10620-012-2377-z.
14. Rosenstock SJ, Møller MH, Larsson H, et al. Improving quality of care in peptic ulcer bleeding: nationwide cohort study of 13,498 consecutive patients in the Danish Clinical Register of Emergency Surgery. Am J Gastroenterol. 2013; 108(9): 1449-57. doi: 10.1038/ajg.2013.162.
15. Tam YH, Lee KH, To KF, et al. Helicobacter pylori-positive versus Helicobacter pylori-negative idiopathic peptic ulcers in children with their long-term outcomes. J Pediatr Gastroenterol Nutr. 2009; 48(3): 299-305. doi: 10.1097/mpg.0b013e31818c5f8b.
16. Drumm B, Rhoads JM, Stringer DA, et al. Peptic ulcer disease in children: etiology, clinical findings, and clinical course. Pediatrics. 1988; 82(3 Pt 2): 410-4.
17. Goggin N, Rowland M, Imrie C, et al. Effect of Helicobacter pylori eradication on the natural history of duodenal ulcer disease. Arch Dis Childhood. 1998; 79: 502-5.
18. El Mouzan MI, Abdullah AM. Peptic ulcer disease in children and adolescents. J Trop Pediatr. 2004; 50(6): 328-30. doi: 10.1093/tropej/50.6.328. PMID: 15537716.
19. Arakawa T, Higuchi K, Fujiwara Y, et al. Has Helicobacter pylori eradication for peptic ulcer been overrated? Intern Med. 2004; 43(3): 179-83. doi: 10.2169/internalmedicine.43.179.
20. Daniel WW, Cross CL. Biostatistics: A Foundation for Analysis in the Health Sciences. 10th ed. USA: John Wiley & Sons Inc; 2013.
21. Deding U, Ejlskov L, Grabas MP, et al. Perceived stress as a risk factor for peptic ulcers: a register-based cohort study. BMC Gastroenterol. 2016; 16(1): 140. doi: 10.1186/s12876-016-0554-9.
22. Anda RF, Williamson DF, Escobedo LG, et al. Self-perceived stress and the risk of peptic ulcer disease. A longitudinal study of US adults. Arch Intern Med. 1992; 152(4): 829-33.
23. Wachirawat W, Hanucharurnkul S, Suriyawongpaisal P, et al. Stress, but not Helicobacter pylori, is associated with peptic ulcer disease in a Thai population. J Med Assoc Thai. 2003; 86(7): 672-85.

24. Gisbert JP, Calvet X. Review article: Helicobacter pylori-negative duodenal ulcer disease. Aliment Pharmacol Ther. 2009; 30(8): 791-815. doi: 10.1111/j.1365-2036.2009.04105.x.
25. Levenstein S. Stress and peptic ulcer: life beyond Helicobacter. BMJ. 1998; 316(7130): 538-41. doi: 10.1136/bmj.316.7130.538.
26. Levenstein S. The very model of a modern etiology: a biopsychosocial view of peptic ulcer. Psychosom Med. 2000; 62(2): 176-85. doi: 10.1097/00006842-200003000-00003.
27. Spicer CC, Stewart DN, Winser DMR. Perforated peptic ulcer during the period of heavy air raids. Lancet. 1944; i: 14.
28. Aoyama N, Kinoshita Y, Fujimoto S, et al. Peptic ulcers after the Hanshin-Awaji earthquake: increased incidence of bleeding gastric ulcers. Am J Gastroenterol. 1998; 93(3): 311-6. doi: 10.1111/j.1572-0241.1998.00311.x.
29. Kanno T, Iijima K, Abe Y, et al. Peptic ulcers after the Great East Japan earthquake and tsunami: possible existence of psychosocial stress ulcers in humans. J Gastroenterol. 2013; 48(4): 483-90. doi: 10.1007/s00535-012-0681-1.
30. Holtmann G, Armstrong D, Pöppel E, et al. Influence of stress on the healing and relapse of duodenal ulcers. A prospective, multicenter trial of 2109 patients with recurrent duodenal ulceration treated with ranitidine. RUDER Study Group. Scand J Gastroenterol. 1992; 27(11): 917-23. doi: 10.3109/00365529209000163.
31. Levenstein S, Prantera C, Varvo V, et al. Long-term symptom patterns in duodenal ulcer: psychosocial factors. J Psychosom Res. 1996; 41(5): 465-72. doi: 10.1016/s0022-3999(96)00196-1.
32. Levenstein S, Prantera C, Scribano ML, et al. Psychologic predictors of duodenal ulcer healing. J Clin Gastroenterol. 1996; 22(2): 84-9. doi: 10.1097/00004836-199603000-00002.
33. Wilhelmsen I, Berstad A. Reduced relapse rate in duodenal ulcer disease leads to normalization of psychological distress: twelve-year follow-up. Scand J Gastroenterol. 2004; 39(8): 717-21. doi: 10.1080/00365520410006044.
34. Raghuram A, Holub JL, Lieberman DA. Lower prevalence of peptic ulcer disease in the era of over-the-counter proton pump inhibitors. Gastroenterology. 2011; 140(5): S726-7.
35. Van Oudenhove L, Vandenberghe J, Geeraerts B, et al. Determinants of symptoms in functional dyspepsia: gastric sensorimotor function, psychosocial factors or somatisation? Gut. 2008; 57(12): 1666-73. doi: 10.1136/gut.2008.158162.
36. Cohen S, Janicki-Deverts D. Who's stressed? Distributions of psychological stress in the United States in probability samples from 1983, 2006, and 2009. J Appl Soc Psychol. 2012; 42: 1320-34. doi: https://doi.org/10.1111/j.1559-1816.2012.00900.x
37. MacLaren R. A review of stress ulcer prophylaxis. J Pharmacy Practice. 2002; 15(2): 147-57. doi: 10.1106/EAD9-P8RX-D7J7.

E-mail: taqi.atyia@nahrainuniv.edu.iq
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Detection of Hepatitis E Virus and Toll-Like Receptor 4 (rs4986790 and rs4986791) Genotypes Among A Sample of Hemodialysis Patients

Haleema S. Salih[1] *PhD*, **Ahmed A. Abbas**[1] *PhD*

[1]Dept. of Microbiology, College of Medicine, Al-Nahrain University, Baghdad, Iraq

Abstract

Background Hepatitis E virus (HEV) is one of the prevalent nosocomial transmitted agents among patients on maintenance hemodialysis. Innate immunity's main actors, Toll-like receptors (TLRs), are able to identify the chemical patterns linked to pathogens.

Objective To investigate the seroprevalence of HEV in hemodialysis (HD) patients and study the TLR4 polymorphism (rs4986790 and rs4986791) in association with HEV in HD patients.

Methods One hundred and fifty patients on maintenance HD attending the HD centers of Al-Karama Hospital and Al-Yarmouk Teaching Hospital in the period from March to November 2021. Using enzyme-linked immunosorbent assay (ELISA) kit, serum samples were examined for the existence of anti-HEV (IgG and IgM) antibodies and conformation by using molecular technique quantitative reverse transcription polymerase chain reaction (qRT-PCR). The selected single nucleotide polymorphisms (SNPs) (rs4986790 and rs4986791) in TLR4 were amplified by using conventional PCR and then confirmed by sequencing their polymorphism.

Results Out of 150 hemodialysis patients, the seropositive result for HEV-IgG and IgM was 10 and 6, respectively. While 14 patients were positive by PCR. On the other hand, the result of IgM was negative for all control group. The analysis of TLR4 rs4986790 SNPs were 24 (80%) AA and 6 (20%) AG in patients while 6 (60%) AA and 4 (40%) AG in control group with insignificant difference. In addition, the TLR4 rs4986791 SNPs were 24 (80%) CC and 6 (20%) CT in patients while 6 (60%) CC and 4 (40%) CT in control group with insignificant difference.

Conclusion Patients undergoing HD are susceptible to HEV infection, the sero-prevalence of HEV in patients considered as risk factor. The genotypes of TLR4 SNPs (rs4986790 and rs4986791) have no significant association with HEV in HD patients.

Keywords HEV, seropositivity, hemodialysis, TLR4

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List of abbreviations: ELISA = Enzyme-linked immunosorbent assay, ERSD = End-stage renal disease, HD = Hemodialysis, HEV = Hepatitis E virus, HLA = Human leukocyte antigen, PCR = Polymerase chain reaction, qRT-PCR = Quantitative reverse transcription polymerase chain reaction, SNPs = Single nucleotide polymorphisms, TLRs = Toll-like receptors

Introduction

The single-stranded RNA virus known as hepatitis E virus (HEV), which causes hepatitis E, belongs to the genus Orthohepevirus in the family Hepeviridae [(1)]. Although the fecal- oral route is the predominant method of HEV transmission, alternative methods include hemodialysis (HD)

is a part of blood transfusions, and organ donation are some other possible routes of HEV transmission [2]. Patients receiving maintenance HD frequently contract HEV, one of the common nosocomial transmitted diseases. The parenteral transmission of HEV and immunocompromised state of continuous HD patients are the causes of this elevated exposure risk. Therefore, HEV infection is a risk for HD patients [3].

This susceptibility is confirmed by the high prevalence of HEV infection among HD patients around the world [4]. However, HEV prevalence among HD patients in various regions can also be influenced by the levels of safety precautions in HD centers and the frequency of HEV in the population [5].

Despite the fact that HEV infection is typically moderate and self-limiting in its clinical manifestation, people with chronic kidney disease, particularly those receiving HD, often have severe bouts of the infection [6]. However, its significance, HD patients are not frequently checked for HEV in HD centers, particularly in endemic countries [3].

Toll-like receptors 4 (TLR4) is involved in the pathogenesis of several viral diseases, including hepatitis B, C, and E [7]. The single nucleotide polymorphisms (SNPs) rs4986790 (A>G, Asp299Gly) and/or rs4986791 (C>T, Thr399Ile) in TLR4 are well-known. Receptor hypo-responsiveness, not receptor expression, is correlated with increased TLR 4 expression (at protein and gene level) and reduced cytokine response upon stimulation of peripheral blood mononuclear cells with lipopolysaccharides in HEV infected patients. These alter the molecule's extracellular domain that found in the fourth exon [8].

This study focused on identifying of HEV, and estimate the TLR4 polymorphism as useful in revealing of disease progression and treating of HD patients.

Methods

One hundred and fifty patients on regular HD (79 males and 71 females) from two dialysis centers in Al-Karama Hospital and Al-Yarmouk Teaching Hospital were enrolled in this study. The control group consisted of 150 apparently healthy individuals from the donor and blood transfusion center and volunteers. The samples were collected from March to November 2021.

Hepatitis E virus-IgG and IgM antibodies were detected by using HEV IgG, IgM enzyme-linked immunosorbent assay (ELISA) kit (Acon, USA). The whole blood was further tested for detection of HEV RNA using quantitative reverse transcription polymerase chain reaction (qRT-PCR) (SacaceBiotechnologies, Italy). Hepatitis E virus RNA was extracted from the samples using the guanidiniumisothiocyanate (GIT) method and a modified proteinase K (PK) method (BioinGentech, Italy).

To synthesize single-stranded cDNA from total RNA using the cDNA Reverse Transcription kit and the primers that used (R 5'-CCCTTRTCYTGCTGMGCATTCTC-3'), F(5'-ATTATGCYCAGTAYCGRGTTG -3') [9].

Polymorphisms of TLR4 (rs4986790 and (rs4986791) was performed by PCR (F:5'-TCTGGCTGGTTTAGAAGTCCA-3', R:(5'-ATTGCCAGCCATTTTCAAG-3') [10] and Sanger sequencing method, by Macrogen Corporation of Korea's ABI3730XL automated DNA sequencer.

After receiving the results through email, knowledgeable software was used in data analysis. All patients and the control group have given their consent. The Institutional Review Board (IRB), College of Medicine at Al-Nahrain University gave approval to this study.

Statistical analysis

Statistical package for the social sciences (SPSS) Software v19.0 was used to examine the data. The bivariate analysis was carried out using the chi-square test in order to identify the risk factors associated with the seropositivity of microorganisms. P-values lower than 0.05 were regarded as statistically significant.

Results

Out of 150 HD patients, 79 (52.7%) were males and 71 (47.3%) were females, while in control

group there were 80 (53.3%) males and 70 (46.7%) were females. The median age of patients and controls were 41.35 and 42.50 years, respectively with no significant difference (Table 1).

Table 1. Age and sex of patients and control groups

Parameter		Patients	Controls	P value
Age (yr)	Median (5-95 percentile)	41.35 (21-63)	42.50 (20-65)	>0.05
Sex	Female No. (%)	71 (47.3%)	70 (46.7%)	>0.05
	Male No. (%)	79 (52.7%)	80 (53.3%)	

Regarding the result of RT-PCR, there were 14 (9.3%) of patients positive for HEV, while all the control group were negative, with highly significant difference (Table 2).

Table 2. Detection of hepatitis E virus by quantitative reverse transcription polymerase chain reaction

HEV PCR		Patients	Control
Positive	No. %	14 9.3%	0 0.0%
Negative	No. %	136 90.7%	150 100%
Total	No. %	150 100.0%	150 100%
P value		≤0.001**	
Odd ratio (95%CI)		2.103	1.86-2.38

** Highly significant

Of the 150 HD patients 10 (6.7%) were seropositive for anti- HEV IgG antibody and 6 (4.0%) had anti-HEV IgM antibody, while among control group all samples were negative for anti-HEV IgG and IgM antibodies (Table 3 and 4).

Table 3. Anti-HEV IgG seropositivity rates in study groups

Anti-HEV IgG		Patients	Control
Positive	No. %	10 6.7%	0 0.0%
Negative	No. %	140 93.3%	150 100%
Total	No. %	150 100%	150 100%
P value		≤0.001**	

** Highly significant

Table 4. Anti-HEV IgM seropositivity rates in study groups

Anti-HEV IgM		Patients	Control
Positive	No.	6	0
	%	4.0%	0.0%
Negative	No.	144	150
	%	96.0%	100%
Total	No.	150	150
	%	100%	100%
P value		0.015*	

* Significant

The majority of HD patients seropositivity of HEV were in the age groups 51-60 years with insignificant difference (Table 5).

Table 5. Association of HEV seropositivity and quantitative reverse transcription polymerase chain reaction results with age groups

Age groups		HEV PCR		Anti-HEV IgG		Anti-HEV IgM	
		Positive	Negative	Positive	Negative	Positive	Negative
≤ 30 years	No.	4	30	3	31	1	33
	%	11.80%	88.20%	8.80%	91.20%	2.90%	97.10%
31-40 years	No.	2	28	3	29	1	31
	%	6.70%	84.80%	10.10%	87.90%	0.00%	93.90%
41-50 years	No.	1	33	0	34	0	33
	%	2.90%	97.10%	0.00%	100%	2.90%	97.10%
51-60 years	No.	5	28	4	27	2	30
	%	15.20%	93.30%	12.00%	90.00%	2.90%	100.00%
61-70 years	No.	2	17	0	19	2	17
	%	10.50%	89.50%	0.00%	100%	10.50%	89.50%
Total	No.	14	136	10	140	6	144
	%	9.30%	90.70%	6.70%	93.30%	4.00%	96.00%
P value		0.48		0.188		0.417	

Concerning the sex, there were 8 (10.1%) females and 6 (8.5%) males positive for HEV by PCR, while 6 (8.5%) females and 4 (5.1%) males were positive anti-HEV IgG, and 3 (4.2%) female and 3 (3.8%) males were positive for anti-HEV IgM by ELISA with no significant difference (Table 6).

According to the analysis of TLR4-rs4986790 SNPs using Sanger sequencing this polymorphism appeared in only two genotypes in both patients and control group, these were AA and AG the frequency of the heterozygous genotype (AG) was equal in patients and control group (50% in each them) (P=0.393).

At allelic level, the frequency of mutant allele (allele G) also was equal in patients and control group (50% in each them) (Table 7).

TLR4 rs4986791 similar to TLR4 rs4986790, this polymorphism also had only two genotypes CC and CT (Table 8).

Table 6. Association of HEV seropositivity with sex

Sex		HEV PCR		Anti-HEV IgG		Anti-HEV IgM	
		Positive	Negative	Positive	Negative	Positive	Negative
Female	No.	8	65	6	65	3	68
	%	10.10%	91.50%	8.50%	91.50%	4.20%	95.80%
Male	No.	6	71	4	75	3	76
	%	8.50%	89.90%	5.10%	94.90%	3.80%	96.20%
Total	No.	14	136	10	140	6	144
	%	9.30%	90.70%	6.70%	93.30%	4.00%	96.00%
P value		0.725		0.307		0.607	

Table 7. Frequency of genotypes and alleles of TLR-4 rs4986790 in study groups

TLR4rs4986790			Study groups		P value
			Patients	Controls	
Genotype	AA	No.	24	6	0.232^{NS}
		%	80.00%	60.00%	
	AG	No.	6	4	
		%	20.00%	40.00%	
Allele	A allele	No.	54	16	0.257^{NS}
		%	90.00%	80.00%	
	G allele	No.	6	4	
		%	10.00%	20.00%	

NS: Non-significant

Table 8. Frequency of genotypes and alleles of TLR-4 rs4986791 in study groups

TLR4 rs4986791			Study groups		P value
			Patients	Controls	
Genotype	AA	No.	24	6	0.232^{NS}
		%	80.00%	60.00%	
	AG	No.	6	4	
		%	20.00%	40.00%	
Allele	A allele	No.	54	16	0.257^{NS}
		%	90.00%	80.00%	
	G allele	No.	6	4	
		%	10.00%	20.00%	

NS: Non-significant

As well as there was no significant association between HEV results by ELISA and PCR with TLR4 SNPs (table 9 and 10).

Table 9. Association of genotypes and alleles of TLR4 rs4986790 with HEV in patients' group

TLR4 rs4986790		HEV PCR Positive	HEV PCR Negative	Anti-HEV IgG Positive	Anti-HEV IgG Negative	Anti-HEV IgM Positive	Anti-HEV IgM Negative	Total
AA	No.	7	17	4	20	2	22	24
	%	29.17%	70.83%	16.67%	83.33%	8.33%	91.67%	100%
AG	No.	3	3	2	4	1	5	6
	%	50.00%	50.00%	33.33%	66.67%	16.67%	83.33%	100%
A	No.	17	37	10	44	5	49	54
	%	31.48%	68.52%	18.52%	81.48%	9.26%	90.74%	100%
G	No.	3	3	2	4	1	5	6
	%	50.00%	50.00%	33.33%	66.67%	16.67%	83.33%	100%
P value		0.393		0.586		0.484		

Table 10. Association of genotypes and alleles of TLR4 rs4986791 with HEV in patients' group

TLR4 rs4986791		HEV PCR Positive	HEV PCR Negative	Anti-HEV IgG Positive	Anti-HEV IgG Negative	Anti-HEV IgM Positive	Anti-HEV IgM Negative	Total
CC	No.	7	17	4	20	2	22	24
	%	29.17%	70.83%	16.67%	83.33%	8.33%	91.67%	100%
CT	No.	3	3	2	4	1	5	6
	%	50.00%	50.00%	33.33%	66.67%	16.67%	83.33%	100%
C	No.	17	37	10	44	5	49	54
	%	31.48%	68.52%	18.52%	81.48%	9.26%	90.74%	100%
T	No.	3	3	2	4	1	5	6
	%	50.00%	50.00%	33.33%	66.67%	16.67%	83.33%	100%
P value		0.393		0.586		0.484		

Discussion

This case control study revealed insignificant difference in median age among patients and control group, because the ages of control group were selected according to patients' group. Most studies worldwide showed that older ages patients (≥50 years) were more likely to have infections by microorganisms. However, some studies did not find such association [11].

The current study revealed that 6.7% of patients were seropositive of anti-HEV IgG antibodies while among control group, all samples were negative with significant difference.

On the other hand, the IgM anti-HEV seropositive rates in hemodialysis patients were 4%, while all samples of the control group were negative with no significant difference.

The HEV seroprevalence reported among HD patients were (39.6%) in Egypt [12]. Alavian et al. [13] found anti-HEV IgG antibodies in 28.3% of HD patients in Isfahan, Iran, as opposed to 9.9% in their control group. Similar to this, Argentinian dialysis patients had considerably

greater seroprevalence than control group (10.2% and 4.3%, respectively) [14].

Other study from England showed that HD patients (36.8%) have a considerably greater seroprevalence of anti-HEV IgG than control group (18.8%) [15]. However, other studies revealed that HD patients' HEV prevalence is not appreciably higher than that of healthy people. The same HEV isolates were discovered in the patient's serum and the transfused viremic blood in a study by Mitsui et al. [16] that demonstrated how an HD patient became positive for HEV RNA by transfusion of HEV-viremic blood one month after HD onset.

This enormous regional variation in the prevalence of HEV seroprevalence may be brought on by variations in the severity of safety precautions and preventive measures implemented in HD facilities, as well as variations in the burden of HEV infection in the general population, risk factors, routes of HEV transmission, and the state of public health and hygiene in various regions. But some of this heterogeneity might be attributable to variations in the specificity and sensitivity of ELISA kits, research period, sample size, timing of sampling, length of disease, and socio-demographic features of the study population in other studies [17].

Other risk factor is the potential for HEV transmission in hemodialysis patients through tainted blood transfusions and heparin. The older individuals have frequent exposure to the outside environment, including contaminated food and water, and this frequent exposure increases the chances of contracting the virus. The virus dose may not be sufficient to cause infection, but it can induce the immune system to produce IgG antibodies against HEV. Anti-HEV IgG seropositive of hemodialysis patients in the current study was higher with age range 51-60 yrs. Numerous studies have found a relationship between older age and increased HEV seropositivity [18].

It is possible that more parenteral exposures or cumulative exposure over time are to blame for this rising incidence with age [19].

Infections with HEV frequently progress asymptomatically or without typical symptoms, resulting in a seroconversion of IgG antibodies that is unremarkable clinically. Consuming raw or undercooked meat, drinking tap water, and receiving blood products other than red blood cells in transfusions have all been mentioned as contributing factors to HEV infection [20].

The nutritional state is another element (micronutrient deficiencies). A high burden of viral diseases and micronutrient deficiencies have been found to contribute to immunologic compromise, including decreased mucosal immunity and dysregulated cytokine production. The immune system may become compromised as a result of this imbalance, raising the risk of HEV infection [21].

It has been documented that infectious HEV has been identified in a variety of sources, including animal feces, sewage water, inadequately-treated water, contaminated shellfish, and animal meat. In-house breeding of domesticated animals or close proximity to human houses are a few factors that may contribute to increased rates of HEV infection among women [22].

Numerous studies have searched for genetic factors associated with HD risk, the role of human leukocyte antigen (HLA)-A2 in Iraqi Arab patients and HLA-B35 in Iraqi Kurdish patients could be considered as highly significant risk factors for end-stage renal disease (ESRD) [23].

This study showed no association of TLR4 rs4986790 and rs4986791 polymorphism with HEV infection. An earlier study found that the TLR4 polymorphism was linked to high viral loads, delayed antiviral therapy, and patients with HCV-induced hepatocellular cancer [24]. Additionally, a different study found a strong correlation between the TLR4 polymorphism and the development of HEV illness [25].

In conclusion: Patients undergoing hemodialysis are susceptible to HEV infection, the seroprevalence of HEV in patients considered as risk factor. The genotypes of TLR4 SNPs (rs4986790 and rs4986791) have no significant association with HEV in HD patients.

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Author contribution
Dr. Salih: Contributed to the design and implementation of the research also wrote the manuscript. Dr. Abbas: Conceived of the presented idea and supervised the project.

Conflict of interest
The authors declare there is no conflict of interest.

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References
1. Taherkhani R, Makvandi M, Farshadpour F. Development of enzyme-linked immunosorbent assays using 2 truncated ORF2 proteins for detection of IgG antibodies against hepatitis E virus. Ann Lab Med. 2014; 34(2): 118-26. doi: 10.3343/alm.2014.34.2.118.
2. Taherkhani R, Farshadpour F, Makvandi M, et al. Cytokine profiles and cell proliferation responses to truncated ORF2 protein in Iranian patients recovered from hepatitis E infection. J Trop Med. 2015; 2015: 523560. doi: 10.1155/2015/523560.
3. Taherkhani R, Farshadpour F. Epidemiology of hepatitis E virus in Iran. World J Gastroenterol. 2016; 22(22): 5143-53. doi: 10.3748/wjg.v22.i22.5143.
4. Uçar E, Cetin M, Kuvandik C, et al. Hatay'da hemodiyaliz tedavisi alan hastalarda hepatit e virus seropozitifliği [Hepatitis E virus seropositivity in hemodialysis patients in Hatay province, Turkey]. Mikrobiyol Bul. 2009; 43(2): 299-302. Turkish.
5. Ben-Ayed Y, Hannachi H, Ben-Alaya-Bouafif N, et al. Hepatitis E virus seroprevalence among hemodialysis and hemophiliac patients in Tunisia (North Africa). J Med Virol. 2015; 87(3): 441-5. doi: 10.1002/jmv.24082.
6. Eini P, Mamani M, Javani M. Seroprevalence of hepatitis e among hemodialysis patients: a report from hamadan, Iran. Hepat Mon. 2015; 15(5): e26260. doi: 10.5812/hepatmon.15(5)2015.26260.
7. Smith DB, Simmonds P, Izopet J, et al. Proposed reference sequences for hepatitis E virus subtypes. J Gen Virol. 2016; 97(3): 537-42. doi: 10.1099/jgv.0.000393.
8. Sridhar S, Teng JLL, Chiu TH, et al. Hepatitis E virus genotypes and evolution: Emergence of camel hepatitis E variants. Int J Mol Sci. 2017; 18(4): 869. doi: 10.3390/ijms18040869.
9. LeDesma R, Nimgaonkar I, Ploss A. Hepatitis E virus replication. Viruses. 2019; 11(8): 719. doi: 10.3390/v11080719.
10. Kamar N, Dalton HR, Abravanel F, et al. Hepatitis E virus infection. Clin Microbiol Rev. 2014; 27(1): 116-38. doi: 10.1128/CMR.00057-13.
11. Cao D, Meng XJ. Molecular biology and replication of hepatitis E virus. Emerg Microbes Infect. 2012; 1(8): e17. doi: 10.1038/emi.2012.7.
12. Abdel Hady SI, El-Din MS, El-Din ME. A high hepatitis E virus (HEV) seroprevalence among unpaid blood donors and hemodialysis patients in Egypt. J Egypt Public Health Assoc. 1998; 73(3-4): 165-79.
13. Alavian SM, Ataei B, Ebrahimi A, et al. Anti-hepatitis E antibody in hemodialysis patients in Isfahan, Iran: Prevalence and risk factors. Hepat Mon. 2015; 15(9): e23633. doi: 10.5812/hepatmon.23633.
14. Pisano MB, Balderramo D, Wassaf MM, et al. Hepatitis E virus infection in patients on dialysis and in solid organ transplant recipients in Argentina: exploring associated risk factors. Arch Virol. 2017; 162(3): 787-92. doi: 10.1007/s00705-016-3171-6.
15. Harrison A, Scobie L, Crossan C, et al. Hepatitis E seroprevalence in recipients of renal transplants or haemodialysis in southwest England: a case-control study. J Med Virol. 2013; 85(2): 266-71. doi: 10.1002/jmv.23463.
16. Mitsui T, Tsukamoto Y, Yamazaki C, et al. Prevalence of hepatitis E virus infection among hemodialysis patients in Japan: evidence for infection with a genotype 3 HEV by blood transfusion. J Med Virol. 2004; 74(4): 563-72. doi: 10.1002/jmv.20215.
17. Khuroo MS, Khuroo MS, Khuroo NS. Transmission of hepatitis E virus in developing countries. Viruses. 2016; 8(9):253. doi: 10.3390/v8090253.
18. Bura M, Łagiedo-Żelazowska M, Michalak M, et al. Comparative seroprevalence of hepatitis A and E viruses in blood donors from Wielkopolska Region, West-Central Poland. Pol J Microbiol. 2018; 67(1): 113-5. doi: 10.5604/01.3001.0011.6151.
19. Naziri H, Tahamtan A, Moradi A, et al. Evaluation of antihepatitis E virus antibody among hemodialysis patients in Gorgan, North of Iran. Iran J Virol. 2016; 10: 13-8.
20. Ding X, Li TC, Hayashi S, et al. Present state of hepatitis E virus epidemiology in Tokyo, Japan. Hepatol Res. 2003; 27(3): 169-73. doi: 10.1016/s1386-6346(03)00233-x.
21. Grabarczyk P, Sulkowska E, Gdowska J, et al. Molecular and serological infection marker screening in blood donors indicates high endemicity of hepatitis E virus in Poland. Transfusion. 2018; 58(5): 1245-1253. doi: 10.1111/trf.14531.
22. Kmush BL, Labrique A, Li W, et al. The association of cytokines and micronutrients with hepatitis E virus

infection during pregnancy and the postpartum period in rural Bangladesh. Am J Trop Med Hyg. 2016; 94(1): 203-11. doi: 10.4269/ajtmh.15-0238.

23. Al-Taie LH, Al-Ghurabi BH, Al-Hassan AA, et al. Frequency of HLA-A and B antigens in Iraqi patients with end-stage renal disease preparing for transplantation. Iraqi Postgraduate Med J. 2012; 11: 642-8.

24. Yugo DM, Meng XJ. Hepatitis E virus: foodborne, waterborne and zoonotic transmission. Int J Environ Res Public Health. 2013; 10(10): 4507-33. doi: 10.3390/ijerph10104507.

25. Wu JF, Chen CH, Ni YH, et al. Toll-like receptor and hepatitis B virus clearance in chronic infected patients: a long-term prospective cohort study in Taiwan. J Infect Dis. 2012; 206(5): 662-8. doi: 10.1093/infdis/jis420.

Correspondence to Dr. Ahmed A. Abbas
E-mail: ahmed26770@yahoo.com
ahmed26770@nahrainuniv.edu.iq
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