Iraqi Journal of Medical Sciences

IRAQI JMS

المجلة العراقية للعلوم الطبية

Volume 17, Number 1, 2019
January - March

P- ISSN 1681-6579
E- ISSN 2224-4719

Volume 17 (1) 2019
DOI: 10.22578/IJMS.17.1.

P-ISSN 1681-6579
E- ISSN 2224-4719

IRAQI JOURNAL OF MEDICAL SCIENCES

Editorial Board Members

Iraqi Journal of Medical Sciences

Aims and Scope

Iraqi Journal of Medical Sciences is published by College of Medicine, Al-Nahrain University. It is a quarterly multidisciplinary medical journal. High quality papers written in English, dealing with aspects of clinical, academic or investigative medicine or research will be welcomed. Emphasis is placed on matters relating to medicine in Iraq in particular and the Middle East in general, though articles are welcomed from anywhere in the world.

Iraqi Journal of Medical Sciences publishes original articles, case reports, and letters to the editor, editorials, investigative medicine, and review articles.

Mission and Vision

Mission of Iraqi JMS

To establish rapid review processes aiming to puplish scientific papers that help to augment knowledge and highlight discoveries in the field of medical sciences to be a world-wide forum in assisting the distribution of medical reasearches to career readers.

Vision of Iraqi JMS

To be pioneer national medical journal interesting in increasing the understanding of diseases and treatment.

All correspondence and subscription information requests should be addressed to:
The Editor of **Iraqi Journal of Medical Sciences**
College of Medicine
Baghdad, Iraq
Tel. + 964 7717516090
P.O. Box 70044, Kadhimiya, Baghdad, Iraq.
E-mail: iraqijms@colmed-alnahrain.edu.iq
http://www.iraqijms.net

Iraqi National Library and Archives, 709 Baghdad

Iraqi JMS FORMAT

INSTRUCTION TO AUTHORS

Iraqi Journal of Medical Sciences (Iraqi JMS) is a periodic, peer-reviewed journal published quarterly by College of Medicine, Al-Nahrain University. Iraqi JMS publishes manuscripts in all fields of health and medicine written in English.

Types of Contributions: Original articles, review articles, case studies, editorials, medical education, history of medicine, ethics, practical points, medical quiz, conferences, meetings and letters to the Editor.

Manuscripts:

- Submission of a manuscript implies that is not being considered for publication anywhere.
- The author should provide the following:

A. A document officially state that the current work was carried out at the site, which provides the certification. The document should be signed by the highest authorized member at that location.

B. Document stated clearly that his current work is in agreement with the medical ethics provided either from the local ethical committee in the place where he did his work or from the Ministry of Health, Department of Training and Improving skill - Research and Educational facilities, the approval has to be stated separetly in the method section.

C. Publication fees are 100,000 IDs in addition to 20,000 IDs for checking of plagiarism. Other extra fees will be taken for extra pages (6000 IDs for each additional page (more than six pages) and up to 24000 IDs only and 10,000 IDs For any Figure).

- Manuscripts submitted to Iraqi JMS are subject to editorial evaluation and revision by three referees after being checked electronically for any plagiarism.
- The format of IJMS complies with the uniform requirements for manuscripts submitted to Biomedical Journals, published by the International Committee of Medical Journals Editors (ICMJE) (Vancouver, British Colombia, 1979) and its last update in October 2001, available on the web site www.icmje.org.
- Manuscript should be typewritten font size 14, double spaced on size A4 (29.5x21 cm) paper with wide margins and line- numbered. Page should be numbered consecutively. One original and three photocopies including figures, tables, and photographs should be submitted. Begin each of following sections on separate page in the following sequence: Title page, abstract and keywords, text, acknowledgments, references, tables, and legends for illustration.
- Manuscript and figures will not be returned to the authors whether the editorial decision is to accept, revise or reject.
- Manuscripts must be accompanied by a covering paper signed by all authors that the paper has not been published in and will not be submitted to any other journal if accepted in Iraqi JMS.

- The title page should contain (a) title of the manuscript, (b) names of each author (first name, middle initial and family name) including highest academic degree, (c) official academic and/or clinical title and affiliation (d) name and address of the institution where the work was done (e) name and address (E-mail if available) of the author to whom correspondence should be sent.
- Authors can also submit the scientific publication through the official Iraqi JMS web site at (http://submit.Iraqijms.com/). Users must register when accessing the Iraqi JMS online submission system for the first time, by clicking on "Register." Three steps are involved in obtaining a personal account.

Abstract: Manuscript should include an abstract of not more than 250 words. Structured abstract typed on a separate sheet and consist of background, objective, method, results, and conclusion.

Keywords: Three to ten keywords should be provided on the same page as the abstract in English. As far as possible, be selected from the National Library of Medicine, Medical Subject Headings.

Manuscript format: It should be divided into the following parts: introduction, methods, results and discussion.

References: All references should be listed in consecutive numerical order by English numerical, in the order of citation in the text **and each reference must be followed with its DOI link.** Once a reference is cited all subsequent citations should be to the original number.

Examples

1. Standard Journal Article: use et al when the number of authors exceeds 3.
Halliwell B, Gutteridge JMC. Oxygen toxicity, Oxygen radicals, transition metals and disease. Biochem J. 1984; 219(1): 1-14.
2. Books: Mann JI, Pyorala K, Teuscher A. Diabetes in epidemiological perspective. London: Churchill Livingstone; 1983. p. 1-5.
3. Chapter in book: Phillips SJ, Whisnant JP. Hypertension and strock. In: Laragh JH, Brenner BM. editors. Hypertension: Pathophysiology, diagnosis, and management. 2nd ed. NewYork: Raven Press; 1995. p. 465-78.

- **How to find DOI for the references of your submitted article to Iraqi Journal of Medical Sciences (IJMS)**

1. First, click on this link http://www.crossref.org/guestquery/
2. Go to "search on article title"
3. Fill in the author name and the title of the reference
4. Copy and paste the found DOI (if any: as some references have no DOI) to the end of each reference in the reference list in your article to be submitted to IJMS.

That's it!!

Tables: Each table should be typed on a separate page double-spaced, including all headings, number all tables with Arabic numerals and include a short title. Vertical lines between columns are to be avoided.

Figures: All figures must be suitable for reproduction without being retouched or redrawn. Photographs must be supplied as glossy black and white prints. The top of the figures should be indicated clearly.

Legends: Captions for figures must be typed; double spaced, and must not appear on the figure.

Acknowledgments: Collate acknowledgments in a separate section at the end of the article before the references and do not, therefore, include them on the title page, as a footnote to the title or otherwise. List here those individuals who provided help during the research (e.g., providing language help, writing assistance or proof reading the article, etc.).

Conflict of interest: All authors must disclose any financial and personal relationships with other people or organisations that could inappropriately influence (bias) their work. **Example** of potential conflicts of interest include employment, consultancies, stock ownership, honoraria, paid expert testimony, patent applications\registrations, and grants or other funding. See also http://www.elsevier.com\conflictsofinterest .
Please complete and upload the conflict of interest and author declaration form with your manuscript.

Author contributions: Each author is required to declare his or her individual contribution to the article: all authors must have materially participated in the research and\or article preparation, so roles for all authors should be described. The statement that all authors have approved the final author's article should be true and included article in the disclosure.

Role of the funding source: You are requested to identify who provided financial support for the conduct of the research and\or preparation of the article and to briefly describe the role of the sponsor (s), if any, in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the article for publication. If the funding source (s) had no such involvement then this should be stated.

List of abbreviation: Any abbreviations used should be listed after the abstract and defined at first use in the main body of the article. Use only widely accepted and conventional abbreviations. Avoid abbreviations in the title and abstract.

Proof Reading will be done by the secretarial office of the journal. The principal author will receive a copy of the journal. The authors are responsible for accuracy of all statements, data, and references included in the manuscript.

- After the manuscript has been accepted for publication, authors are required to supply the final version of the manuscript on CD in MS word version 6 or later.

Iraqi Journal of Medical Sciences

A Medical Journal Encompassing All Medical Specializations

Issued Quarterly

CONTENTS

Iraqi Journal of Medical Sciences

A Medical Journal Encompassing All Medical Specializations

Issued Quarterly

CONTENTS

Iraqi JMS

Published by Al-Nahrain College of Medicine
P-ISSN 1681-6579
E-ISSN 2224-4719
Email: iraqijms@colmed-alnahrain.edu.iq
http://www.colmed-alnahrain.edu.iq
http://www.iraqijms.net
Iraqi JMS 2019; Vol. 17(1)

Medicinal Fungi

Azhar A.F. Al-Attraqchi *PhD*

Dept. of Microbiology, College of Medicine, Al-Nahrain University, Baghdad, Iraq

Abstract

Medicinal fungi are those fungi, which produce medically significant metabolites or can be induced to produce such metabolites using biotechnology. The range of medically active compounds that have been identified include antibiotics, anti-cancer drugs, cholesterol inhibitors, psychotropic drugs, immunosuppressants and even fungicides. Although initial discoveries centered on simple molds of the type that cause spoilage of food, later work identified useful compounds across a wide range of fungi.

Keywords Medicinal, fungi

Citation Al-Attraqchi AAF. Medicinal fungi. Iraqi JMS. 2019; 17(1): 1-3. doi: 10.22578/IJMS.17.1.1

List of abbreviation: None

Although fungi products have been used in traditional and folk medicines, probably since pre-history, the ability to identify beneficial properties and then extract the active ingredient started with the discovery of penicillin by Alexander Fleming in 1928. Since that time, many additional antibiotics have been discovered and the potential for fungi to synthesize biologically active molecules, useful in a wide range of clinical therapies, has been extensively exploited.

Pharmacological research has now isolated antifungal, antiviral, and antiprotozoan, isolates from fungi [1].

The fungus with probably the longest record of medicinal use, *Ganoderma lucidum*, is known in Chinese as língzhī "spirit plant", and in Japanese as mannentake "10,000-year mushroom". Traditional Chinese medicine. Notable medicinal mushrooms with a well-documented history of use include *Agaricus subrufescens* [2].

Studies have shown another species of genus *Ganoderma*, *G. applanatum*, contains compounds with anti-tumor and anti-fibrotic properties.

Inonotus obliquus was used in Russia as early as the 16th century, and it featured in Alexandr Solzhenitsyn's 1967 novel Cancer Ward [3].

Applications

Cancer

Paclitaxel is synthesized using *Penicillium raistrickii* and plant cell fermentation. Fungi can synthesize other mitotic inhibitors including vinblastine, vincristine, podophyllotoxin, griseofulvin, aurantiamine, oxaline, and neoxaline [4].

11,11'-Dideoxyverticillin A, an isolate of marine *Penicillium*, was used to create dozens of semi-synthetic anticancer compounds [5]. 11,11'-Dideoxyverticillin A, andrastin A, barceloneic acid A, and barceloneic acid B, are farnesyl transferase inhibitors that can be made by *Penicillium* [6]. 3-O-Methylfunicone, anicequol, duclauxin, and rubratoxin B, are anticancer/cytotoxic metabolites of *Penicillium*. *Penicillium* is a potential source of the leukemia medicine asparaginase [7].

Antibacterial agents (antibiotics)

Alexander Fleming led the way to the beta-lactam antibiotics with the *Penicillium* mold and penicillin. Subsequent discoveries included alamethicin, aphidicolin, brefeldin A, cephalosporin, cerulenin, citromycin, eupenifeldin, fumagillin, fusafungine, fusidic acid, itaconic acid, MT81, nigrosporin B, usnic acid, verrucarin A, vermiculine and many others. Antibiotics retapamulin, tiamulin, and valnemulin are derivatives of the fungal metabolite pleuromutilin. Plectasin, austrocortilutein, austrocortirubin, coprinol, oudemansin A, strobilurin, illudin, pterulone, and sparassol are antibiotics isolated from basidiomycete species. Rene Dubos had reported the discovery of the first naturally derived antibiotic, tyrothricin, a compound of 20% gramicidin and 80% tyrocidine, from B. brevis. It was one of the first commercially manufactured antibiotics and was very effective in treating wounds and ulcers during World War II [8].

Cholesterol biosynthesis inhibitors

Statins are an important class of cholesterol-lowering drugs; the first generation of statins were derived from fungi [9]. Lovastatin, the first commercial statin, was extracted from a fermentation broth of *Aspergillus terreus* [9]. Industrial production is now capable of producing 70 mg lovastatin per kilogram of substrate [10]. The red yeast rice fungus, *Monascus purpureus*, can synthesize lovastatin, mevastatin, and the simvastatin precursor monacolin J. Nicotinamide riboside, a cholesterol biosynthesis inhibitor, is made by *Saccharomyces cerevisiae*.

Antifungals

Some antifungals are derived or extracted from other fungal species. Griseofulvin is derived from a number of *Penicillium* species, caspofungin is derived from *Glarea lozoyensis* [11]. Strobilurin, azoxystrobin, micafungin, and echinocandins, are all extracted from fungi. Anidulafungin is a derivative of an *Aspergillus* metabolite.

Immunosuppressants

Ciclosporin, was discovered *in Tolypocladium inflatum*. Bredinin was discovered in *Eupenicillium brefeldianum*. Mycophenolic acid was discovered in *Penicillium stoloniferum*. Thermophilic fungi were the source of the fingolimod precursor myriocin. *Aspergillus* synthesizes immunosuppressants gliotoxin and endocrocin. Subglutinols are immunosuppressants isolated *from Fusarium subglutinans* [12]. Other compounds include mizoribine.

Malaria

Codinaeopsin, efrapeptins, zervamicins, and antiamoebin [13] are made by fungi.

Diabetes

Many fungal isolates act as DPP-4 inhibitors, alpha-glucosidase inhibitors, and alpha amylase inhibitors in vitro. Ternatin is a fungal isolate that suppresses hyperglycemia [14]. Aspergillusol A is an alpha-glucosidase inhibitor made by *Aspergillus*. Sclerotiorin is an aldose reductase inhibitor made by *Penicillium*.

Psychotropic effects

A number of fungi have well documented psychotropic effects, some of them severe and associated with sometimes acute and life-threatening side-effects [15]. Well known amongst these is *Amanita muscaria*, the fly agaric. More widely used informally are a range of fungi collectively known as "magic mushrooms", which contain psilocybin and psilocin.

The history of bread-making is also peppered with references to deadly ergotism caused by ergot, most commonly *Claviceps purpurea*, a parasite of cereal crops. A number of therapeutically useful drugs have subsequently been extracted from ergot including ergotamine, pergolide and cabergoline [16].

Psychotropic compounds created from ergot alkaloids also include dihydroergotamine, methysergide, methylergometrine, hydergine, nicergoline, lisuride, bromocriptine, cabergoline, pergolide. Polyozellus

multiplexsynthesizes prolyl endopeptidase inhibitors polyozellin, thelephoric acid, kynapcins. Neurotrophic fungal isolates include L-theanine, tricholomalides, scabronines, termitomycesphins. Many fungi synthesize the partial, non-selective, serotonin receptor agonist/analog psilocin.

A number of other fungal species, including species of *Aspergillus* and *Penicillium*, have been induced to produce ergot alkaloids.

Vitamins

Fungi are a source of ergosterol, which can be converted to vitamin D upon exposure to ultraviolet light to synthesize vitamins D2 (ergocalciferol), D4 (22-dihydroergocalciferol), and D1 (Lumisterol+D2) [17,18].

References

1. Engler M, Anke T, Sterner O. Production of antibiotics by Collybia nivalis, Omphalotus olearis, a Favolaschia and a Pterula species on natural substrates. Z Naturforsch C. 1998; 53(5-6): 318-24. doi:10.1515/znc-1998-5-604.
2. Hetland G, Johnson E, Lyberg T, et al. Effects of the medicinal mushroom Agaricus blazei Murill on immunity, infection and cancer. Scand J Immunol. 2008 Oct;68(4):363-70. doi: 10.1111/j.1365-3083.2008.02156.x.
3. Zheng W, Miao K, Liu Y, et al. Chemical diversity of biologically active metabolites in the sclerotia of Inonotus obliquus and submerged culture strategies for up-regulating their production. Appl Microbiol Biotechnol. 2010; 87(4): 1237-54. doi: 10.1007/s00253-010-2682-4.
4. Nicoletti R, Ciavatta ML, Buommino E, et al. Antitumor extrolites produced by Penicillium species. Int J Biomed Pharmaceut Sci. 2008 2(1): 1-23.
5. Tratfon A, MIT News Office. Research update: Chemists find help from nature in fighting cancer. URL: https://news.mit.edu/2013/chemists-find-help-from-nature-in-fighting-cancer-0227 Retrieved 2013-12-17.
6. Overy DP, Larsen TO, Dalsgaard PW, et al. Andrastin A and barceloneic acid metabolites, protein farnesyl transferase inhibitors from Penicillium albocoremium: chemotaxonomic significance and pathological implications. Mycol Res. 109 (Pt 11): 1243-9. doi:10.1017/S0953756205003734.
7. Shrivastava A, Khan AA, Shrivastav A,. Kinetic studies of L-asparaginase from Penicillium digitatum. Prep Biochem Biotechnol. 2012; 42(6): 574-81. doi: 10.1080/10826068.2012.672943.
8. Van Epps HL. René Dubos: unearthing antibiotics. J Exp Med. 2006; 203(2): 259. doi: 10.1084/jem.2032fta
9. Tolbert JA. Lovastatin and beyond: the history of the HMG-CoA reductase inhibitors. Nat Rev Drug Discov. 2003; 2(7): 517-26. doi:10.1038/nrd1112 .
10. Jahromi MF, Liang JB, Ho YW, et al. Lovastatin production by Aspergillus terreus using agro-biomass as substrate in solid state fermentation. J Biomed Biotechnol. 2012; 2012: 196264. doi: 10.1155/2012/196264.
11. Richardson MD, Warnock DW. Fungal infection diagnosis and management. 3rd ed. USA: Blackwell Publishing; 2003.
12. Kim H, Baker JB, Park Y, et al. Total synthesis, assignment of the absolute stereochemistry, and structure-activity relationship studies of subglutinols A and B. Chem Asian J. 2010; 5(8): 1902-10. doi: 10.1002/asia.201000147.
13. Nagaraj G, Uma MV, Shivayogi MS, et al. Antimalarial activities of peptide antibiotics isolated from fungi. Antimicrob Agents Chemother. 2001; 45(1): 145-9. doi: 10.1128/AAC.45.1.145-149.2001 .
14. Lo HC, Wasser SP. Medicinal mushrooms for glycemic control in diabetes mellitus: History, current status, future perspectives, and unsolved problems (review). Int J Med Mushrooms. 2011; 13(5): 401-26. doi: 10.1615/intjmedmushr.v13.i5.10 .
15. Hoegberg LC, Larsen L, Sonne L, et al. Three cases of Amanita muscaria ingestion in children: two severe courses. Clin Toxicol. 2008; 46(5): 407-8. doi: 10.1080/15563650802071703 .
16. Schade R, Andersohn F, Suissa S, et al. Dopamine agonists and the risk of cardiac-valve regurgitation. N Engl J Med. 2007; 356(1): 29-38. doi: 10.1056/NEJMoa062222 .
17. Keegan RJ, Lu Z, Bogusz JM, et al. Photobiology of vitamin D in mushrooms and its bioavailability in humans. Dermatoendocrinol. 2013; 5(1): 165-76. doi: 10.4161/derm.23321.
18. Kamweru PK, Tindibale EL. Vitamin D and Vitamin D from Ultraviolet-Irradiated Mushrooms (Review). Int J Med Mushrooms. 2016; 18(3): 205-14. doi: 10.1615/IntJMedMushrooms.v18.i3.30.

E-mail: tariq_963@yahoo.com
dr.azhar.ibrahim@colmed-alnahrain.edu.iq

Iraqi JMS

Published by Al-Nahrain College of Medicine
P-ISSN 1681-6579
E-ISSN 2224-4719
Email: iraqijms@colmed-alnahrain.edu.iq
http://www.colmed-alnahrain.edu.iq
http://www.iraqijms.net
Iraqi JMS 2019; Vol. 17(1)

The Vitro Study Effect of Ginger Extracts on Fungal Isolated from A Suppurative Otitis Media and Externa

Azhar A.F. Al-Attraqchi[1] *PhD*, **Hayder B. Sahib**[2] *PhD*, **Jaafer M.K. Al-Hasseni**[3] *FICMS*, **Maha M. Mohammed** *MSc*

[1]Dept. of Microbiology, College of Medicine, Al-Nahrain University, Baghdad, Iraq, [2]Dept. of ????, College of Pharmacy, Al-Nahrain University, Baghdad, Iraq, [3]Dept. of Surgery, College of Medicine, Al-Nahrain University, Baghdad, Iraq

Abstract

Background Otitis media is a group of complex inflammatory disorders affecting the middle ear, which can be acute or chronic. Otitis externa is an inflammation on the skin of the external auditory canal usually associated with secondary bacterial and/or fungal infection of macerated skin and subcutaneous cellular tissue.

Objective To investigate the effect of ginger extracts on fungal isolates from patients suffering from otitis.

Methods Two hundred patients suffering from suppurative otitis media and externa who attended to ENT Department, Al-Imamein Al-Kadhimein Medical City enrolled in this study from November 2016 to the end of April 2017, included patients all age groups with discharging ear. All specimens were transported to the laboratory for processing and investigations at the same day. The powder of ginger rhizomes soaked with the solvent and left in a shaking water bath at 40 °C for 24 hours, and then filtered using Whatmann's filter paper No.1 for clear extract. Each extract was concentrated using a rotary evaporator with vacuum to get the final crude extract\; after the procedure of ginger extract was done, this extract was taken and tested for bacterial and fungal isolates from patients with otitis.

Results Results revealed that acute otitis media consisted of about 96 (48%), while chronic suppurative otitis media about 75 (37.5%). Otitis externa was less common infection among the other types of otitis 29(14.50%). The most fungal isolates were *Candida parapsilosis*. In addition, there is a significant effect of (chloroform, methanol, and aqueous) extract of ginger on pathogenic fungi.

Conclusion Ginger extracts have been showed evident zones of inhibition effect on pathogenic fungi by chloroform more than ethanol, with less effect by aqueous extract.

Keywords Otitis externa, otitis media, ginger extract

Citation Al-Attraqchi AAF, Sahib HB, Al-Hasseni JMK, Mohammed MM. The vitro study effect of ginger extracts on fungal isolated from a suppurative otitis media and externa. Iraqi JMS. 2019; 17(1): 4-11. doi: 10.22578/IJMS.17.1.2

List of abbreviations: AOM = Acute otitis media, CSOM = Chronic suppurative otitis media, OE = Otitis externa, DMSO = Dimethyl sulfoxide

Introduction

The infection of ear is a common clinical problem throughout the world and the major cause of preventable hearing loss in the developing world. Ear infection can be classified as acute otitis media (AOM), chronic suppurative otitis media (CSOM), and otitis externa (OE) [(1)]. Otitis media is an infection of middle ear caused by bacteria, fungi and virus resulting in inflammation of mucosal lining [(2)]. AOM is inflammation of the middle ear with the production of otorrhea and other symptoms but with less than two weeks of duration [(3)]. In

developing countries, the natural course of the disease is different, leading to purulent otitis often with perforation and further complications [4]. CSOM is a name given to long standing inflammatory disease affecting mucoperiosteal lining of the middle ear [5]. Inflammation of the cutis and sub cutis of the external auditory canal is a primary symptom in acute otitis externa [6]. OE, also called swimmer's ear, involves diffuse inflammation of the external ear canal that may extend distally to the pinna and proximally to the tympanic membrane [7]. The treatment for ear infection mainly depends on antibiotic therapy. Within consideration the major public health challenge for antibiotic resistant bacteria [8]. So herbal medicine and homeopathy are interchangeable practiced together and sometimes confused [9]. Plant derived products have been used for medicinal purposes for centuries. In traditional Indian medicine *Zingiber officinale* and many other herbs have been used as medicine [10]. Today, the interest for use of herbs instead of chemical drugs is increasing because of lesser side effects [11]. Rhizome part of ginger genus: Zingiber is extensively employed in medicine for the management of different diseased conditions [12]. *Zingiber aceae* is among the plant families that are widely distributed throughout the tropics, particularly in Southeast Asia [13]. Ginger is a strong antioxidant substance and may either mitigate or prevent generation of free radicals. This natural herbal product is known to have powerful antifungal properties [14]. It is also having broad spectrum of biological activities includes antioxidant, antimicrobial, antitumor or antidiabetic effects [15].

The objective of this study was to investigate the effect of ginger extracts on fungal isolates from patients suffering from otitis.

Methods

Samples collection:

Two hundred patients suffering from CSOM and OE who attended to Al-Imamein Al-Kadhimein Medical City were enrolled in this study from November 2016 to the end of April 2017, included patients in all age groups who attended ENT Department with ear symptoms. The majority of patients were from Baghdad and its suburbs. All swabs specimens were collected from each patient in clean sterile swabs. These were labeled with code number and name. All swabs samples were taken at same day that had been transported to laboratory for investigations.

Preparation of ginger extract

Five hundred grams of dried rhizomes of ginger (*Zingiber officinale*) were purchased from local markets in Baghdad –Iraq and was identified by the National Iraqi Institute for Herbs. The dried rhizomes grind into very fine powder using a heavy-duty grinder. The powder of ginger rhizomes, then divided into 12 portions, each portion extracted sequentially with three solvents beginning with the non-polar solvent and ascending to the most polar solvent respectively (chloroform, methanol and distilled water) with a ratio of 1:7 W/V (30 gm of powder/ 210 ml of solvent); the extraction repeated twice for each solvent and the process of extraction used was the cold method, i.e., Maceration. The powder of ginger rhizomes soaked with the solvent according to the ratio mentioned previously and left in a shaking water bath at 40 °C for 24 hours and then filtered using Whatmann's filter paper No. 1 for clear extract. Each extract was concentrated using a rotary evaporator with vacuum to get the final crude extract. The extract powder was weighed and kept in sterile bottles, labeled accordingly and stored in the refrigerator, according to Muslim et al. (2012) [16]. One gm of the crude extracts of chloroform, methanol and aqueous extracts were dissolved in 10 ml Dimethyl sulfoxide (DMSO) as stock solutions.

Antifungal test of extracts using agar well diffusion method

The antifungal activity of different extracts against fungi was evaluated by using agar well diffusion method [17]. Isolated colonies were selected from Sabouraud's dextrose agar plate cultures and transferred to 3ml of 0.85 % normal saline of a density equivalent to the

turbidity of the (0.5) McFarland standards. A sterile cotton swab was dipped into the fungal suspension; excess fluid inoculum from the swab was removed by pressing the swab firmly on the side wall of the tube above the fluid level, streaking of the inoculum was done over the entire sterile agar surface. This procedure was repeated by streaking 2 more times, rotating the plate approximately 60 ° each time to ensure an even distribution of inoculum as a final step, the rim of the agar was swabbed. The plates were left at room temperature for 15 minutes to allow any excess surface moisture to be absorbed. Wells of 5 mm were punctured with the help of a sterilized cork-porer into the pre-solidified Mueller Hinton agar plates containing the test organism. Using the micropipette, 20 µl of each extract (chloroform, methanol, and aqueous) was poured into the different wells of the inoculated plates. DMSO well used as a negative control, fungal plates were incubated at 37 °C for 72 hrs. The diameters of zones of inhibition were measured, later on.

Statistical analysis

Data of this study samples were entered using EPI INFO7 Windows Version and analyzed by using statistical package for social sciences (SPSS) version 20. Descriptive statistics were presented as frequencies, percentage (%), means and standard deviation (SD). Chi square test was used to estimate the association between two categorical variables. Level of significance of ≤ 0.05 was considered as significant. Analysis of variance (ANOVA) used for comparison among more the two groups. A paired samples T test used for comparison between two groups.

Results

Gender and age distribution of patients with ear infection

A total of 200 patients suffering from otitis were enrolled in this study. The mean age of patients was (30.04), ranged from 7 days-80 years old. It was found that a half of patients were males as 109 (54.50%) and 91 (45.50%) were females. Two hundred patients with otitis were classified into Seven age groups per decade (Table 1).

Table 1. Classification of patients with otitis regarding age groups

Age group	No.	Percentage %
<10 years	50	25.0
11-12 years	23	11.5
21-30 years	35	17.5
31-40 years	25	12.5
41-50 years	30	15.0
51-60 years	21	10.5
>60 years	16	8.0
Total	200	100%

Isolation and identification of fungi

Two hundred ear swab samples were collected from patients with otitis have been cultured on Sabouraud's dextrose agar, the results revealed that thirty-two samples were positive for fungi (Table 2).

Fungal isolates from patients with otitis:

Table (3) summarized fungal isolates from patients with otitis as discussed below.

Table 2. Fungal species isolated from ear discharge

Age group	Frequency	Percentage %
Aspergillus spp.	4	2.0
Candida parapsilosis	20	10.0
Candida glabrata	1	0.5
Microsporum audouinii	2	1.0
Penicillium spp.	1	0.5
Trichophytone mentogrophte	4	2.0
No growth	168	84.0
Total	200	100%

Table 3. Percentages of fungal isolates from patients with otitis

Fungal isolate	AOM	CSOM	OE	Total
Aspergillus spp.	2 (2.1%)	2 (2.7%)	0 (0.0%)	4 (2.0%)
Candida parapsilosis	8 (8.4%)	7 (9.3%)	5 (17.2%)	20 (10.0%)
Candida glabrata	1 (1.0%)	0 (0.0%)	0 (0.0%)	1 (0.5%)
Microsporum audouinii	2 (2.1%)	0 (0.0%)	0 (0.0%)	2 (1.0%)
Penicillium spp	1 (1.0%)	0 (0.0%)	0 (0.0%)	1 (0.5%)
Trichophytone mentogrophte	3 (3.1%)	1 (1.3%)	0 (0.0%)	4 (2.0%)
No growth	79 (82.3%)	65 (86.7%)	24 (82.8%)	168 (84.0%)
Total	96 (100%)	75 (100%)	29 (100%)	200 (100%)
p value		**0.733**		

Antifungal activity of ginger extracts fungal isolates

The antifungal activities of ginger extract with (chloroform, methanol, and aqueous) have been tested against pathogenic fungal species isolates from patients with otitis (Table 4). In case of *Aspergillus spp.*, chloroform extract revealed the larger mean of diameter of inhibition zones as (6.00±7.12 mm) followed by aqueous extract of ginger when the mean of diameter of inhibition zones as (4.00±4.62 mm), while methanol extract showed no effect. In *Candida parapsilosis*, chloroform extract was the strongest among the others when the mean of diameter of inhibition zones was (11.14±5.05 mm), followed by methanol extract when the mean of diameter of inhibition zones was (7.57±4.08 mm), then aqueous extract of ginger as (4.86±4.51 mm). In *Candida glabrata*, chloroform extract was the strongest among the others when the mean of diameter of inhibition zones was (12 mm), followed by methanol extract, the mean of diameter of inhibition zones was (8 mm), then aqueous extract of ginger as (7 mm). *Microsporum audouinii* was inhibited by chloroform extract when the mean of diameter of inhibition zones as (22.50±16.26 mm), followed by this obtained by aqueous extract of ginger as (12.50±0.71 mm), and then by this obtained by methanol extract as (7.00±0 mm). Chloroform extract was the only effective agent against *Trichophytone mentogrophte* when the mean of diameter of inhibition zone was (8.50±5.7 4mm), while each of aqueous extract of ginger and methanol extract showed no effect.

Table 4. Antifungal activities of each of aqueous, methanol, and chloroform extract of ginger against pathogenic fungi isolated from patients with otitis

Fungal culture	Mean of the diameter of inhibition Zones (mm)			
	Chloroform	Methanol	Aqueous	Negative control
Aspergillus spp.	6.00±7.12	0.00±0.00	4.00±4.62	0.00±0
Candida parapsilosis	11.14±5.05	7.57±4.08	4.86±4.51	0.00±0
Candida glabrata	12.0	8.0	7.0	0.00
Microsporum audouinii	22.50±16.26	7.00±0	12.50±0.71	0.00±0
Trichophytone mentogrophte	8.50±5.74	0.00±0	0.00±0	0.00±0

Discussion

Gender and age distribution of patients with ear infection

In the present study, results indicated the percentage of infected males were 109 (54.50%) and females were 91(45.50%) out of 200 patients with otitis, hence there was significant difference between males and females infection rate upon existing both in different condition, geographical variation, male may be more exposed to different conditions in work such as dust, humidity, and may be more actively involved in outdoor activities, hence to be more exposed to contaminated environment, in females wearing of scarfs may be considered an important factor to decrease infection, in addition to the differences in the No. of each involved in the study. This result agrees with other obtained by Almamory and Kamal in 2014 [17] who mentioned that the rate of ear infection in males was higher than females, while disagree with this obtained by Khammas and Abbas in 2010 [18] who mentioned that the rate of ear infection in females was higher than those of males. In the current study and according to the result of age of patients with ear infection revealed that all age groups could be developed otitis with significant differences, the highest infection rate was (50) cases occurred in the age group (≤10) years, the plausible explanation of these result that children and infants may have low resistance to infection, and because of relative short and straight Eustachian tube [19], the lower immune system of children compared to adults, and the fact that bacteria adhere better to epithelial cells of children than adults [20]. This result agrees with other study, done by Jayakar et al in 2014 [21] who proved that there were significant differences in the distribution of age in ear infection.

Fungal isolates from patients with otitis

Through this work it was found that fungi have a good role in otitis. The highest percentage of infection which caused by *Candida parapsiolosis* as 20 (10.0%), this agree with Al Husaini and Abu-serag in 2016 [22] who proved that *Candida parapsilosis* was the predominant species as (31.95%). *Trichophytone mentogrophte* consist of 4 (2.0%), then *Microsporum audouinii* as 2 (1.0%), followed by *Penicillium spp*. and *Candida glabrata* when the percentage of infection was 1 (0.5%). These results agree with Aremu and Alabi in 2011 [23] who found the most fungi caused CSOM was *Candida spp.* followed by *Aspergillus spp*. In case of acute otitis media, it was found that the most common fungi were *Candida spp*. followed by *Aspergillus spp*., this disagree with that obtained by Almamory and Kamal in 2014 [17] who proved the highest frequency of infection by *Aspergillus spp*. and the lowest percentage was due to *Alternaria spp*. When there were no any isolates of *Candida spp*. The plausible explanation of this phenomenon that humidity, low hygienic condition may have a role of developing these cases of infection. Regarding *Penicillium spp*. the percentage of infection was only 1 (0.5%) and this agree with Kiakojuri et al in 2015 [24] who found only one case of otitis caused by *Penicillium spp*. Also, it was found that each of

Trichophytone mentogrophte and *Microsporum audouinii* were responsible of AOM when there were no previous compatible studies to compare this result with. Ear self-cleaning is the most common predisposing factor for infection because this will lead to remove the protective ear wax [25].

Antifungal activity of ginger extract versus chloroform, methanol, and aqueous

Table (4), showed *Aspergillus spp*. it has been found that chloroform extract had the larger inhibition zones of mean of diameters as (6.00±7.12 mm) followed by aqueous extract with a mean of diameters of inhibition zones as (4.00±4.62 mm), while methanol extract showed no effect at all. This study disagrees with this by Ikegbunam et al in 2015 [26] who found that the aqueous extract of ginger had no effect on *Aspergillus spp*. and agree with Abd El-khalek et al in 2016 [27] who mentioned that ginger aqueous extract have an effect on *Aspergillus spp*., these incompatibilities in results may due the differences in the local prepared ginger, technique used into specific variation among the local isolates of *Aspergillus spp*. In *Candida parapsilosis*, chloroform extract was the strongest among the other when the mean of the diameter of inhibition zones was (11.14±5.05 mm), followed by methanol extract when the mean of diameter of inhibition zones was (7.57±4.08 mm), then aqueous extract of ginger as (4.86±4.51 mm). In *Candida glabrata*, chloroform extract was the strongest among the other when the mean of the diameter of inhibition zones was (12 mm), followed by methanol extract the mean of diameter of inhibition zones was (8 mm), then aqueous extract of ginger as (7 mm), these results disagree with those obtained by Jasim et al (2013) [28] who mentioned that aqueous extract of ginger have no effect on each of *Candida parapsilosis*, and on *Candida glabrata* at all. This discrepancy may due to the above reasons. The growth of *Microsporum audouinii* was inhibited by chloroform extract when the mean of diameter of inhibition zones as (22.50±16.26 mm), flowed by this obtained by aqueous extract of ginger as (12.50±0.71 mm), then by this obtained by methanol extract as (7.00±0 mm), this study with the first applying this agents against *Microsporum audouinii* as antifungal, the higher activity of the chloroform extract could be attributed to the presence of more phytochemicals than in this methanol extract. Chloroform extract was the only effective agent against *Trichophytone mentogrophte* when the mean of diameter of inhibition zone was (8.50±5.74 mm), while each of aqueous extract and methanol extract of ginger showed no effect at all and this may because the nature of the structure of hyphae of *Trichophytone mentogrophte* that may be resist to methanol and aqueous extract and sensitive to chloroform extract because chloroform extract has more phytochemicals. There is no such compatible study to compare these results with.

This study had concluded that the most frequent fungi isolated from patients with otitis it was *Candida parapsilosis*, followed by *Aspergillus spp*. The percentage of AOM is the highest among the other types of infection and the lower one is otitis externa. The age group ≤10 years old were the highest among other groups in developing otitis. Chloroform extract of ginger is the most effective as an antifungal followed by methanol extract, while aqueous extract to less evident of inhibition zones.

This study recommended that in vivo Study of ginger extracts to evaluate its less toxicity to be used as a drug of choice in otitis for human in future instead of antimycotics because it has a dual activity (antibacterial and *Microsporumaudouinii* antifungal) and because it is less toxic than chemicals.

Acknowledgments

The authors are grateful to all staff member of Medical Microbiology Department, College of Medicine, Al-Nahrain University for their help and cooperation. Special thanks for the staff member of ENT Department in Al-Imamein Al-Kadhimein Medical City for their kind assistance in samples collection.

Author contribution

Mohammed: Msc student, Dr. Al- Attraqchi: Supervision, Dr. Al-Hassani: Sample collection,

Dr. Sahib: consultation of the pharmaceutical part of research.

Conflict of interest

The author declares that they have no competing interests.

Funding

Self-finding.

References

1. Argaw-Denboba A, Abejew AA, Mekonnen AG. Antibiotic-resistant bacteria are major threats of otitis media in wollo area, northeastern Ethiopia: A ten-year retrospective analysis. Int J Microbiol. 2016; 2016: 8724671. doi: 10.1155/2016/8724671.
2. Mittal R, Grati M, Gerring R, et al. In vitro interaction of Pseudomonas aeruginosa with human middle ear epithelial cells. PLoS One. 2014; 9(3): e91885. doi: 10.1371/journal.pone.0091885.
3. Seid A, Deribe F, Ali K, Kibru G. Bacterial otitis media in all age group of patients seen at Dessie referral hospital, North East Ethiopia. Egypt J Ear, Nose, Throat Allied Sci [Internet] 2013; 14(2): 73-8. doi: https://doi.org/10.1016/j.ejenta.2013.02.005
4. Winn W, Allen S, Janda W, et al. Koneman's Color atlas and textbook of diagnostic microbiology. 6th ed. Philadelphia USA: Lippincot's Williams and Wilkins; 2006, p. 211-302, 439-40.
5. Poorey VK, Iyer A. Study of bacterial flora in CSOM and its clinical significance. Indian J Otolaryngol Head Neck Surg Vol 2002; 54(2): 91-5. doi: 10.1007/BF02968724.
6. Mösges R, M Nematian-Samani M, Eichel A. Treatment of acute otitis externa with ciprofloxacin otic 0.2% antibiotic ear solution. Ther Clin Risk Manag. 2011; 7: 325-36.
7. Schaefer P, Baugh RF. Acute otitis externa: an update. Am Fam Physician. 2012; 86(11): 1055-61.
8. Li HB, Tai XH, Sang YH, et al. Association between vitamin D and development of otitis media: A PRISMA-compliant meta-analysis and systematic review. Medicine (Baltimore). 2016 Oct; 95(40): e4739. doi: 10.1097/MD.0000000000004739.
9. Marom T, Marchisio P, Tamir SO, et al. Complementary and alternative medicine treatment options for otitis media a systematic review. Medicine (Baltimore). 2016; 95(6): e2695. doi: 10.1097/MD.0000000000002695.
10. Supreetha S, Mannur S, Simon SP, Jain J, Tikare S, Mahuli A. Antifungal activity of ginger extract on Candida Albicans : An In-vitro Study. J Dent Sci Res. 2011; 2(2): 18-21.
11. Benlafya K, El Farsaoui M, Chatouani S, et al. Antimicrobial potentials of aqueous and methanolic crude extracts of Zingiber officinale and Foeniculum vulgare. J Chem Pharm Res. 2015; 7(8): 964-6.
12. Riaz H, Begum A, Raza SA, et al. Antimicrobial property and phytochemical study of ginger found in local area of Punjab, Pakistan. Int Curr Pharm Journal, 2015; 4(7): 405-9. doi: https://doi.org/10.3329/icpj.v4i7.23591.
13. Hindi NKK, Al-Mahdi ZKA, Adil Z, Chabuck AG. Antibacterial activity of the aquatic extract of fresh, dry powder ginger, apple vineger extract of fresh ginger and crud oil of ginger (Zingber officinale) against different types of bacteria in Hilla city, IRAQ. Int J Pharm Pharm Sci. 2014; 6(5): 414-7.
14. Ogbonna AI, Tanko JS, Falemara BC, et al. Studies on the effects of Zingiber officinale Roscoe (Ginger) aqueous and ethanolic extracts on some fungal and bacterial species. IOSR J Pharm Biol Sci. 2014; 9(5): 16-23.
15. Mošovská S, Nováková D, Kaliňák M. Antioxidant activity of ginger extract and identification of its active components. Acta Chim Slovaca. 2016; 8(2): 115-9. doi: 10.1515/acs-2015-2020.
16. Muslim NS, Nassar ZD, Aisha AFA, et al. Anti-angiogenesis and antioxidant activity of ethanol extracts of *Pithecellobium jiringa*. BMC Compl Alternat Med. 2012; 12: 210. doi: https://doi.org/10.1186/1472-6882-12-210.
17. Almamory IAAS, Kamal SAA. Bacteria and fungi associated with acute otitis media. J Biol Agric Healthcare. 2014; 4(20): 41-6.
18. Khammas AH, Abbas AK. Isolation and identification of fungi associated with otomycosis. Iraqi J Comm Med. 2010; 3: 186-9.
19. Mansoor T, Musani MA, Khalid G, et al. Pseudomonas areugenosa in chronic suppurative otitis media: Sensitivity spectrum against various antibiotics. J Ayub Med Coll Abbottabad. 2009; 21(2): 120-3.
20. Osazuwa F, Osazuwa E, Osime C, et al. Etiologic agents of otitis media in Benin city, Nigeria. N Am J Med Sci. 2011; 3(2): 95-8. doi: 10.4297/najms.2011.395.
21. Jayakar R, Sanders J, Jones E. A study of acute otitis at Wellington Hospital, 2007–2011. Australas Med J. 2014; 7(10): 392-9. doi: 10.4066/AMJ.2014.2094.
22. Al Husaini IM, Abu-serag NA. Diagnostic study of otomycosis in Hilla city / Iraq. Int J PharmTech Res 2016; 9(9): 314-20.
23. Aremu SK, Alabi BS. Tuberculous otitis media: a case presentation and review of the literature. BMJ Case Rep. 2010; 2010: bcr0220102721. doi: 10.1136/bcr.02.2010.2721
24. Kiakojuri K, Rajabnia R, Jalili B, et al. Otomycosis in adolescent patients referred to the therapeutic centers in Babol City, Iran. Jundishapur J Microbiol. 2015; 8(5): e17138. doi: 10.5812/jjm.8(5)2015.17138.
25. Adegbiji WA, Aremu SK, Olatoke F, et al. Epidemiology of otitis Externa in developing country. Int J Recent Sci Res. 2017; 8(6): 18023-7. doi: http://dx.doi.org/10.24327/ijrsr.2017.0806.0437.
26. Ikegbunam M, Ukamaka M, Emmanuel O. Evaluation of the antifungal activity of aqueous and alcoholic extracts of six spices. Am J Plant Sci. 2016; 7(1): 118-25. doi: 10.4236/ajps.2016.71013.
27. Abd El-khalek AB, El-Sayed HS, Ibrahim GA, et al. Phenolic compounds, microbial content and sensory

evaluation of synbiotic labneh containing ginger and probiotic. Int J ChemTech Res. 2016; 9(2): 238-47.

28. Jasim A, Kumar Y, Benjamin J, et al. Studies on antifungal properties of some plant extracts (garlic, fenugreek, ginger) against of clinical isolate Candida species. Int J Sci Eng Technol Res. 2013; 2(19): 2180-5.

Correspondence to Maha M. Mohammed
E-mail: mahamicrobiology@yahoo.com
Received Jan. 23rd 2018
Accepted Dec. 12th 2018

Iraqi JMS

Published by Al-Nahrain College of Medicine
P-ISSN 1681-6579
E-ISSN 2224-4719
Email: iraqijms@colmed-alnahrain.edu.iq
http://www.colmed-alnahrain.edu.iq
http://www.iraqijms.net
Iraqi JMS 2019; Vol. 17(1)

Pediatric Hodgkin Lymphoma in Iraq-KRG-Sulaimani

Basil K. Abdallah[1] *MD*, **Nawshirwan G. Rashid**[2] *MD*, **Shwan A. Tawfiq**[2] *MD*

[1]Dept. of Pediatric Hematology and Oncology, Hiwa Hemato-Oncology Hospital, Iraq-KRG-Sulaimani, [2]Dept. of Clinical Hematology, Hiwa Hemato-Oncology Hospital, Iraq-KRG-Sulaimani

Abstract

Background Hodgkin lymphoma (HL) is a highly curable malignancy. It is a unique neoplasm, in which the malignant cell is the Reed-Stenberg cell (RSC); it constitutes approximately 40% of all lymphomas that present during childhood.

Objective To obtain local data on the pattern of childhood and adolescent HL in our community at presentation and then compare it with the international figure.

Methods Eighty-five patients with newly diagnosed pediatric HL were admitted to Hiwa Hematology/Oncology Hospital in Sulaimaniya province of Iraqi Kurdistan were included in this study, they were studied prospectively from March 2006 to March 2014. The patients staged by Ann Arbor Staging system and categories into three risk group depending on the Stage and Number of Nodal Sites and the presence of Bulky Disease. Data analyzed using SPSS software; version 13 and P-value obtained by Chi-square test.

Results The median age at diagnosis was about 13.7 years with peak age of incidence was between 15-18 year and with male predominance. Most common site of the primary tumor was cervical lymph nodes; nodular sclerosis was the most common histopathological subtype and the majorities were presented in stage IIA.

Conclusion The stage in this study was age dependent but neither there was relation between the site of the primary tumor and the risk categories nor relation between risk categories and sex.

Keywords Hodgkin lymphoma, stage, sex distribution, risk category

Citation Abdallah BK, Rashid NG, Tawfiq SA. Pediatric Hodgkin lymphoma in Iraq-KRG-Sulaimani. Iraqi JMS. 2019; 17(1): 12-17. doi: 10.22578/IJMS.17.1.3.

List of abbreviations: HL = Hodgkin lymphoma, LDHL = Lymphocytic depletion Hodgkin lymphoma, LRCHL = Lymphocytic rich cellularity Hodgkin lymphoma, MCHL = Mixed cellularity Hodgkin lymphoma, NLPHL = Nodular lymphocytic predominant Hodgkin lymphoma, NSHL = Nodular sclerosis Hodgkin lymphoma

Introduction

Formally known as Hodgkin's disease, Hodgkin lymphoma (HL) is a highly curable malignancy. It is a unique neoplasm in which the malignant cell, the Reed-Stenberg cell (RSC), represents only a small proportion of cells constituting the bulk of the tumor. It also has very particular clinical characteristics and distinct biological behavior. HL is a rather rare malignancy in the pediatric population; however, it constitutes approximately 40% of all lymphomas that present during childhood and is the most common malignancy in adolescents and young adults. In all age groups, HL is highly sensitive to chemotherapy and irradiation. In fact, HL was the first cancer to be cured with radiation therapy alone or with a combination of several chemotherapeutic agents. The cure rate for children and adolescents with HL has steadily improved over the years, particularly with the introduction of combined radiation and multiagent chemotherapy [(1)]. This therapeutic success has come at the price of serious long-

term toxicities, such that a 30-year survivor of HL is more likely to die of therapy-related complications rather than from HL. Therefore, the therapeutic paradigm has shifted toward reducing treatment-associated toxicity while maintaining high cure rates. This new paradigm has led to the current risk-adapted, response-based approach to the treatment of HL [2-4].

This study aimed to obtain local data on the pattern of childhood and adolescent Hodgkin lymphoma in our community at presentation and then to compare it with the international figure.

Methods

Eighty-five patients with newly diagnosed pediatric and adolescent HL were admitted to Hiwa Hematology/Oncology Hospital in the Sulaimaniya province of Kurdistan/ Iraq. They were studied prospectively from March 2006 to March 2014.

Inclusion criteria

1. All children and adolescents aged 18 years or younger.
2. Randomly collected regardless to the gender.
3. Histopathologically proved HL.
4. Newly diagnosed patients who were not treated previously by chemotherapy.

Exclusion criteria

1. Age more than 18 years.
2. Relapsed HL or previously treated with chemotherapy.

Study requirements

All the targeted patients had their Sulaimaniya facilities of hitopathological diagnosis, complete blood count (CBC), blood film, erythrocyte sedimentation rate (ESR), liver function test, serum alkaline phosphates, hepatitis B serology, hepatitis C serology, human immunodeficiency virus serology, renal function test, serum electrolyte, serum lactic dehydrogenase (LDH), serum ferritin level, abdominal ultrasound, chest X-ray, echocardiography, computerized tomography (CT)-scan and/or magnetic resonance imaging (MRI) of the primary site, chest and abdomen, bone marrow aspirate and trephine biopsy was done for those with one or more of the following criteria:

1. Patients with clinical stage III or stage IX.
2. Patients with B symptoms.
3. Patients with cytopenia on CBC.
4. Patients with elevated serum alkaline phosphates.

Positron emission tomography (PET) scan and Epstein-Barr (EB) virus Study were not done for the majority of the case because it was not available.

The patients were staged according to the Modified Ann Arbor Staging system [1,5,6].

Stage I

Involvement of a single lymph node region or lymphoid structure.

Stage II

Involvement of two or more lymph node regions or localized involvement of one extranodal site and one or lymph node regions, all on the same side of the diaphragm.

Stage III

Involvement of lymph node regions or structures on both sides of the diaphragm.

Stage IV

Diffuse or disseminated involvement of one or more extralymphatic organs, or isolated extralymphatic organ involvement without adjacent regional lymph node involvement, but with disease in distant site(s), or any involvement of the liver, bone marrow, pleura or cerebrospinal fluid (CSF).

According to their HL risk categories depending on the stage and number of nodal sites and the presence of bulky disease, favorable-risk pediatric HL (stage IA or IIA with < 3 nodal sites, and some IIIA without bulky disease), intermediate-risk disease (stage IIA bulky disease with extension or =3 nodal sites, stage IB, IIB, stage IIIA, stage IVA) and advanced or unfavorable pediatric HL (all other patients that were not included in the favorable or the intermediate risk groups).

Data analyzed using Statistical package for social sciences (SPSS) software; version 13 and P-value obtained by Chi-square test, P value less than 0.05 considered as significant.

Results

Among 85 patients with HL studied, 59 patients (69.41%) were males and 26 patients (30.58%) were females. The male:female ratio was 2.27:1.

A median age at diagnosis was about 13.7 years with peak age of incidence was between 15-18 years. Table 1 shows the age distribution.

Table 1. Age distribution of Hodgkin's lymphoma at diagnosis

Age in years	No. of patients	Percent (%)
0-4	4	4.7
5-9	17	20.0
10-14	25	29.4
15-18	39	45.9

The most common site of the primary tumor was the cervical lymph nodes, which were the primary site of the tumor in 48 out of 85 patients (56.47%), followed by isolated mediastinal primary which occurred in 13 patients (15.29%), axillary lymph nodes primary in 8 patients (9.41%), primary inguinal lymph nodes involvement occurred in 7 patients (8.23%), retroperitoneal lymph nodes primary in 5 patients (5.88%) and isolated splenic involvement occurred in 4 patients (4.7%).

Among the 85 patients with HL studied, 44 patients (51.76%) had nodular sclerosis HL (NSHL), 23 patients (27.05%) had mixed cellularity HL (MCHL), 12 patients (14.11%) had lymphocytic rich cellularity HL (LRCHL), 1 patient (1.17%) had Lymphocytic depletion HL (LDHL) and 5 patients (5.88%) were nodular lymphocytic predominant HL (NLPHL).

Regarding modified Ann Arbor Staging of the studied patients, 9 patients (10.59%) were in Stage I, three of them (3.53%) with B symptoms, 37 patients (43.53%) were in Stage II, 12 of them (14.12%) with B symptoms, 31 patients (36.47%) were in Stage III, 9 of them (10.59%) with Stage III B and 8 of patients (9.41%) were in Stage IV, three of them with Stage IV B.

According to HL risk categories that depend on the stage and number of nodal sites and the presence of bulky disease, favorable-risk pediatric Hodgkin lymphoma (stage IA or IIA with < 3 nodal sites, and some IIIA without bulky disease) were occurred in 31 patients (36.47%), intermediate-risk disease (stage IIA bulky disease with extension or = 3 nodal sites, stage IB, IIB, stage IIIA, stage IVA) occurred in 42 patients (49.41%) and advanced or unfavorable pediatric Hodgkin lymphoma that included all other patients that who were not included in the favorable or the intermediate risk groups) occurred in 12 patients (14.12%).

This study showed that there is significant correlation between age and risk categories so that favorable risk category occurred more in younger age patients and high-risk category found in older children and adolescent (Table 2).

This study found that there was statistic significant correlation (P-value = 0.03408) between age and sex as there was male predominance in young patients less than 10 years, while male to female ratio tend to be decrease with increasing age.

Table 3 shows age in relation to the sex (P-value = 0.03408).

Also, there was significant correlation between Age and Histopathological subtypes of HL, showing that MCHL found to be more common among children less than 10 years old, whereas NSHL found more in patients above 10 years old (P-Value = 0.05).

This study showed that there was significant statistic relation between histopathological

subtypes and risk categories, showing that larger number of LDHL and NSHL fall in high risk group, whereas larger number of LRCHL and NLPHL fall in the favorable risk group (Table 4).
There was no significant statistic relation between the site of the primary tumor and the risk categories in the current study (P-Value = 0.78195).
Also, there was no significant statistic relation between risk categories and sex (P-Value = 0.66731).

Table 2. Relation between age and the risk categories

Age in Years	Risk Categories Number/(Percentage)		
	Favorable Risk Group	Intermediate Risk Group	High Risk Group
0-4	2 (50.0%)	1 (25.0%)	1 (25.0%)
5-9	12 (70.59%)	4 (23.53%)	1 (5.88%)
10-14	9 (36.0%)	13 (52.0%)	3 (12.0%)
15-18	8 (20.51%)	24 (61.54%)	7 (17.95%)

Chi-square test P-value = 0.02997

Table 3. Age in relation to the sex

Age in years	Male No. / total (%)	Female No. / total (%)
0-4	3/4 (75.0%)	1/4 (25.0%)
5-9	15/17 (88.24%)	2/17 (23.53%)
10-14	20/25 (80.0%)	5/25 (20.0%)
15-18	21/39 (53.85%)	18/39 (46.15%)

Chi-square test P-value = 0.03408

Table 4. The relation of histological subtype and risk categories risk categories NSHL

Risk Categories	NSHL No./Total (%)	MCHL No./Total (%)	LRCHL No./Total (%)	LDHL No./Total (%)	NLPHL No./Total (%)
Favorable Risk Group	17/31 (54.84%)	2/31 (6.45%)	9/31 (29.0%)	0/27 (0.0%)	2/31 (6.45%)
Intermediate Risk Group	21/42 (50.0%)	15/42 (35.71%)	3/42 (7.14%)	0/42 (0.0%)	3/42 (7.14%)
High Risk Group	6/12 (50.0%)	5/12 (41.7%)	0/12 (0.0%)	1/12 (8.3%)	0/12 (0.0%)

Chi-square test P-value = 0.00827

Discussion

Pediatric and adolescent HL cancer continues to be the leading cause of death in children younger than 15 years old, and lymphomas are among the most common cancers seen in children. Fortunately, survival rates for childhood cancers have increased significantly over the years. Children respond to and deal with chemotherapy better than adults. Today, 96% of children diagnosed with Hodgkin's disease will survive 5 or more years [7]. However;

those with high-risk disease continue to have poor outcomes.

The total number of cases studied was eighty-five, over a period of eight years. It found that males were affected more than females with The male:female ratio of 2.27:1; also our study found that there was significant correlation between age and sex as there was more male predominance in young patients less than 10 years, while male to female ratio tend to be decrease with increasing age, this figure is similar to male: female ratio in other studies which showed that children younger than 5 years show a strong male predominance (M:F = 5:3) and children aged 15 to 19 years show a slight female predominance (M:F = 0.8) [3,7].

Peak age incidence at presentation in present study was 15-18 years which was represent 45.9% of cases, In the USA, the incidence of HL is age-related and is highest among adolescents aged 15 to 19 years (29 cases per million per year), whereas children ages 10 to 14 years, 5 to 9 years, and 0 to 4 years having approximately threefold, eightfold, and 30-fold lower rates, respectively. In non-European Union countries, there is a similar rate in young adults but a much higher incidence in childhood [7].

In the current study the median age at diagnosis was about 13.7 years which was lower than that showed by study done in USA (median age: 15.6) [8,9].

This younger median age at diagnosis in the present study might be due to the high incidence of EB virus infection in earlier age in our region; however, this concept should be confirmed by studying the EB virus genomes in the HL cells.

This study had concluded that pediatric HL was higher in male than female in our community. A median age at diagnosis was about 13.7 years with peak age of incidence between 15-18 years and the majority of the patients presented with cervical lymph nodes primary at the time of diagnosis. Nodular sclerosis histopathology is the most common.

Most of the patients had stage II with Intermediate risk category.

There was strong correlation between age and advanced stage which meant that stage is age dependent and significant correlation between age and sex as there was more male predominance in young patients less than 10 years. Also, there was significant correlation between age and histopathology of HL in our study with mixed cellularity found to be more common among children less than 10 years old. Neither there was relation between the site of the primary tumor and the risk categories nor relation between risk categories and sex.

Despite absence of PET scan for the majority of patients for proposed risk stratification system for purpose of risk categories, significant number of our patients got intermediate and high-risk category.

Acknowledgments

None.

Author contribution

Dr. Abdallah: conducted obtaining consent, data collection, statistical analysis, and writing the manuscript. Dr. Rashid and Dr. Tawfiq: data collection, editing, and finalizing the writing of the study.

Conflict of interest

Authors declare no conflict of interest.

Funding

There is no funding source for this research.

References

1. Arya LS, Dinand V. Current strategies in the treatment of childhood Hodgkins disease. Indian Pediatr. 2005; 42(11): 1115-28.
2. Wikipedia - Hodgkin's lymphoma. URL: https://en.wikipedia.org/wiki/Hodgkin%27s_lymphoma. Accessed 21-6-2008.
3. Percy CL, Smith MA, Linet M, et al. Lymphomas and Reticuloendothelial Neoplasms. In: SEER Pediatric Monograph. Cancer incidence and survival among children and adolescents: united states SEER Program 1975-1995. National Cancer Institute. 1999.
4. Staal SP, Ambinder R, Beschorner WE, et al. A survey of Epstein-Barr virus DNA in lymphoid tissue. Frequent detection in Hodgkin's disease. Am J Clin Pathol. 1989; 91(1): 1-5.
5. Hjalgrim H, Askling J, Rostgaard K, et al. Characteristics of Hodgkin's lymphoma after infectious mononucleosis. N Engl J Med. 2003; 349(14): 1324-32. doi: 10.1056/NEJMoa023141.
6. Weiss LM, Chen YY, Liu XF, et al. Epstein-Barr virus and Hodgkin's disease. A correlative in situ hybridization

and polymerase chain reaction study. Am J Pathol. 1991; 139(6): 1259-65.
7. Ries LA, Kosary CL, Hankey BF, et al., eds.. SEER Cancer Statistics Review 1973-1995. Bethesda, Md: National Cancer Institute, 1998.
8. Macfarlane GJ, Evstifeeva T, Boyle P, et al. International patterns in the occurrence of Hodgkin's disease in children and young adult males. Int J Cancer. 1995; 61(2): 165-9.
9. Best T, Li D, Andrew D Skol AD, et al. Variants at 6q21 implicate PRDM1 in the etiology of therapy-induced second malignancies after Hodgkin's lymphoma Nat Med. 2011; 17(8): 941-3. doi: 10.1038/nm.2407.

Correspondence to Dr. Basil K. Abdallah
E-mail: basilonc@yahoo.com
Received Jan. 23rd 2018
Accepted Feb. 27th 2019

Iraqi JMS

Published by Al-Nahrain College of Medicine
P-ISSN 1681-6579
E-ISSN 2224-4719
Email: iraqijms@colmed-alnahrain.edu.iq
http://www.colmed-alnahrain.edu.iq
http://www.iraqijms.net
Iraqi JMS 2019; Vol. 17(1)

Incidence of Neurotrophic Keratopathy in Association with Diode Endolaser Retinal Photocoagulation

Najah k. M. Al-Quraishi[1] *FICS, H Dip Laser (Ophth)*, **Abeer A. Mohammad**[2] *FICS,* **Hind A. Mahdi**[2] *FICS*

[1]Chairman of Iraqi Board in Ophthalmology, Baghdad, Iraq, [2]Dept. of Surgery, Medical College, Al-Nahrain University, Baghdad, Iraq

Abstract

Background Neurotrophic keratopathy as a consequence of laser retinal photocoagulation is not uncommon problem but unfortunately and unfortunately it is not well evaluated in Iraq as well as the world.

Objective To estimate the incidence of neurotrophic keratophathy in association with diode endolaser retinal photocoagulation.

Methods This cross-sectional descriptive study was conducted in vitreoretinal clinic at Ibn-Alhaithum Teaching Eye Hospital in Baghdad. Patients were selected during a period from April 2013 to January 2014, all patients were examined by slit- lamp microscope, and condensing lens + 78 Diopter for dilated fundus examination and cotton wisp to assess corneal sensation. Positive cases documented by anterior segment photograph.

Results The study sample consisted of 108 vitrectomizd eyes of 108 patients, their ages ranged between 5-65 years old with mean of (35.5 years), 42 of them were females and 66 of them were males. By follow up of those patients; 13 of their eyes (12%) developed neurotrophic keratopathy. It had been found that 61.5% of those eyes manifested as stage II, while 30.8% presented as stage I and only 7.7% presented as stage III.

Conclusion Diode endolaser retinal photocoagulation may be an unrecognized reason for neurotrophic keratopathy

Keywords neurotrophic keratophathy, diode endolaser retinal photocoagulation, pars plana vitrectomy, diode laser 810 nm

Citation Al-Quraishi NKM, Mohammad AA, Mahdi HA. Incidence of neurotrophic keratopathy in association with diode endolaser retinal photocoagulation. Iraqi JMS. 2019; 17(1): 18-23. doi: 10.22578/IJMS.17.1.4

List of abbreviations: BIOM = Binocular indirect ophthalmo microscope, Ca=Calcium CO_2 = Carbonic dioxide, MMPs = Matrix metalloproteinases, PPV=Pars plana vitrectomy, SDM=Sub visible diode micro pulse, Zn = Zinc

Introduction

The cornea is the principal refractive surface of the human eye and along with the sclera forms the outermost coat of the eyeball. It constitutes up to one-sixth of the entire eyeball. The corneal epithelium is derived from the surface ectoderm and the mesoderm gives rise to Bowman's layer, stroma, Descemet's membrane and endothelium. Tear film keeps the corneal surface moist and prevents the adherence of microbes. It also has many biologically active substances such as histamines, interleukins, prostaglandins and growth factors. Some of these factors modulate corneal epithelial migration, proliferation and differentiation [(1)].

The cornea is primarily supplied by the sensory nerves derived from the ciliary nerves of the

ophthalmic branch of the trigeminal nerve. The long ciliary nerves supply the perilimbal nerve ring. Nerve fibers penetrate the cornea in the deep peripheral stroma radially and then course anteriorly forming a terminal sub epithelial plexus. Presence of corneal sensation is vital to the maintenance of the integrity of the cornea. In cases of herpes simplex, herpes zoster and diabetes, corneal sensations are diminished and this may lead to persistent epithelial defects or delayed epithelial wound healing [1,2].

The diode lasers emit a wavelength of 810 nm infrared in continuous wave mode. In the eye, diode laser light is absorbed only by melanin and consequently is most commonly used for retinal photocoagulation. Low scattering of this wavelength ensures good penetration of the ocular media and of edematous retina. The 810 nm wavelength also penetrates the sclera [3]. Common clinical protocols use intra-operatively visible endpoints that cause iatrogenic chorioretinal damage. For this reason, laser therapy is normally limited to levels of disease severity for which the benefit-to-risk ratio justifies its application. The use of 810 nm diode laser in the Micro Pulse mode offers the surgeon the possibility to minimize iatrogenic retinal damage [4].

Pars plana vitrectomy (PPV)

Small instruments, inserted through the pars plana, are used to cut and remove vitreous, peel membranes, and laser photocoagulate providing treatment to various posterior segment pathologies [5].

Neurotrophic keratitis

Neurotrophic keratitis is a degenerative disease of corneal epithelium characterized by impaired healing. Absence of corneal sensitivity is the hallmark of the condition, which may end in corneal stromal melting and perforation. The causes of decreased corneal sensation are myriad and may affect sensory nerve supply from the trigeminal nucleus to the corneal nerve endings. Reduced corneal sensation renders the corneal surface prone to occult injury and decreases reflex tearing; it also appears to decrease healing rates of corneal epithelial injuries. Vulnerability and poor healing secondary to corneal sensory denervation favor the formation of nonhealing epithelial defects that tend to ulcerate and ultimately perforate if not appropriately treated in a timely fashion [6].

Clinical stages of neurotrophic keratitis

Stage I: punctate epithelial staining with fluorescein

Stage II: Acute loss of epithelium

Stage III: Stromal lysis

This study was conducted to estimate the incidence of neurotrophic keratophathy in association with diode endolaser retinal photocoagulation.

Methods

This cross-sectional descriptive study was conducted in Ibn-Alhaithum Teaching Hospital, the Vitreoretinal Clinic, from April 2013 to January 2014. A one hundred eight sample of patients received diode laser retinal photocoagulation during pars plana vitrectomy done by three vitreoretinal surgeons. The patients who were enrolled in this study include all vitrectomizd patients who were attending the vitreoretinal clinic of the three vitreoretinal surgeons.

Exclusion criteria

Patients with diabetes, corneal surgery, herpetic eye disease, chronic glaucoma medication, patients with pars plana vitrectomy but without endolaser and age above 65 years (old age group).

Collection of data

Data were collected using a pre-constructed data collection form, which was formulated for purpose of this study and it was validated by the supervisor. The data collected included:

1. Name
2. Age
3. Gender
4. History of diabetes
5. Previous corneal surgery

6. History of herpetic infection
7. History of chronic drug use (especially glaucoma medication)
8. History of contact lens use
9. Duration of operation
10. Scraping of corneal epithelium
11. Pattern of laser (localized or 360 degree)
12. Visual acuity
13. Treatment

The followings were taken from the case sheet of the patients:
Name of senior
Reference number
Date of operation

All patients were examined using slit- lamp microscope, condensing lens +78 diopter for dilated fundus examination and cotton wisp to assess corneal sensation. Positive cases documented by anterior segment photograph.

Ethical issue

This research has been approved by scientific council of ophthalmology.
Verbal consent has been obtained from the patients.

Statistical analysis

Statistical analysis was conducted by using Excel Microsoft Office 2013.

Results

The study sample consisted of 108 vitrectomizd eyes of 108 patients, their ages ranged between 5-65 years old with mean of (35.5 years), 42 of them were related to females and 66 were related to males as shown in table 1.

Table 1. Baseline characteristics of study group

Gender	Number	Percentage
Female	42	38.8
Male	66	61.2
Total	108	100

The higher incidence of neurotrophic keratopathy occurs among age group range from 25years to less than 35 years as shown in table 2.
By follow up of those patients; 13 of their eyes (12%) develops neurotrophic keratopathy as shown in figure 1.

It had been found that 61.5% of those eyes manifested as stage II, while 30.8% presented as stage I and only 7.7% presented as stage III as shown in table 3.

Table 2. The incidence of neurotrophic keratopathy among different age groups

Age (years)	Frequency	Percentage
5-14	2	15.39
15-24	1	7.69
25-34	5	38.47
35-44	2	15.39
45-54	2	15.39
55-64	1	7.69

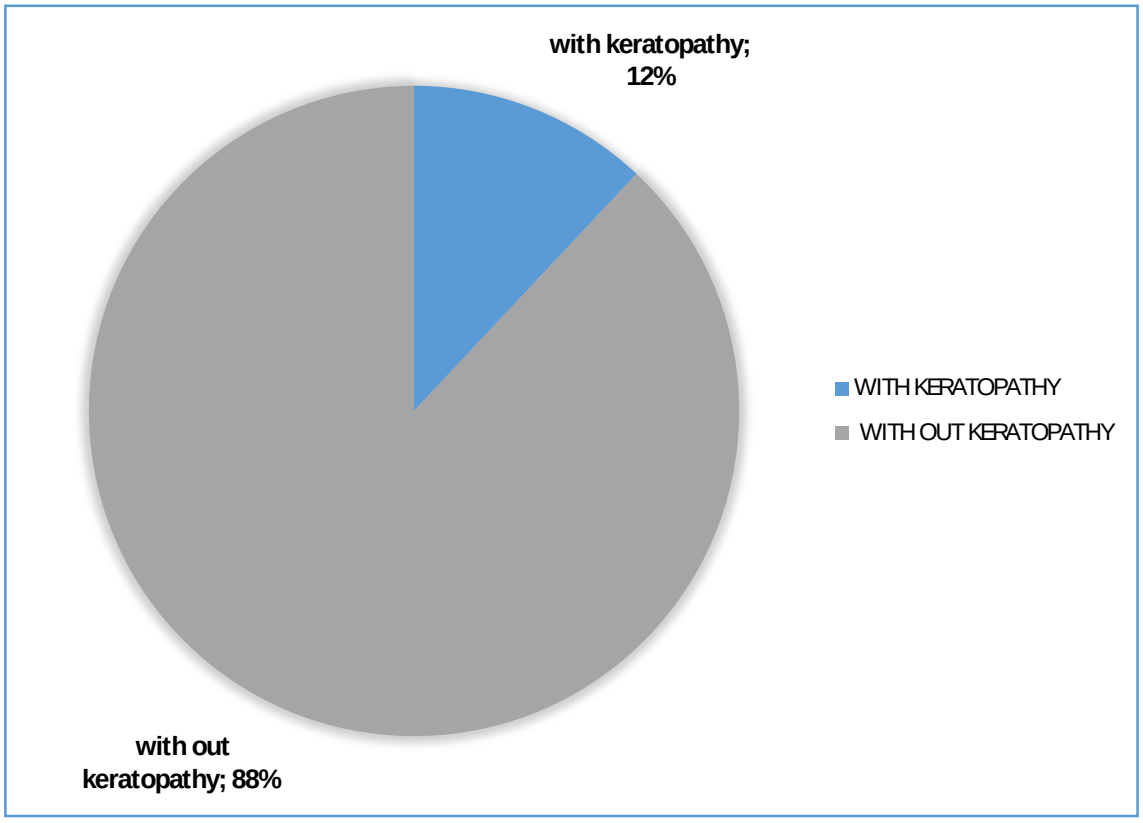

Figure 1. Incidence of neurotrophic keratopathy in association with diode endolaser retinal photocoagulation

Table 3. Frequency distribution and percentage of clinical stage

Clinical stage	Number of eyes	Percentage
Stage 1 *	4	30.8
Stage 2 **	8	61.5
Stage 3 ***	1	7.7
Total	13	100

* stage I: Punctate epithelial staining with fluorescein
** stage II: Epithelial defect
*** stage III: Stromal lysis, sometimes resulting in corneal perforation

Discussion

Neurotrophic keratitis is a degenerative disease of corneal epithelium characterized by impaired healing. Absence of corneal sensitivity is the hallmark of the condition. The causes of decreased corneal sensation are myriad and may affect sensory nerve supply from the trigeminal nucleus to the corneal nerve endings [7]. One of the iatrogenic causes of neurotrophic keratophathy is trauma to ciliary nerves by laser [1,6,8].

The diode lasers emit a wavelength of 810 nm infrared in continuous wave mode. Low scattering of this wavelength ensures good penetration of the ocular media and of edematous retina. The 810 nm wavelength also

penetrates the sclera [4]. It is of high power and deeper penetration into the choroids [9]. Sub visible diode micro pulse (SDM) is applied for non-damaging retinal phototherapy by using shorter bursts with small spot size (100-200 micrometer) [10].

In the present study, 108 patients; who had undergone pars plana vitrectomy with diode endolaser retinal photocoagulation; 13 (12%) of them developed neurotrophic keratopathy. Other causes of neurotrophic keratopathy were excluded to illustrate the association with diode endolaser retinal photocoagulation. Laser retinal photocoagulation well known to cause this condition and the diode laser specifically has physical ability to be absorbed by the fundus to somewhat unpredictable way [10]. Diode laser pan retinal photocoagulation associated with neurotrophic keratopathy especially while using it in high power [11].

Unfortunately, there are no studies similar to this study to compare with. Recently, two studies were published that support this study. Banerjee et al report a series of 5 cases of patients without diabetes who developed neuropathic corneal ulceration presumed secondary to long ciliary nerve compromise following vitrectomy surgery with endolaser and silicone oil tamponade for retinal detachment [12]. Doyle et al found that pars plana vitrectomy with pan retinal photocoagulation may be an unrecognized cause of neurotrophic keratopathy [13].

So, the question that could be answered is that the diode endolaser retinal photocoagulation while used in continuous mode may be the cause of neurotrophic keratopathy.

The limitations of the study are:

- Small sample size
- The examination of corneal sensation was not done by esthesiometer as it is not available which is more reliable and give quantitative results.
- There was no documentation in the case sheet of all patients for epithelial scraping which is needed in some patients intraoperatively, so it can't be correlated as risk factor for neurotrophic keratopathy.
- Also the number of laser shoots and the power used not documented in the case sheets.
- Follow up period is not the same for all patients.

This study concluded that diode endolaser retinal photocoagulation may be an unrecognized reason for neurotrophic keratopathy.

The recommendations of this study are:

- Studying of a larger sample size
- Studying of two groups, one with epithelial scraping and other without.
- Studying of two groups, one treated with diode laser 810 nm and the other group should be treated with less penetrating photocoagulater laser e.g. Nd:YAG laser 530 nm.
- Also using high power density laser blamed for appearance of complications, so make sure to use as minimum as possible power density especially at 3.00 and 9.00 o'clock of the retinal periphery.
- SDM is showing promise as it is applied for non-damaging retinal phototherapy by using shorter bursts with small spot size (100-200 micrometer).
- Documentation of epithelial scraping and number of laser shoots and the power used in the case sheets.

Acknowledgments

The author would like to express his gratitude to his supervisor Assistant Prof. Dr. Najah Al-Quraishi for his great assistance, kind advice & scientific guidance. Also, to thank Assistant Prof. Dr. Faiz Al-shakarchi for his kind support, Dr. Ali Al-Fazaa for his help in data collection. Also, the author is grateful to the staff of statistics unit of the Ibn AL Haitham teaching eye hospital.

Author contribution

All authors have contributed equally to this article.

Conflict of interest

The authors declare no conflict of interest.

Funding

None.

References

1. Sharma N, Vajpayee RB. Corneal ulcers diagnosis and management. New Delhi: Jaypee Brothers Medical Publishers Ltd; 2008. p. 3-5, 16, 154-61.
2. Remington LA, Goodwin D. Clinical anatomy and physiology of the visual system. 3rd ed. Missouri: Elsevier Butter worth Heinemann; 2012. p. 219.
3. Elkington AR, Frank HJ, Greaney MJ. Clinical optics. 3rd ed. UK: Blackwell Science; 1999. p. 221-4.
4. Dorin G. Subthreshold and micropulse diode laser photocoagulation. Sem Ophthalmol. 2003; 18(3), 147-53. doi: https://doi.org/10.1076/soph.18.3.147.29812.
5. Bernardino CR. Yale guide to ophthalmic surgery. Lippincott Williams & Wilkins; 2011. p. 153-4.
6. Krachmer JH, Mannis MJ, Holland EJ. Cornea fundamental, diagnosis and manegment. 3rd ed. MOSBY Elsevier; 2011. p. 90.
7. Bhagat N, Zarbin MA. Subthreshold micropulse diode laser for DME. Retinal Physician. 2011 (accessed 3 May 2014).
8. Davis EA, Dohlman CH. Neurotrophic Keratitis. Int Ophthalmol Clin. 2001; 41(1): 1-11.
9. Bhattacharyya B. Step by step laser in ophthalmology. New Delhi: Jaypee Brothers Medical Publishers Ltd; 2009. p. 8.
10. Garge A, Lin JT, Aloi JL, et al. Mastering the technique of LASER application in ophthalmology. New Delhi: Jaypee Brothers Medical Publishers; 2008. p. 476, 744.
11. Bandello F, Brancato R, Trabucchi G, et al. Diode versus argon-green laser panretinal photocoagulation in proliferative diabetic retinopathy: a randomized study in 44 eyes with a long follow-up time. Graefes Arch Clin Exp Ophthalmol. 1993; 231(9): 491-4. doi: 10.1007/BF00921112.
12. Banerjee PJ, Chandra A, Sullivan PM, et al. Neurotrophic corneal ulceration after retinal detachment surgery with retinectomy and endolaser: a case series. JAMA Ophthalmol. 2014; 132(6): 750-2. doi: 10.1001/jamaophthalmol.2014.280.
13. Doyle JI, Hall T, Michelson MA. Neurotrophic keratopathy following pars plana vitrectomy and indirect ophthalmoscopic laser. ASCRS.ASOA Boston Symposium and Congress; 2014.

Correspondence to Dr. Hind A. Mahdi
E-mail: H.daffodel@gmail.com
aberamir@colmed-alnahrain.edu.iq
Received Jun. 6th 2018
Accepted Dec. 18th 2018

Iraqi JMS

Published by Al-Nahrain College of Medicine
P-ISSN 1681-6579
E-ISSN 2224-4719
Email: iraqijms@colmed-alnahrain.edu.iq
http://www.colmed-alnahrain.edu.iq
http://www.iraqijms.net
Iraqi JMS 2019; Vol. 17(1)

Risk Factors of Chronic Kidney Disease among Patients Attending Ibn Sina Teaching Hospital in Mosul City

Fanar F. Khaleel[1] *FABHS/ Family Medicine*, **Shaima S. Hussain**[2] *FICSM/Family Medicine*, **Azzam H. Hmood**[3] *FICSM/Family Medicine*

[1]University Primary Health Care Center, Mosul, Iraq, [2]Al-Qudis Primary Health Care Center, Mosul, Iraq, [3]Ibn-Sina Teaching Hospital, Mosul, Iraq

Abstract

Background Chronic kidney disease (CKD) is a long-term health condition that in many cases is preventable. Many people do not know they have kidney disease, because up to 90% of kidney function can be lost before symptoms are evident. Fortunately, simple tests performed by a general practitioner can identify most cases of CKD when the disease is in its early stages, enabling treatment to prevent or slow progress.

Objective To evaluate the risk factors for the development of CKD.

Methods A case-control study conducted to 300 persons (150 cases and 150 controls) selected from Medical Wards, Dialysis Kidney Unit and in Outpatient Clinic at Ibn Sina Teaching Hospital in Mosul City.

Results Diabetes mellitus (DM) had 12.9 folds risk for developing of CKD and both type 1 and type 2 D.M. had significant role for developing CKD. Diabetic patients for (> 15 years) had 29.8 times risk for development of CKD. Hypertension (HT) had 3.5 folds risk for CKD and those having HT more than 15 years had 12.9 times risk for CKD. Cardiovascular disease (CVD) had 13.6 folds risk for CKD and having more than one type of CVD had 37 times risk for CKD than heart failure, ischemic heart disease. Family history of renal diseases had 7.1 times risk for CKD and there was statistically significant role of having family history of CKD that had more than 22 times risk for CKD. Statistically significant role of having proteinuria with 383 times risk for CKD.

Conclusion There are many risk factors significantly contributing to the development of CKD in Mosul especially DM, HT, CVD, family history of renal disease, family history of chronic diseases, and from this study it has been concluded that proteinuria plays a major role for the development and progress of CKD.

Keywords Chronic kidney disease, risk factors

Citation Khaleel FF, Hussain SS, Hmood AH. Risk factors of chronic kidney disease among patients attending Ibn Sina Teaching Hospital in Mosul City. Iraqi JMS. 2019; 17(1): 24-31. doi: 10.22578/IJMS.17.1.5

List of abbreviations: ACEI = Angiotensin converting enzyme inhibitor, AKI = Acute kidney injury, ARB = Angiotensin receptor blocker, BPH = Benign prostatic hyperplasia, CAD = Coronary artery disease, CKD = Chronic kidney disease, NSAID = Non-steroidal anti-inflammatory drugs, RA = Rheumatoid arthritis, SLE = Systemic lupus erythematosus, UTI = Urinary tract infection

Introduction

Chronic kidney disease (CKD) refers to all conditions of the kidney, lasting more than three months, where a person has had evidence of kidney damage and/or reduced kidney function. Evidence of kidney damage manifests as either proteinuria or albuminuria, hematuria or scarring detected by imaging tests [1,2] (Table 1).

KDOQI guidelines have classified CKD into five stages (Table 2).

Table 1. Criteria for definition of chronic kidney disease [3]

CKD is defined as abnormalities of kidney structure or function, present for more than 3 months, with implications for health. These may include the following:	
Markers of kidney damage	❖ Albuminuria (Alb. Excretion Rate ≥ 30 mg/24 h; Alb. To Creatinine Ratio ≥ 30 mg/g (≥ 3 mg/mmol]) ❖ Urine sediment abnormalities (Pyuria, haematuria, cast) ❖ Electrolyte and other abnormalities caused by tubular disorders ❖ Abnormalities detected through histology ❖ Structural abnormalities detected through imaging ❖ History of kidney transplantation
Decreased GFR (Glomerular Filtration Rate)	GFR <60 ml/min/1.73 m^2

Table 2. Stages of chronic kidney disease based on estimated glomerular filtration rate [3,4]

eGFR (mL/min/1.73m^2)	**Description**	**Clinical Action Plan**
90	Stage 1 CKD - Kidney damage with normal kidney function	Establish etiology of CKD Diagnose and treat cardio vascular disease (CVD) risk factors and comorbid conditions
60-89	Stage 2 CKD - Kidney damage with mild decrease in kidney function	Estimate CKD progression rate Diagnose and treat CVD risk factors and comorbid conditions
45 - 59	Stage 3a CKD - mild-moderate decrease in kidney function	As above, plus: Kidney imaging study, e.g., US or CT Consider nephrology consultation
30-44	Stage 3b CKD - moderate-severe decrease in kidney function	As above plus: Refer patients with diabetes to nephrology
15 - 29	Stage 4 CKD - severe decrease in kidney function	Nephrology consultation with transition of management and care Initiate decisions regarding kidney replacement therapy, vascular access, and kidney transplant Diagnose and treat CVD risk factors and comorbid conditions Adjust drug dosing for CKD stage
< 15	Stage 5 CKD - ESKD	As above plus: Referral to a nephrologist

The risk factors for progression to CKD are divided into non modifiable & modifiable factors.

A- Non modifiable risk factors:

- Age (older age)
- Gender (generally worse in males)
- Race or Ethnicity (non-Caucasian)
- Genetics like adult polycystic kidney disease
- Family history of renal disease
- Family history of chronic disease (Diabetes mellitus (DM), hypertenstion (HT), dyslipidemia & heart disease)

B- Modifiable risk factors:

- Obesity or metabolic syndrome
- Smoking
- Residence
- Low socioeconomic status & low levels of education
- Diabetes mellitus
- Systemic hypertension
- Cardiovascular disease
- Dyslipidemia
- Nephrotoxic agents: NSAIDs, analgesics, aminoglycosides, ACEI, and radiographic contrast media.
- Autoimmune disease: SLE, RA, vasculitis
- Previous episode of acute renal failure
- Proteinuria
- Abnormal urinary sediment
- Structural abnormalities of the urinary tract
- History of obstructive uropathy (renal stone, BPH, malignancy)
- History of recurrent UTI and pyelonephritis

The progression of CKD is demonstrated in the figure 1.

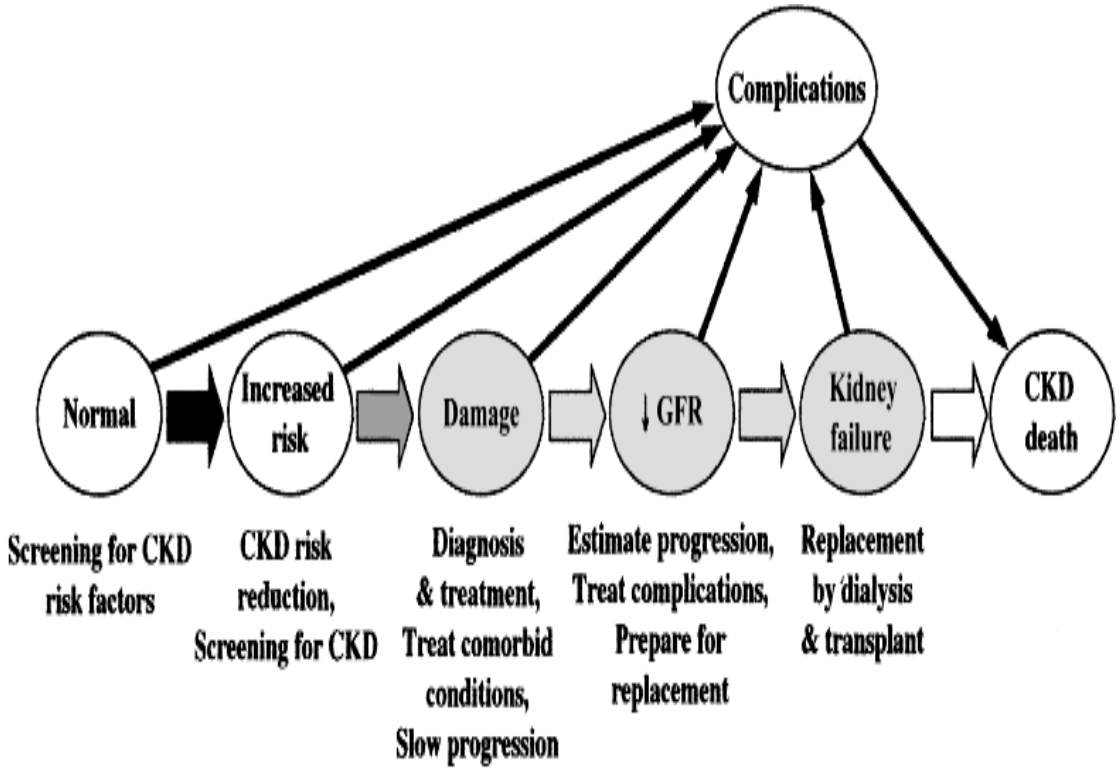

Figure 1. Stages in progression of chronic kidney disease and therapeutic strategies [5]

This study aimed to assess the risk factors that contribute to the development & progression of CKD.

Methods

Settings & study design

The present study was conducted in the Medical Wards and Dialysis Kidney Unit at Ibn Sina Teaching Hospital in Mosul city. This hospital is located in the right bank of Tigris River and it delivers services to many patients in Mosul city. This hospital has 3 medical wards for male and female adults' patients, each medical ward has 66 beds, also include dialysis kidney unit.

A Case control design was adopted to 300 persons (150 cases and 150 controls) in order to achieve the objective of the present study.

Ethical consideration

An official agreement was obtained from the Ministry of Health and Directorate of Health in Mosul before conduction of the study. A verbal consent was taken from all the cases and controls included in the study.

Case and control definition

Case: patient, aged > 18 years old, admitted to the Medical Wards or Dialysis Kidney Unit at Ibn Sina Teaching Hospital who is a known case of CKD diagnosed by his specialist.

Control: patient, aged > 18 years old, admitted to the Medical Wards at Ibn Sina Teaching Hospital & proved that he is not having CKD by estimation of GFR.

Both cases and controls were randomly collected (in a lottery method) during the study period (from 1st of February 2016 to 31st of July 2016).

Inclusion criteria

Those patients with CKD & aged > 18 years old.

Exclusion criteria

Those with present AKI (Acute kidney Injury).

Retrieving data

The main source of data was obtained directly from the cases and controls by the investigator through direct interview with the patients, from their case sheets & from the laboratory results (blood & urine) of each case or control and filling the questionnaire form which was prepared to record all relevant information related to cases and controls in the study sample.

Procedure:

The questionnaire form was specially prepared in order to collect all the relevant information related to the study sample. The questionnaire form included information in regard to:

Patient sociodemographic factors, previous history of DM, previous history of HT, previous history of CVD, family history of renal diseases, GUE (general urine examination), blood Urea, serum creatinine, serum total cholesterol, eGFR (estimated GFR).

(This questionnaire information was gathered from multiple recent resources including comprehensive clinical nephrology 2015, Harrison nephrology & recent researches about CKD).

Analysis of data

Data were entered to a Word-Excel 2010 worksheet and statistical analysis of data was carried out by using Pentium five computer through the use of (SYSTAT 12) statistical software.

Results

Table 3 shows that diabetes mellitus has 12.9 risk for developing of CKD and that type 2 has 12.4 risk and more than 15 years of diabetes has 30 risks for developing of CKD.

Table 3. Distribution of the cases and controls according to diabetes mellitus

DM	Case		Control		O.R.	P-Value	95% of C.I.
	No.	%	No.	%			
Yes	58	38.67	7	4.0	12.879	0.000	5.733 – 28.857
No	92	61.33	143	96.0			
Total	150	100	150	100			
Types of DM							
Type 1	7	12.9	1	14.29	7.294	0.032	1.150 – 45.872
Type 2	51	87.93	6	85.71	12.364	0.000	5.222– 29.187
Total	58	100	7	100			
Duration of DM							
< 10 years	9	15.52	4	57.14	2.330	0.156	0.742 – 7.292
10-15 years	24	41.38	2	28.57	14.095	0.000	3.611 – 54.726
>15 years	25	43.10	1	14.29	29.800	0.000	5.035 – 175.33
Total	58	100	7	100			

Table 4 reveals that hypertension has 3.4 risks and having hypertension for more than 15 years has 13 risks for developing of CKD.

Table 5 reveals that CVD has 13.7 risks and having more than one type of CVD has 37.25 risks for developing of CKD.

Table 6 reveals that Family history of renal disease has 7 risks and family history of CKD has 23 risks for developing of CKD.

Table 7 reveals that proteinuria has 383 risks for developing of CKD.

Table 4. Distribution of the cases and controls according to hypertension

HT	Case		Control		O.R.	P-Value	95% of C.I.
	No.	%	No.	%			
Yes	91	60.67	46	30.67	3.487	0.000	2.166 – 5.613
No	59	39.33	104	69.33			
Total	150	100	150	100			
Duration of HT							
< 10 years	39	42.85	39	85.0	1.000	1.000	0.598 – 1.671
10-15 years	40	43.96	6	13.0	8.727	0.000	3.654 – 20.783
>15 years	12	13.19	1	2.0	12.96	0.002	2.124 – 78.430
Total	91	100	46	100			

Table 5. Distribution of the cases and controls according to cardiovascular disease

CVD	Case		Control		O.R.	P-Value	95% of C.I.
	No.	%	No.	%			
Yes	48	31.33	5	3.33	13.647	0.000	5.401 – 34.370
No	102	68.67	145	96.67			
Total	150	100	150	100			
Types of CVD							
IHD	11	22.9	3	60.0	3.878	0.029	1.136 – 13.167
HF	7	14.5	1	20.0	7.294	0.032	1.150 – 45.872
>one type	30	62.5	1	20.0	37.25	0.000	6.323 – 218.22
Total	48	100	46	100			

Table 6. Distribution of the cases and controls according to family history of renal diseases

Family history of renal disease	Case		Control		O.R.	P-Value	95% of C.I.
	No.	%	No.	%			
Yes	43	28.67	8	4.67	7.133	0.000	3.269 – 15.527
No	107	71.33	142	95.33			
Total	150	100	150	100			
Types of Family history of renal al diseases							
Polycystic kidney disease	2	4.65	1	12.5	2.014	0.562	0.260 – 15.507
CKD	20	46.51	1	12.5	22.92	0.000	3.845 – 135.75
kidney stone	12	27.91	5	62.5	2.522	0.080	0.900 – 7.040
>one type	9	20.93	1	12.5	9.511	0.010	1.530 – 58.617
Total	43	100	8	100			

Table 7. Distribution of the cases and controls according to proteinuria

Family history of renal disease	Case		Control		O.R.	P-Value	95% of C.I.
	No.	%	No.	%			
Yes	108	72.0	1	0.67	383.143	0.000	65.202 – 2231.9
No	42	28.0	149	99.33			
Total	150	100	150	100			

Figure 2 shows that the highest rates of the risk factors of CKD, which had statistically significant association for the developing of CKD were: proteinuria (72%) (P=0.000), family history of chronic diseases (68%) (P=0.000), hypertension (60.67%) (P=0.002), urinary cast (61.33%) (P=0.000), pyuria (58.67%) (P=0.000), cigarettes smoking (50%) (P=0.000), dyslipidemia (46 %) (P=0.000) and showed that hematuria, recurrent pyelonephritis and illiterate had (43.30%) (P=0.000) and for DM (38.7%) (P=0.000) and for Nephrotoxic drugs and agents (34.67%) (P=0.000).

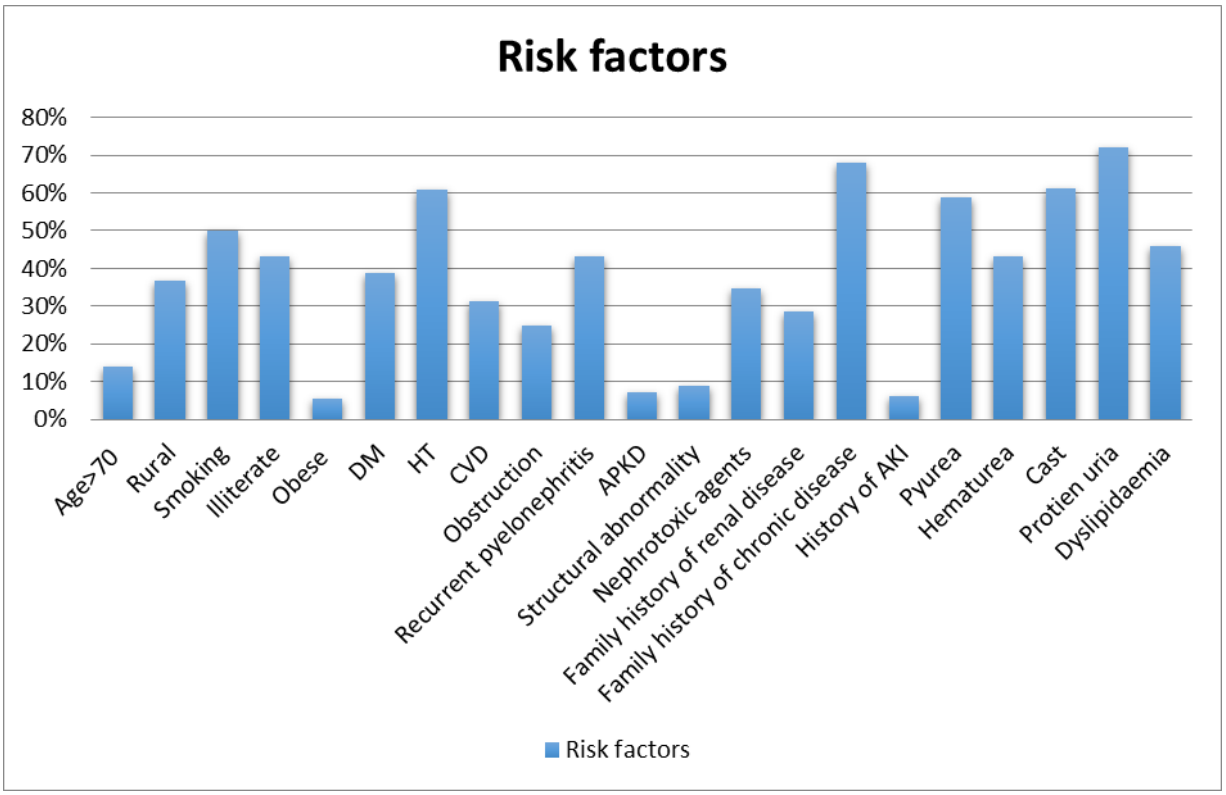

Figure 2. Distributions of the rates of the risk factors of CKD in the case group

Discussion

From this study it has been revealed that diabetes has 38.67% of the adult cases with CKD. Nevertheless, 20-40% of diabetics will develop diabetic nephropathy during the end stage of their disease; therefore, with the increase of cases of diabetic patients, the incidence of CKD is expected to rise. This result was similar to the result of the study done in 2003 (and to the result in the study by Remuzzi et al in 2012 [6]. Hypertension is very common in patients with CKD, and the level of blood pressure is associated with the rate of loss of kidney function. Control of blood pressure slows the rate of decline of kidney function. This result was similar to the result of the study in 2016 [7]. The presence of CVD like IHD (ischemic heart disease) and HF (heart failure) is independently associated with kidney function decline, increased risks of serum creatinine elevation, eGFR decline and development of CKD. This result was similar to the result of a study in united states in 2005 [8]. Family history of CKD/ESRD has been considered a significant risk factor for CKD. Genetic predisposition plays a key role in many forms of CKD. This result was similar to the result of the study done in Beijing 2008 [9]. Proteinuria is the strongest single predictor of GFR decline. Therapies that decrease proteinuria generally slow GFR

decline. Proteinuria-induced glomerular and renal tubular injury is a key mechanism of natural progression. The threshold for natural progression attributable to proteinuria appears to be crossed when proteinuria exceeds 500 mg/day. Proteinuria magnitude is the strongest single risk factor CKD progression. This result was similar to the result of a study in 2003 [5].

This study concluded that there are many risk factors significantly contributing to the development of CKD in Mosul especially diabetes mellitus, hypertension, family history of renal disease, from this study it has been also concluded that proteinuria plays a major role for the development and progression of CKD. Application of further prevention and control are highly recommended to reduce the burden of CKD.

Acknowledgments

Authors great thanks and appreciation are to all the doctors, medical staffs and parastaffs in Medical Wards and Dialysis Kidney Unit at Ibn-sina Teaching Hospital where data were collected, for their cooperation and assistance during data collection.

Also, authors are very grateful to all the patients and their relatives that admitted to Medical Wards and Dialysis Kidney Unit at Ibn-sina Teaching Hospital that included in the study for their kind cooperation, which made this work possible.

Author contribution

Dr. Khaleel: collection of data, statistics and conclusion. Dr. Hussain: helped in the collection of data. Dr. Hmood: helped by giving clinical notes in collecting the data of the study.

Conflict of interest

There is financial conflict in doing the investigation for the study and collecting of data.

Funding

Self-funding.

References

1. National Kidney Foundation. KDOQI clinical practice guidelines for chronic kidney disease: Evaluation, classification, and stratification. Am J Kidney Dis. 2012; 39(suppl 1): S1-S266.
2. Coresh J, Astor BC, Greene T, et al. Prevalence of chronic kidney disease and decreased kidney function in the adult U.S. population: Third National Health and Nutrition Examination Survey. Am J Kidney Dis. 2003; 41(1): 1-12. doi: 10.1053/ajkd.2003.50007
3. Carroll LE. The stages of chronic kidney disease and the estimated glomerular filtration rate. J Lancaster Gen Hosp. 2016; 1: 70-74.
4. Caskey F, Davenport A, Dawnay A, et al. UK renal registry 2013 16th annual report of the renal association," Nephron Clin Pract. 2013; 125: 1-4.
5. Writing Team for the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Research Group. Sustained effect of intensive treatment of type 1 diabetes mellitus on development and progression of diabetic nephropathy: the Epidemiology of Diabetes Interventions and Complications (EDIC) study. JAMA. 2003; 290(16): 2159-67. doi: 10.1001/jama.290.16.2159.
6. Remuzzi G, Schieppati A, Ruggenenti P. Clinical practice. Nephropathy in patients with type 2 diabetes. N Engl J Med. 2012; 346(15): 1145-51. doi: 10.1056/NEJMcp011773.
7. Klag MJ, Whelton PK, Randall BL, et al. Blood pressure and end-stage renal disease in men. N Engl J Med. 2016; 334: 13-8. doi: 10.1056/NEJM199601043340103.
8. Foley RN, Murray AM, Li S, et al. Chronic kidney disease and the risk for cardiovascular disease, renal replacement, and death in the United States Medicare population, 1998 to 1999. J Am Soc Nephrol. 2005; 16(2): 489-95. doi: 10.1681/ASN.2004030203
9. Zhang L, Zhang P, Wang F, et al. Prevalence and factors associated with CKD: a population study from Beijing. Am J Kidney Dis. 2008; 51(3): 373-84. doi: 10.1053/j.ajkd.2007.11.009.

Correspondence to Dr. Fanar F. Khaleel
E-mail: mr.photoshop2@gmail.com
Received Jun. 21st 2018
Accepted Dec. 18th 2018

Iraqi JMS

Published by Al-Nahrain College of Medicine
P-ISSN 1681-6579
E-ISSN 2224-4719
Email: iraqijms@colmed-alnahrain.edu.iq
http://www.colmed-alnahrain.edu.iq
http://www.iraqijms.net
Iraqi JMS 2019; Vol. 17(1)

Effect of Progesterone in Lowering Maternal Plasma Corticotropin-Releasing Hormone in Patients with Preterm Labor

Enas M. Yaseen[1] *CABOG, FICOG*, **Athraa Fadhil Abd**[2] *FICOG*

[1]Dept. of Obstetrics and Gynecology, College of Medicine, Tikrit University, Tikrit, Iraq, [2]Dept. of Obstetrics and Gynecology, Samaraa General Hospital, Samaraa, Iraq

Abstract

Background Preterm labor is a major obstetrics problem because it associated with high morbidity and mortality to the born baby. Many studies were done to study different aspects of its risk factors, diagnosis and treatment to decrease its incidence, bad sequel to the fetus and recurrence.

Objective To evaluate the role of progesterone in lowering CRH level in plasma of patient with preterm labor.

Methods This study is a case control study. Forty-five pregnant women with preterm labor were included in the study and basal plasma Corticotropin releasing hormone (CRH) level was done for each patient. All patients were given oral tocolytic drug, with an initial bolus of 20 mg Nifedipine followed by 10 mg three times daily. They received betamethasone injection (12 mg) 24 hours after hospitalization. Then after stabilization of each patient with random selection, 25 patients were included in group (1) and were given progesterone injection. Twenty patients were included in group (2) or control group because no progesterone injection was given to them. After 24 hours of admission to the hospital, plasma CRH level was measured for both groups with evaluation of the outcome of each patient with preterm labor.

Results Progesterone is more effective in lowering CRH level in patients with preterm labor. In study of 45 cases of preterm labor, the mean CRH level of group (1) decreased from 33.41 ng/ml to 22.12 ng/ml, while the mean level of it in group (2) increased from 27.44 ng/ml to 28.54 ng/ml.

Conclusion Progesterone treatment is effective in lowering CRH level in patients with preterm labor. This would have a positive effect in prolonging the pregnancy period in these patients.

Keywords Preterm labor, progesterone, CRH

Citation Yaseen EM, Abd AF. Effect of progesterone in lowering maternal plasma corticotropin-releasing hormone in patients with preterm labor. Iraqi JMS. 2019; 17(1): 32-42. doi: 10.22578/IJMS.17.1.6

List of abbreviations: ACTH = Adrenocorticotropin hormone, 17OHPC = 17 Alpha hydroxyprogesterone caproate, CRH = Corticotropin releasing hormone, CRH1,CRH2 = Corticotropin releasing hormone receptors, CS = Cesarean section, DCs = Dendritic cells, HCG = Human chorionic gonadotropin, NCIU = Neonatal care intensive unit, NK = Natural killer, PGDH = Prostaglandin dehydrogenase, PR-A,PR-B = Progesterone receptors, PTL = Preterm labor, RDS = Respiratory distress syndrome, Th2 = T-helper cell type 2

Introduction

Preterm labor occurs when there is frequent, regular uterine contraction and cervical dilation before 37 weeks of gestation. The incidence of preterm labor in developed world between 7 to 12% [(1)]. There has been a small gradual rise in the incidence of preterm labor associated with assisted reproduction and an increased tendency to obstetric intervention. The rate of preterm labor prior to 32 weeks has remained relatively stable at 1-2% [(1,2)].

The mortality, morbidity and costs of preterm labor are higher at lower gestational ages, there is a high risk of short - and long-term morbidity [(3)].

Three potential causal pathways leading to preterm delivery have been identified: infection - cervical pathway, placental - vascular pathway and stress - strain pathway. these factors

provide a direction for the identification of patient at risk and the application of potential interventions [4,5]. Pathogenesis of it includes idiopathic: preterm labor of unknown etiology accounts for 60-70% of it [6]. Labor due to premature activation of hypothalamic/pituitary/adrenal axis. Infection: there is a strong correlation between infection within the uterus and the onset of spontaneous labor [7,8], hemorrhage, placental abruption and uterine over distension [2].

Corticotropin-releasing hormone (CRH) is a 41 amino acid peptide hormone in the hypothalamus. It regulates adrenocorticotropin hormone (ACTH) secretion in the anterior pituitary which signals adrenal gland to release cortisol that has powerful metabolic and anti-inflammatory effects [9,10]. Physical or psychological events stimulate secretion of CRH, which co-ordinates neuroendocrine response to stress because it acts as a neuro-transmitter in some regions of the central nervous system. It produced in a variety of tissues outside the central nervous system, including the gut, ovary, testis, fetal membranes maternal and fetal blood, amniotic fluid and placenta during pregnancy with large amounts of CRH in comparison to non-pregnant [11].

It is now well established that the concentrations of CRH in maternal blood rise progressively during pregnancy [12,13]. This rise correlates with increased levels of CRH mRNA and CRH peptide in placental tissue [14]. In the circulation, CRH is largely associated with a high-affinity circulating CRH-binding protein (CRH-BP) produced in the liver, placenta and at other sites, including the brain. CRH-BP effectively blocks the action of placental CRH on the maternal pituitary gland and on the myometrium. Near term, and in association with preterm labor, CRH-BP concentrations fall, coincident with the increase in circulating CRH [13].

Many factors regulate placental CRH output and has been reviewed extensively [12]. Estrogens, progesterone, and nitric oxide inhibit CRH production, while number of neuropeptides exert a stimulatory effect [12], also, glucocorticoids, prostaglandins, cytokines and catecholamine increase it. Karalis and Majzoub suggested that the inhibitory effect of progesterone is exerted through binding to CRH receptor in trophoblastic cells. At term, increased levels of cortisol displace progesterone bound to GRH receptors and this is will lead to an increase in CRH output. Thus, the mechanism of interaction between progesterone and cortisol in the regulation of CRH is similar to that proposed for the regulation of enzyme 15-hydroxy prostaglandin dehydrogenase (PGDH) [15].

In spite of the fact that CRH binding protein (CRHBP) decreases in maternal circulation before labor, placental CRHBP mRNA expression remain unchanged. This leads to the hypothesis that another source of CRHBP exists, such as a fetal source that may be responsible for this decrease [16,17].

The functional target of placental CRH is not the maternal pituitary adrenal axis but the fetal pituitary adrenal axis [18]. Placental CRH stimulate ACTH production from the fetal pituitary. ACTH stimulates fetal adrenals to produce dehydroepiandrosterone (DHEA), dehydroepiandrosterone-sulphate (DHEA-S), and cortisol. ACTH is also produced in the placenta through paracrine mechanisms. Fetal adrenal DHEA is metabolized to estrogens in the placenta that favor parturition [11,19,20]. The produced cortisol exerts a stimulatory effect on the placenta to further produce CRH, thus a positive loop is established that causes placental CRH to rise exponentially as pregnancy advances [11].

CRH has two receptors, specific receptors, termed CRH-R1 and CRH-R2 [21]. The expression of CRH-R2 protein increased toward onset of labor increases myometrial contractility [22]. Both types of receptors have been identified in the upper and lower uterine segment of contracting human myometrium, but during labor CRH-R1 decreases significantly at the upper segment but not at the lower one. This will explain the fact during labor the fundus of

the uterus switches to a highly contractile state, while the lower segment remains relatively quiescent [23].

Studies of preterm labor aimed to give time to steroid to take its action and current evidence shows that a single course of maternal steroids given between 24 and 34 weeks gestation and received within 7 days of delivery results in markedly improved neonatal outcomes, with a significant reduction in rate of respiratory distress syndrome, neonatal death, intraventricular hemorrhage [1,2].

Progesterone is a vital gestational-support steroid hormone that belongs to the C21 group of progestagens produced in the adrenal glands, corpus luteum in non-pregnant and in pregnant till 10 weeks of gestational age, brain and placenta [24].

Progesterone modulates immune reaction of maternal body against embryo and fetus by anti-inflammatory effects throughout pregnancy. It inhibits the activity of dendritic cells (DCs) that generate proinflammatory responses and help the process of tolerogenic DCs. It limits the action of natural killer (NK) cells and the differentiation of T cells into T-helper cell type 2 (Th2) like clones which maintain pregnancy [25,26].

According to the "progesterone block" hypothesis, proposed by Csapo, progesterone blocks myometrial contraction and maintains pregnancy, while its withdrawal transforms the myometrium to the delivery state [27].

However, in humans, progesterone levels remain high throughout pregnancy and during labor [28]. This has led to the hypothesis of a "functional" progesterone withdrawal that may occur [29]. In human myometrial cells, the ratio of PR-A:PR-B mRNA increases 2- to 3-fold compared with the non- laboring state, mainly due to over expression of PR-A which induces "functional estrogen activation" through increased estrogen receptor α (ERα) expression [30]. PR-A may also suppress the transcriptional activity of PR-B, which is the main receptor for the nuclear signal transduction of progesterone [31,32]. Apart from myometrial contractions, the functional progesterone withdrawal due to the altered expression of PR-A, PR-B isoforms may also contribute to the cervical changes during labor [33,34].

While the exact mechanism of action of progestogens in preventing preterm labor is unknown, several possibilities have been proposed; in summary by two mechanisms; either anti-inflammatory effect, or local increase in progesterone in gestational tissues [35-41] as it may act on prevention of gap junctions formation which inhibits myometrial contractions and prevent spontaneous early miscarriage and preterm labor (PTL) [42].

Progestins are available in natural or synthetic formulations for oral intramuscular or vaginal rout in the form of suppository or gel. Natural (micronized) progesterone is an exact duplicate of the progesterone produced in the corpus luteum and placenta. It is therefore more readily metabolized by the body and is associated with minimal side effects.

17 alpha hydroxylprogesterone caproate (17OHPC) is 17-hydroxyprogesterone derivative; it is the most commonly used synthetic progestin given intramuscularly to prevent PTL. It has been isolated from both adrenal glands and corpora lutea. The synthetic caproat ester works as a long-acting progestin when administrated intramuscularly for 7-8 days with peak plasma concentration is bout 2-8 hours. The most common undesirable side effects were injection site pain, injection site swelling urticarial, pruritis, nausea, contusion, injection site nodule and vomiting [43].

The aim of this study is to evaluate the role of progesterone in lowering CRH level in plasma of patient with preterm labor.

Methods

This study is a case control study. It was conducted at Tikrit Teaching Hospital – Department of Obstetrics and Gynecology from January 2013 to January, 2014. This study was approved by the Ethical Committee of the Iraqi Scientific Council for Medical Specialization-Department of Obstetrics and Gynecology. The

informed consent was taken from each patient. Women with preterm labor between 24-34 weeks of gestation were included in the study.

Inclusion criteria

- Single life pregnancy.
- Intact membranes.
- No cerclage.
- Cervical dilation of equal or <2 cm.

Exclusion criteria

- Signs of infection (urinary tract infection, chorioamnionitis).
- Medical diseases.
- Contraindication to tocolysis.
- Adverse reaction to progesterone or any component of the formulation (by history).
- Progesterone treatment within 4 weeks before enrollment.
- History or suspicion of breast or genital tract malignancy.
- Evidence of intra-uterine reconstructions, or congenital anomalies in ultrasound.

At admission full history, general and obstetrical examination, maternal vital signs (blood pressure, temperature, pulse rate, respiratory rate) and cardiotocography (fetal heart rate assessment, uterine contraction) was done to each patient.

All patients had urine examination, high vaginal swab for culture and sensitivity to exclude genital tract infection and complete blood picture. CRH level was measured in each patient in both groups by: drawing venous blood (4-5 mL) into a tube that contained the anticoagulant sodium citrate. This assay employed by the competitive inhibition enzyme immune assay technique. The kit manufactured by CUSABIO.

All patients were given oral tocolytic, with an initial bolus of 20 mg Nifedipine followed by 10 mg three times daily. After hospitalization they received betamethasone injection (12 mg) in two divided doses during 24 hours. After patient's condition stabilization with random selection, 25 of them received intramuscular Hydroxyprogesterone Caproate 250 mg (Bayer) and they were classified as group (1). They received this injection weekly till 36 completed weeks or earlier if they delivered. The remaining patients who received no progesterone injection were classified as group (2) or control groups.

Observation of each patients were done including: blood pressure, pulse rate, uterine contraction, fetal heart rate; and any other maternal side effects as headache hypotension, nausea, vomiting, injection site reaction, purities, and vaginal discharge. After 24 hours of progesterone injection, plasma CRH level was measured gain.

All patients followed up till delivery in outpatient clinic. Detection Range was between 1.6- 40 ng/ml.

Statistical analysis and data management

The Statistical Package for Social Sciences (SPSS, version 18) was used for data entry and analysis. Chi ($\chi 2$) square test, unpaired Student t test, Paired t- test, one –way ANOVA and Pearson correlation was used to complete statistical analysis. Odds ratio used to test the risk. P value of ≤ 0.05 was regarded as statistically significant.

Results

Patient's general characteristics are shown in (Table -1).

Figure (1) and (2) show the relation between parity, gravida and basal plasma CRH levels. The level of them increases in a significant moderate positive linear correlation with the parity and gravida increment.

Table 1. General characteristics of patient with preterm labor

Study group characteristics		Mean	SD
Gravid		3.69	2.29
Para		2.13	1.59
Abortion		0.56	1.06
Gestational age		31.13	2.63
Gestational age of delivery		34.93	3.45
Basal plasma CHR level		30.76	8.41
Plasma CHR after 24 hr		24.97	12
		Frequency	**Percent**
History of preterm labor	Yes	6	13.3%
	No	39	86.7%
Cause of preterm	Negative	39	86.6%
	Infection	3	6.7%
	Idiopathic	3	6.7%
Antenatal care	Yes	29	64.4%
	No	16	35.6%
Mode of delivery	NVD	35	77.8%
	CS	10	22.2%
Total		45	100%

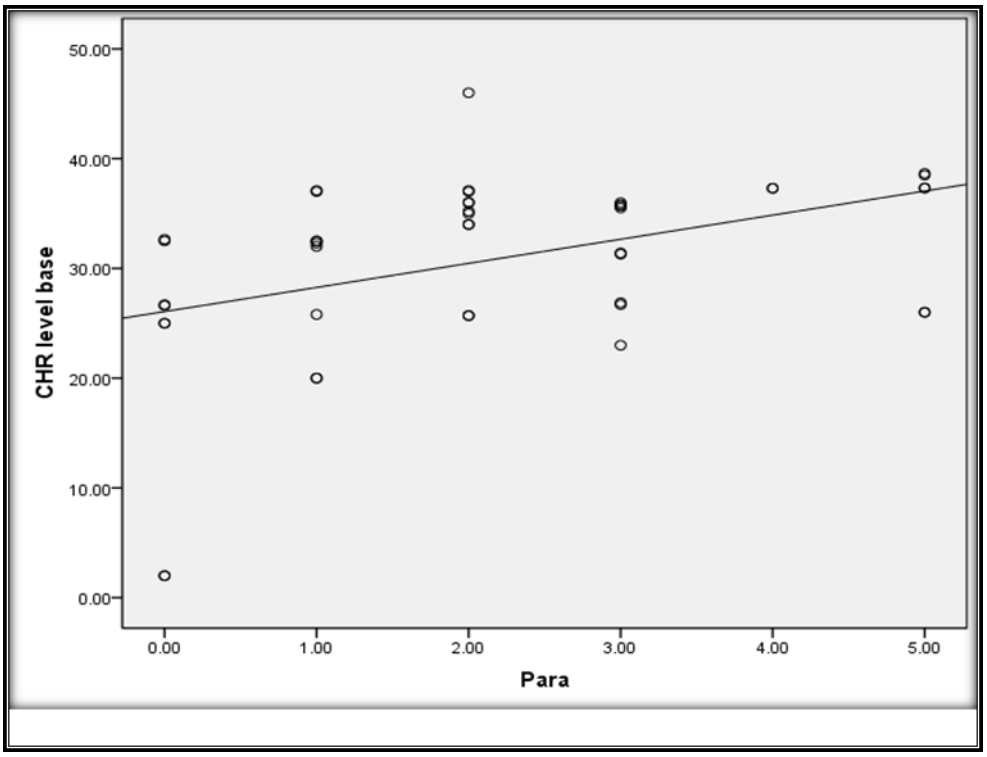

Figure 1. Correlation between parity and basal plasma CHR level

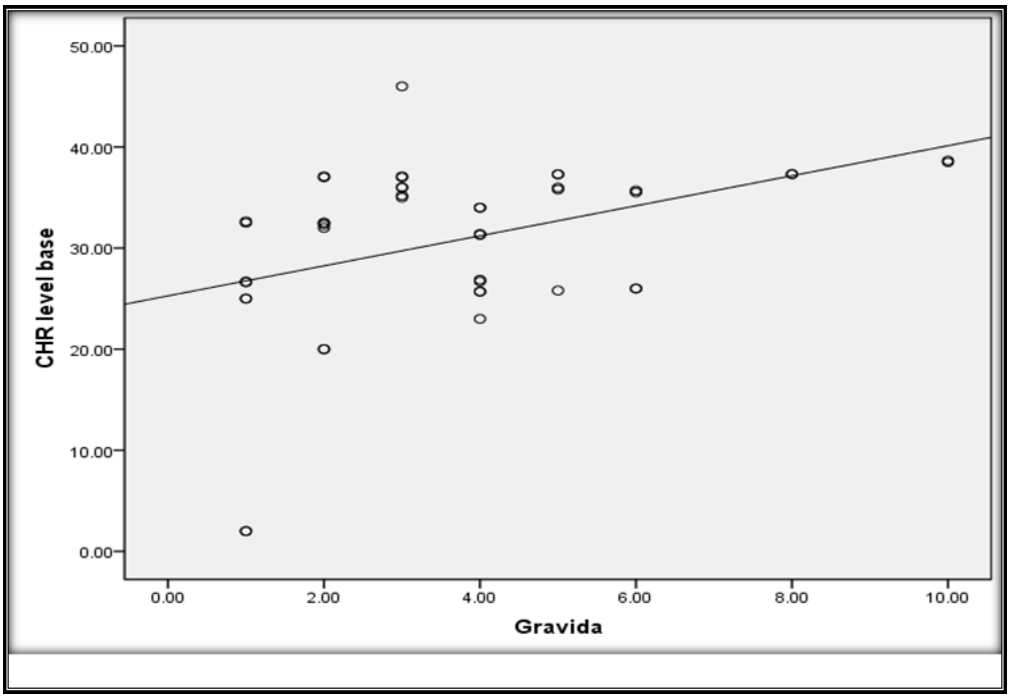

Figure 2. Correlation between Gravida and basal plasma CRH level

The mean level of CRH level decreased after 24hr due to progesterone injection in group (1), while it increased in group (2) (Table 2).

Sixty eight percent of group (1) delivered at term or near term while only 45% of group (2) delivered at this time. The odds ratio to deliver within 2 weeks for the group (2) was 2.597 times more than group (1). The relative risk of group (2) was 1.7 to deliver within 2 weeks, while for the group (1) was 0.6 (Table 3).

Table (4) shows a decrease in mean plasma CRH level after 24hr of treatment in all patients of group (1). Its mean is increasing in group (2) for those who delivered within 2weeks, while it is decreasing slightly for those who delivered at term or near term.

Table 2. The mean level of CRH level among study groups at base line and after 24 hr of treatment

CRH levels	Group (1)			Group (2)			Independent
	Mean	SD	SE	Mean	SD	SE	t-test P value
Basal CRH level	33.41	4.40	0.88	27.44	10.89	2.43	<0.05
CRH (after 24 hr)	22.12	12.88	2.58	28.54	10.00	2.24	>0.05
Paired t-test P value		<0.05			>0.05		

Table 3. The risk and odds ratio of preterm labor

	Value	95% Confidence Interval	
		Lower	Upper
Odds Ratio for time delivery (within 2 week / reach term or near term)	2.597	0.769	8.775
Risk of deliver within 2 weeks for group (1)	0.644	0.355	1.170
Risk of deliver within 2 weeks for group (2)	1.673	0.871	3.213

Table 4. The mean plasma CRH level in both groups according to the delivery time

Out -come of treatment		Group (1)			Group (2)		
		Basal plasma CRH level	Plasma CRH level after 24 hr	Paired t- test	Basal plasma CRH level	Plasma CRH level after 24 hr	Paired t- test
Delivery within 2 weeks	Mean	34.54	31.15	<0.05	22.98	25.87	<0.05
	SD	4.98	4.97		11.57	11.26	
	SE	1.76	1.76		3.49	3.39	
	Minimum	26.60	25.30		2.00	4.00	
	Maximum	38.65	37.20		34.00	35.50	
	No.	8	8		11	11	
Reach term/ near term	Mean	32.87	17.87	<0.05	32.89	31.80	<0.05
	SD	4.16	13.34		7.32	7.57	
	SE	1.01	3.23		2.44	2.52	
	Minimum	23.00	2.00		25.70	23.40	
	Maximum	37.33	32.60		46.00	45.00	
	No.	17	17		9	9	
Independent t-test		> 0.05			<0.05		

Table (5) shows no statistical differences in mode of delivery in both groups. While there is significant in neonatal out comes such as: admission to neonatal care intensive unit (NCIU) (40%) of group (1) and only (85%) of group (2) had admission to NCU.

Table 5. The mode of delivery and neonatal outcomes in both groups

		Group (1)		Group (2)		P value
		No.	Percent	No.	Percent	
Mode of delivery	NVD	17	68%	18	90%	>0.05
	CS	8	32%	2	10%	
Neonatal out come	no admission	15	60 %	3	15%	<0.05
	< 7 days	7	28%	10	50%	
	> 7 days	3	12%	7	35%	
Causes of admission	RDS	3	12%	5	25%	>0.05
	hyperbilirubinemia	2	8%	4	20%	
	septicemia	2	8%	3	15%	
	Convulsion	1	4%	2	10%	
	Others	2	8%	3	15%	
Total		25	100%	20	100%	

Discussion

Preterm birth (PTB) occurs when delivery before 37+0 weeks of gestation and it is the most important single determinant of adverse infant outcome in terms of both survival and quality of life. Gestational age determines preterm outcomes such as death or neurosensory defects, huge psychosocial and emotional effects on the family and cost of health services [44].

Many tocolytic drugs suppress myometrium contractions such as beta-agonists, calcium channel blockers, oxytocin receptor antagonists, prostaglandin synthetase inhibitors, nitric oxide donors and magnesium sulphate. There is little reliable information about current clinical practice but it is likely that the use of the beta-agonist ritodrine hydrochloride, which was widespread in the past, has declined. Magnesium sulphate is popular for tocolysis in the USA and some other parts of the world but has rarely been used for this indication in the UK [45].

Of all treatments evaluated for the prevention of spontaneous PTB to date, progestational agents have demonstrated the greatest promise. Progesterone supplement therapy is one of the few proven effective methods to prevent PTB in women with history of spontaneous PTB and in women with short corpus luteal phase. There are 2 types of progesterone therapy currently used for prevention of PTB: weekly intramuscular injection of 17-alpha hydroxyprogesterone caproate and daily administration of natural micronized progesterone vaginal gel, vaginal suppository, or oral capsule [46].

Progesterone and CRH play an important role in pregnancy and labor, so their changes affect transition from myometrial quiescence to contractility [27]. In this study, evaluation of the effect of progesterone on CRH, which is the main player in initiation of labor was done.

Our study showed that progesterone treatment has more effect in decreasing CRH level than tocolytics. This result affects the time of delivery.

In a new study done by Khazaali (2018) in Iraq the author found that a significant reduction in preterm delivery rate among women receiving progesterone vaginal suppositories and there was significant reduction in the frequency of respiratory distress syndrome, low birth weight neonates and admissions to neonatal intensive care unit in women taking vaginal progesterone pessary compared to the control [47].

Also, there are several studies demonstrates the relation of CRH and progesterone on labor. Vrachnis et al in 2012 explained immune and myometrial effects of progesterone and CRH in Labor because both modify immune response during pregnancy and progesterone withdrawal encourages inflammatory pathways. In labor, withdrawal of progesterone occurs by with metabolic changes of progesterone, receptors changes, and other factors or enzymes that

stimulate or inhibit progesterone. Placental CRH acts on the fetal pituitary-adrenal axis to stimulate adrenal production of androgens and cortisol and also acts directly on myometrial cells via its receptors [27].

Regmi et al in 2012 conducted a study of progesterone for Prevention of recurrent preterm labor after arrested it. Progesterone reduces preterm labor recurrence significantly but neonatal outcomes unchanged in between groups [42].

A meta-analysis of nine studies by Coomarasamy et al in 2006 showed the effectiveness of progesterone in the prevention of preterm labor by in suspected patients and reduction in neonatal respiratory complications [48].

Mackenzie et al in 2006 conducted a meta-analysis evaluating the use of progesterone for high risk women with PTB. Again, progesterone prophylaxis reduces incidence of preterm labor without significant reduction in neonatal complications [49]. This agree with our results.

Kurki et al in 1991 measured maternal plasma CRH in preterm patients before and after given indomethacin or nylidrin, they showed a 10% decrease in the indomethacin group and 10-20% decrease in the nylidrin group, but these changes were not statistically significant [50].

Our results were promising regarding clinical and biochemical effectiveness of progesterone in lowering the level of CRH in patients of PTL. The changing in CRH level has positive effect in delaying the labor till term or near term.

We recommend larger studies are needed to study the level of CRH according to different maternal characteristics in preterm labor. Using progesterone in combination with other tocolytic drugs as new combinations of drugs in hope that increasing their effectiveness in treatment of PTL and possibilities in using this combination in multiple pregnancies.

Acknowledgments

Authors would like to thanks all the doctor and staff of Tikrit Teaching Hospital for their help.

Author contribution

Dr. Abd: collection of data, statistical analysis and writing the first draft of manuscript, and both authors made the final draft of manuscript.

Conflict of interest

No conflict of interest.

Funding

No financial support to this study.

References

1. Bennett P, Edmond K. Preterm Labor. Dewhurst's textbook of obstetrics and gynecology for postgraduates. 7th ed. Blackwell Science Ltd; 2007. p. 177-91.
2. Taylor M, Rundle S. Preterm labor. In: Luesly DM, Kilby MD (eds). An evidence-based text for MRCOG in obstetrics and gynecology, 3rd ed. CRC Press; 2006. p. 327-37.
3. Cunningham FG, Leveno K, Bloom S, et al. Williams Obstetric. Textbook, 23rd ed. MacGraw Hill; 2010. p. 804-8.
4. Hacker NP, Moore JC. Obstetrics complications: Obstetric Complications: Preterm Labor, PROM, IUGR, Postterm Pregnancy, and IUFD. In: Hacker NP, Gambone JC (eds). Hacker and Moore Essential of Obstetrics and Gynecology. Philadelphia: Elsevier, Saunders; 2004. p. 167-82.
5. Romero R, Espinoza J, Erez O, et al. The role of cervical cerclage in obstetric practice: can the patient who could benefit from this procedure be identified? Am J Obstet Gynecol. 2006; 194(1): 1-9. doi: 10.1016/j.ajog.2005.12.002.
6. ACOG Committee on Practice Bulletins--Obstetrics. ACOG practice bulletin. Management of preterm labor. Number 43, May 2003. Int J Gynaecol Obstet. 2003; 82(1): 127-35.
7. Goldenberg RL, Andrews WW, Hauth JC. Choriodecidual infection and preterm birth. Nutr Rev. 2002; 60(5 Pt 2): S19-25. doi: 10.1301/00296640260130696.
8. Goncalves LF, Chaiworapongsa T, Romero R. Intrauterine infection and prematurity. Ment Retard Dev Disabil Res Rev. 2002; 8(1): 3-13. doi: 10.1002/mrdd.10008.
9. Challis JRG. Fetal endocrine signals and preterm labor. Biol Neonate. 2001; 79(3-4): 163-7. doi: 10.1159/000047085.
10. Whittle WL, Patel FA, Alfaidy N, et al. Glucocorticoid regulation of human and ovine parturition: The relationship between fetal hypothalamic-pituitary-adrenal axis activation and intrauterine prostaglandin production. Biol Reprod. 2001; 64(4): 1019-32.
11. Smith R: Parturition. N Engl J Med. 2007; 365: 271-83. doi: 10.1056/NEJMra061360.
12. Petraglia F, Florio P, Nappi C, et al. Peptide signaling in human placenta and membranes: autocrine,

paracrine and endocrine mechanisms. Endocr Rev. 1996; 17(2): 156-86. doi: 10.1210/edrv-17-2-156.
13. Linton EA, Perkins AV, Woods RJ, et al. Corticotropin releasing hormone- binding protein (CRH-BP); plasma levels decrease during the third trimester of normal human pregnancy. J Clin Endocrinol Metab. 1993; 76(1): 260-2. doi: 10.1210/jcem.76.1.8421097.
14. Frim DM, Emmanuel RL, Robinson BG, et al. Characterization and gestational regulation of corticotropin-releasing hormone messenger RNA in human placenta. J Clin Invest. 1988; 82(1): 287-92. DOI: 10.1172/JCI113585.
15. Karalis K, Majzoub JA. Regulation of placental corticotrophin-releasing hormone by steroids. Possible implications in labor initiation. Ann N Y Acad Sci. 1995; 771: 551-5.
16. McLean M, Bisits A, Davies J, et al. A placental clock controlling the length of human pregnancy. Nat Med. 1995; 1(5): 460-3.
17. Torricelli M, Giovannelli A, Leucci E, et al. Labor (term and preterm) is associated with changes in the placental mRNA expression of corticotrophin-releasing factor. Reprod Sci. 2007; 14(3): 241-5. DOI: 10.1177/1933719107300971.
18. McLean M, Smith R. Corticotrophin-releasing hormone and human parturition. Reproduction. 2001; 121(4): 493-501. doi: http://dx.doi.org/10.1530/rep.0.1210493.
19. Sirianni R, Mayhew BA, Carr BR, et al. Corticotropin-releasing hormone (CRH) and urocortin act through type 1 CRH receptors to stimulate dehydroepiandrosterone sulfate production in human fetal adrenal cells. J Clin Endocrinol Metab. 2005; 90(9): 5393-400. doi: 10.1210/jc.2005-0680.
20. Mesiano S, Welsh TN. Steroid hormone control of myometrial contractility and parturition. Semin Cell Dev Biol. 2007; 18(3): 321-31. doi: 10.1016/j.semcdb.2007.05.003.
21. Hillhouse EW, Grammatopoulos DK. The molecular mechanisms underlying the regulation of the biological activity of corticotropin-releasing hormone receptors: implications for physiology and pathophysiology. Endocr Rev. 2006; 27(3): 260-86. doi: 10.1210/er.2005-0034.
22. Grammatopulos DK. Placental corticotrophin-releasing hormone and its receptors in human pregnancy and labor: still a scientific enigma. J Neuroendocrinol. 2008; 20(4): 432-8. doi: 10.1111/j.1365-2826.2008.01660.x.
23. Cong B, Zang L, Gao L, et al. Reduced expression of CRH receptor type 1 in upper segment human myometrium during labor. Reprod Biol Endocrinol. 2009; 7: 43. doi: 10.1186/1477-7827-7-43.
24. Speroff L, Glass RH, Kase NG. Clinical gynecologic endocrinology and infertility. 5th ed. Baltimore: Williams and Wilkins; 1994. p. 842.
25. Szekeres-Bartho J, Halasz M, Palkovics T. Progesterone in pregnancy; receptor-ligand interaction and signaling path- ways. J Reprod Immunol. 2009; 83(1-2): 60-4. doi: 10.1016/j.jri.2009.06.262.
26. Raghupathy R. Th1-type immunity is incompatible with successful pregnancy. Immunol Today. 1997; 18(10): 478-82.
27. Csapo A. Progesterone block. Am J Anat. 1956; 98(2): 273-91.
28. Mitchell BF, Taggart MJ. Are animal models relevant to key aspects of human parturition? Am J Physiol Regul Integr Comp Physiol. 2009; 297(3): R525-45. doi: 10.1152/ajpregu.00153.2009.
29. Mendelson CR. Mini review: fetal-maternal hormonal signaling in pregnancy and labor. Mol Endocrinol. 2009; 23(7):947-54. doi: 10.1210/me.2009-0016.
30. Mesiano S, Chan EC, Fitter JT, et al. Progesterone withdrawal and estrogen activation in human parturition are coordinated by progesterone receptor A expression in the myometrium. J Clin Endocrinol Metab. 2002; 87(6): 2924-30. doi: 10.1210/jcem.87.6.8609.
31. Giangrande PH, Kimbrel EA, Edwards DP, et al. The opposing transcriptional activities of the two isoforms of the human progesterone receptor are due to differential cofactor binding. Mol Cell Biol. 2000; 20(9): 3102-15. doi: 10.1128/MCB.20.9.3102-3115.2000.
32. Merlino AA, Welsh TN, Tan H, et al. Nuclear progesterone receptors in the human pregnancy myometrium: evidence that parturition involves functional progesterone withdrawal mediated by increased expression of progesterone receptor-A. J Clin Endocrinol Metab. 2007; 92(5): 1927-33. doi: 10.1210/jc.2007-0077
33. Stjernholm-Vladic Y, Wang H, Stygar D, et al. Differential regulation of the progesterone receptor A and B in the human uterine cervix at parturition. Gynecol Endocrinol. 2004; 18(1): 41-6. doi: http://dx.doi.org/10.1080/09513590310001651777.
34. Vladic-Stjernholm Y, Vladic T, Blesson CS, et al. Prostaglandin treatment is associated with a withdrawal of progesterone and androgen at the receptor level in the uterine cervix. Reprod Biol Endocrinol. 2009; 7: 116. doi: 10.1186/1477-7827-7-116.
35. Renthal NE, Chen CC, Williams KC, et al. miR-200 family and targets, ZEB1 and ZEB2, modulate uterine quiescence and contractility during pregnancy and labor. Proc Natl Acad Sci U S A. 2010; 107(48): 20828-33. doi: 10.1073/pnas.1008301107.
36. Briery CM, Veillon EW, Klauser CK, et al. Women with preterm premature rupture of the membranes do not benefit from weekly progesterone. Am J Obstet Gynecol. 2011; 204(1): 54.e1-5. doi: 10.1016/j.ajog.2010.08.022.
37. Zakar T, Mesiano S. How does progesterone relax the uterus in pregnancy? N Engl J Med. 2011; 364(10): 972-3. doi: 10.1056/NEJMcibr1100071.
38. Peltier MR, Tee SC, Smulian JC. Effect of progesterone on proinflammatory cytokine production by monocytes stimulated with pathogens associated with preterm birth. Am J Reprod Immunol. 2008;

60(4): 346-53. doi: 10.1111/j.1600-0897.2008.00633.x.

39. Xu H, Gonzalez JM, Ofori E, et al. Preventing cervical ripening: the primary mechanism by which progestational agents prevent preterm birth? Am J Obstet Gynecol. 2008; 198(3): 314. e1-8. doi: 10.1016/j.ajog.2008.01.029.

40. Sfakianaki AK, Norwitz ER. Mechanisms of progesterone action in inhibiting prematurity. J Matern Fetal Neonatal Med. 2006; 19(12): 763-72. doi: 10.1080/14767050600949829.

41. O'Brien JM, DeFranco EA, Adair CD, et al. Effect of progesterone on cervical shortening in women at risk for preterm birth: secondary analysis from a multinational, randomized, double-blind, placebo-controlled trial. Ultrasound Obstet Gynecol. 2009; 34(6): 653-9. doi: 10.1002/uog.7338.

42. Regmi MC, Rijal P, Agrawal A, et al. Progesterone for prevention of recurrent preterm labor after arrested preterm labor - a randomized controlled trial. Gynecol Obstet. 2012; 2: 125. doi: 10.4172/2161-0932.1000125.

43. How HY, Sibai BM. Progesterone for the prevention of preterm birth: indications, when to initiate, efficacy and safety. Ther Clin Risk Manag. 2009; 5(1): 55-64.

44. Sigal S, Doyal LW. An overview of mortality and sequel of preterm birth from infancy to adulthood. Lancet. 2008; 371(9608): 261-9. doi: 10.1016/S0140-6736(08)60136-1.

45. Royal College of Gynecology and Obstetrics. Green-top Guideline. Tocolysis for Women in Preterm Labour. No. 1b; 2011: 1.

46. Choi SJ. Use of progesterone supplement therapy for prevention of preterm birth: review of literatures. Obstet Gynecol Sci. 2017; 60(5): 405-420. doi: 10.5468/ogs.2017.60.5.405.

47. Khazaali EAA. Vaginal progesterone pessary for preterm labor prevention in women with short cervix early in the second trimester. Iraqi JMS; 2018; 16(2): 133-43. doi: 10.22578/IJMS.16.2.4.

48. Coomarasamy A, Thangaratinam S, Gee H, et al. Progesterone for the prevention of preterm birth: A critical evaluation of evidence. Eur J Obstet Gynecol. Reprod Biol. 2006; 129: 111-8. doi: 10.1016/j.ejogrb.2006.05.013.

49. Mackenzie R, Walker M, Armson A, et al. Progesterone for the prevention of preterm birth among women at increased risk: A systematic review and meta-analysis of randomized controlled trials. Am J Obstet Gynecol. 2006; 194(5): 1234-42. doi: 10.1016/j.ajog.2005.06.049.

50. Kurki T, Laatikainen T, Salminen-Lappalainen K, et al. Maternal plasma corticotrophin-releasing hormone--elevated in preterm labor but unaffected by indomethacin or nylidrin. Br J Obstet Gynecol. 1991; 98(7): 685-91.

Correspondence to Dr.Enas M. Yaseen
E-mail: enasyaseen51@yahoo.com
Received Jun. 21st 2018
Accepted Dec. 5th 2018

Iraqi JMS

Published by Al-Nahrain College of Medicine
P-ISSN 1681-6579
E-ISSN 2224-4719
Email: iraqijms@colmed-alnahrain.edu.iq
http://www.colmed-alnahrain.edu.iq
http://www.iraqijms.net
Iraqi JMS 2019; Vol. 17(1)

Detection of TTV Antigen in Patients with Hepatitis HBV and HCV

Ealaf A. Khudair[1] *BSc*, **Arwa M.A. Al-Shuwaikh**[2] *PhD*, **Nawal M. Farhan**[3] *CABM, FICMS, GH & H*

[1]Alnahrain Company for Drug Industry, Baghdad, Iraq, [2]Dept. of Microbiology, College of Medicine, Al-Nahrain University, Baghdad – Iraq, [3]Gastroenterology and Hepatology Teaching Hospital, Baghdad, Iraq

Abstract

Background Several lines of evidence have suggested the presence of new hepatitis agents, in addition to established hepatitis viruses A-E. Torque Teno virus (TTV) was more prevalent in patients with hepatitis, so it was thought to have hepatotropic properties.

Objective To detect TTV Ag in apparently healthy blood donors and patients infected with chronic hepatitis B virus (HBV) or chronic hepatitis C virus (HCV) by enzyme linked immunosorbent assay (ELISA) technique. Also, to find out any possible association between the study population demographic data and TTV status.

Methods This study was conducted from the beginning of November, 2017 to the end of March, 2018. Serum samples were collected from 50 patients who had chronic hepatitis HBV or HCV and attended to Gastroenterology and Hepatology Teaching Hospital. Also, sera were collected from a total 43 healthy blood donors from the Blood Donation Center in Al-Imamein Al-Kadhimein Medical City. The clinical characteristics of both patients and controls such as alanine transaminase (ALT), aspartate transaminase (AST), hepatitis C virus antibody (HCV-Ab), hepatitis B surface antigen (HBsAg) and hepatitis B core antibody (HBcAb) were obtained from hospital records. Serum samples were tested by ELISA technique for detection of TTV Ag.

Results TTV was detected in 89.2% (33 out of 37) of the HBV-positive patients and in 30.8% (4 out of 13) of the HCV-positive patients versus 23.3% of the healthy blood donor (10 out of 43). Results of this study showed that the prevalence of TTV in HBV patients is significantly higher than HCV patients and healthy blood donors. Concerning risk factors, it was found that there was statistically significant relationship between TTV positivity and mean level of ALT but results indicated that the presence of TTV was not associated with AST, sex, age and history of transfusion, surgery and tattooing.

Conclusion TTV may play a role in hepatitis and its presence was associated with biochemical signs of liver disease, and among patients infected with chronic HBV or HCV.

Keywords Torque Teno virus, hepatitis B virus, hepatitis C virus, healthy blood donors

Citation khudair EA, Al-Shuwaikh AMA, Farhan NM. Detection of TTV antigen in patients with hepatitis HBV and HCV. Iraqi JMS. 2019; 17(1): 43-49. doi: 10.22578/IJMS.17.1.7

List of abbreviations: ALT = Alanine transaminase, AST = Aspartate Aminotransferase, TTV= Torque Teno virus

Introduction

Torque Teno virus (TTV) is a relatively small (3.8 kb) circular ssDNA virus that has been classified in the newly characterized family Anelloviridae [1,2]. It was suspected of being significantly associated with hepatitis [3].

TTV is characterized by extremely high prevalence, with the relatively high level of genomic heterogeneity. It was detected in the liver and blood of people with hepatic pathologies of unknown etiology. Blood transfusion was initially indicated as the

principal of viral transmission due to direct contact with contaminated blood. However, there are new routes of transmission that have been recognized, due to the presence of the virus in different body fluids such as in feces, saliva and also in river water contaminated by sewage [4].

Wide ranges of TTV virus infection is reported in individuals with liver disease, Human Immunodeficiency Virus (HIV) positive, intravenous drug users, thalassemic patients and patients on maintenance hemodialysis [5]. From preliminary reports, two characteristics of TTV infection have emerged rendering it as a potential cause of liver disease. First, Nishizawa *et al.* [6] reported the presence of TTV-DNA in the sera of patients with non-A-E hepatitis to reveal a close correlation with alanine transaminase (ALT) level. Second, Okamoto et al. [7] discovered that TTV-DNA levels in liver tissue were equal or one hundred times more than those in serum, suggesting that this virus replicated in the liver. The overall TTV viremia rates in hepatitis B virus (HBV) and hepatitis C virus (HCV) positive subjects were 90.75% and 84.9%, respectively as well as 81.4% in healthy individuals in Qatar [3].

In this study, we aimed to determine the frequency of TTV viremia by performing enzyme linked immunosorbent assay (ELISA) in healthy blood donors and patients with chronic HBV or HCV. Also, estimation of demographic data such as age, sex, aspartate aminotransferase (AST) and alanine transaminase (ALT) and history of underlying medical conditions.

Methods

Subjects

This study included a total of 93 blood samples collected from 50 patients with chronic HBV or HCV from the Gastroenterology and Hepatology Teaching Hospital for the period from the beginning November, 2017 to the end of March, 2018. In addition, 43 blood samples were collected from healthy blood donors who attended the Blood Donation Center in Al-Imamein Al-Kadhimein Medical City. Blood sample was drawn from each patients and control by venipuncture, after obtaining informed consent. The protocol for the research project was approved by the Institutional Review Board (IRB) of College of Medicine, Al-Nahrain University.

Specimens collection

Serum samples were collected from all hepatitis patients and healthy blood donors by venipuncture of the median cubital vein. Five (5) ml blood samples were collected in sterile gel tubes without any anticoagulant, allowed to clot in the room temperature within 1 hour of its collection before centrifugation at 3,000 rpm for 10 minutes, divided into aliquots then immediately stored frozen at (-44) ˚C until be used.

Immunoassay

Ninety-three samples were tested for qualitative detection of TTV Ag by ELISA kit (Abbexa, England). The wells of the microtiter plate were coated with an antibody specific to TTV Ag. The procedure was done following manufacturer's instructions. For sample preparation, each sample was diluted (1:5) with sample diluent buffer before adding into the set wells by adding 10µl of serum to 50 µl sample diluents which supplied with the kit. Blank, positive and negative controls were included in ELISA run. The optical densities (O.D.) of each well were measured at wave length (450 nm). The calculated absorptions for the patient and control serum were compared with the cut off value. The cut off value = absorbance of negative control + 0.15, if the absorbance of the sample is equal or higher than the cut off value, the test sample is considered positive, otherwise the test sample is considered negative.

Statistical analysis

Analysis of data was carried out using Statistical Packages for Social Sciences- version 19 (SPSS-19). Categorical data presented as count and percentage; the differences were examined by Chi-square test (χ^2-test). Whereas, numerical data presented as mean, standard deviation and

evaluated by using independent sample T-test, statistical significance was considered whenever the P value for the test of significance was equal or less than 0.05.

Results

This study included fifty hepatitis patients with HBV or HCV infection, with mean age of 36.20 ± 13.4 S.D. year and 43 healthy blood donors that randomly selected persons considered as control group, their mean age was 35.22 ± 9.8 S.D. year. Thirteen out of 50 (26%) patients were hepatitis C positive while 37 out of 50 (74%) patients were hepatitis B positive. Regarding the sex distribution, 24 hepatitis patients and 40 healthy blood donors were males. Females represented 26 and 3 in patients and healthy blood donors, respectively. T-test showed that the mean values of liver function test parameters i.e. ALT and AST were higher in hepatitis patients than in healthy blood donors as shown in Table 1.

Table 1. Characteristics of the subjects

Parameters	HBV or HCV Patients (n=50)	Healthy blood donors (n=43)	P. value
Age	36.20±13.4	35.22 ±9.8	0.679
Sex (M/F)	24/26	40/3	0.000
ALT (U/l)	50.9 ± 49.7	16.0 ± 6.0	0.000
AST (U/l)	52.6 ± 56.5	22.96 ± 7.7	0.001

TTV was detected in 23.3% of the healthy blood donor (10 out of 43) as well as in 30.8% of the HCV-positive individuals (4 out of 13) and in 89.2% of the HBV- positive individuals (33 out of 37), the difference was statistically significant (P<0.05) as shown in Table 2.

Results showed that there was a significant difference in sex between TTV positive and negative individuals. TTV infection was found in higher proportions among females than males (74.1% vs 40.9%) (P = 0.004). Concerning the risk factors for study individuals, there is no significant difference regarding history of blood transfusion, tattooing and surgery in TTV positive and negative individuals (P > 0.05), as shown in Table 2.

Results showed that there was no statistically significant difference between TTV positive and TTV negative subjects as regards AST but there was a significant difference between TTV positive and TTV negative as regards ALT as shown in Table 3. The mean age of TTV positive vs. TTV negative was (34.66 ± 11.651, 36.17 ± 11.361, P > 0.05), so there was no significant difference in the mean age between TTV positive and negative individuals.

Discussion

Despite that the pathogenicity of TTV is not fully clear, but undoubtedly TTV can infect patients already infected with other viruses previously [7]. Since TTV considered as post-transfusion virus that cause hepatitis, the most important high-risk persons are those infected with HBV and HCV [8].

Epidemiological studies have shown that TTV is described worldwide in various populations. Many studies have been done trying to assess whether TTV could cause liver diseases [9,10]. Although TTV has absence of apparent pathogenicity, the phenomenon of co-infection is common in TTV; it has been detected in co-infection with many other viral species and is known to aggravate various infections like liver disorders, cancer of pancreas, rhinitis and asthma [11].

Table 2. Detection of TTV Ag and clinical data in studied population (total number n=93)

Parameters		TTV Ag				P. value
		Positive		Negative		
		Count	%	Count	%	
Condition	Healthy	10	23.3%	33	76.7%	0.000
	HBV	33	89.2%	4	10.8%	
	HCV	4	30.8%	9	69.2%	
	Total	47	50.5%	46	49.5%	
Sex	Male	27	40.9%	39	59.1%	0.004
	Female	20	74.1%	7	25.9%	
	Total	47	50.5%	46	49.5%	
Surgery	Yes	13	43.3%	17	56.7%	0.338
	No	34	54.0%	29	46.0%	
	Total	47	50.5%	46	49.5%	
Tattoo	Yes	7	53.8%	6	46.2%	0.797
	No	40	50.0%	40	50.0%	
	Total	47	50.5%	46	49.5%	
Blood transfusion	Yes	2	50.0%	2	50.0%	0.982
	No	45	50.6%	44	49.4%	
	Total	47	50.5%	46	49.5%	

Table 3. Age, serum ALT and AST level (U/L) in relation to TTV Ag status in the studied populations (total number n=93)

Parameters	TTV Ag	Mean ± S.D.	P. value
Age (Year)	Positive	34.66 ± 11.651	0.527
	Negative	36.17 ± 11.361	
ALT(U/l)	Positive	44.51 ± 50.285	0.041
	Negative	26.11 ± 23.545	
AST (U/l)	Positive	45.94 ± 50.904	0.352
	Negative	32.63 ± 35.063	

In this study, the overall frequency of TTV infection was 50.5%, TTV Ag was detected in 23.3% of the healthy blood donor as well as in 53.8% of the HCV-positive individuals and in 89.2% of the HBV- positive individuals as shown in Table 2 which is higher than the previously reported frequency in patients [12]. This is may be due to differences in diagnostic techniques, study sample size, and geographic distribution. On the other hand, these data were in concordance with those reported in patients with chronic hepatitis or cirrhosis in Japan, Taiwan and Iran [13,14].

In comparison with laboratory parameters of liver injury, no significant correlation was observed in the level of AST in healthy blood donors and hepatitis patient with or without TTV infection but there was a significant

difference between TTV positive and TTV negative as regards ALT as shown in Table 3, suggesting that the presence of TTV could be associated with more severe liver damage in hepatitis patient. This is similar to Nishizawa et al [6] results, who observed that there is an influence of TTV infection on the worsening of liver enzymes in hepatitis patients; hence there is a pathogenic role of TTV in causing liver injury. In contrast, regarding the biochemical parameters another study reported the absence of significant difference in liver enzyme levels between the TTV-positive and negative hepatitis patients [15].

Considering age of studied population most of the TTV positive individuals were with mean age of 34.66 ± 11.651 year. However, there was no significant difference in age between TTV positive and TTV negative in healthy blood donors and hepatitis patients. This result found to be consistent with Chattopadhyay et al. [16] results, who reported a statistically insignificant difference between the age of TTV positive and TTV negative infected individuals. One more study reported that there was a significant correlation between TTV infection and age [17]. Non-significant association of age in this study could be explained by the fact that this kind of viral infection could occur in people with any age [16].

TTV infection was found in nearly higher proportions among females than males (74.1% vs. 40.9%). The most acceptable explanation for this result is the possibility that women being mostly as multiparous, nosocomial way of transmission as well as the accumulative exposure to various sources (family, occupation, hospital). This is in agreement with another previous study, Al-Qahtani et al. [18], who mentioned that infection rate with TTV was slightly higher in females. In contrast, Chattopadhyay et al. [16] described a notable difference in TTV prevalence according to sex with a higher proportion of males among TTV positive patients.

Considering history of blood transfusion, the present study did not report any significant association with TTV infection, this may be due to the limited number of individuals giving a history of blood transfusion in this study (4 out of 100). In addition, many studies reported that TTV was not associated with blood transfusion history indicating that blood transfusion transmission is not the only way for people to be infected with TTV [17,19]. However, another study showed a significant difference between TTV positive and negative patients regarding blood transfusions [17].

The fact that TTV is also observed in healthy blood donors without history of blood transfusion suggests that it could be transmitted by ways other than blood and injection. Possible mechanisms of transmission need consideration include fecal-oral route, saliva, amniotic fluid from TTV-positive women, breast milk, bile and other tissues [7].

This study showed that no significant association was found between TTV positivity and individuals with history of surgery. This is in disagreement with another previous study which suggested nosocomial transmission of the virus, as a consequence of using contaminated fomites and intravascular catheters, as well as with postoperative wounds and elasticized surgical bandages in surgical patients and surgical wound. Also, instruments, equipment, wound dressing, all may act as sources of infection as a result of contamination from blood and blood products [16,20].

Results from epidemiologic studies regarding the risk of viral infections among tattooed individuals are conflicting [21,22], therefore, this study also tried to investigate the relationship between TTV infection and tattoo in order to determine the risk of transmission of TTV infection. The present study didn't find any association between history of tattooing and TTV infection in all study groups ($P > 0.05$), these findings are consistent with a previous study in Japan [17].

To date, solid proof for the association of TTV in chronic liver damage or any involvement with hepatocellular carcinoma, acute sporadic hepatitis, or non-A-E hepatitis has not been confirmed. Another study suggested that these viruses may serve as commensal organisms that have some beneficial role in maintaining homeostasis in the host. These types of “harmless” viruses have been referred to as orphan viruses [23]. These viruses had been

isolated but had not yet been associated with any known disease. So, they considered as "simple guests," although it may be difficult to attribute the term "guest" or "endosymbiont" to viral agents which, due to their biological characteristics, alter normal cell functions [24].

If TTV is a hepatitis agent, it causes illness in only a small minority of infected persons, the difference possibly depending upon immune responsiveness, host susceptibility or viral load. The finding that TTV most likely to be found in serum of an HIV-infected intravenous drug abuser support the possibility that these high viral titers are mainly due to the immunosuppression [17].

This study concluded that the frequency of TTV Ag was significantly higher in HBV patients than HCV patients and healthy blood donors and may play a role in hepatitis. Detection of TTV among blood donors suggests that these viruses are also transmitted by different routes rather than blood transfusion.

Acknowledgments

To the staff members in Blood Donation Center in Al-Imamein Al-kadhimein Medical City, Gastroenterology and Hepatology Teaching Hospital for their assistance in samples collection.

Author contribution

All authors contributed to this manuscript. Dr. Al-Shuwaikh: design, interpreted and arranged this manuscript, khudair: performed all the laboratory work, implementation and progress of this study, Dr. Farhan: helps in clinical aspect and collection of samples.

Conflict of interest

There is no conflict of interest.

Funding

This work was partially supported by L'Oreal-UNESCO for Women in Science Levant and Egypt.

References

1. Muljono DH, Nishizawa T, Tsuda F, et al. Molecular epidemiology of TT virus (TTV) and characterization of two novel TTV genotypes in Indonesia. Arch Virol. 2001; 146(7): 1249-66.
2. Biagini P. Human circoviruses. Vet Microbiol. 2004; 98(2): 95-101.
3. AbuOdeh R, Al-Mawlawi N, Al-Qahtani AA, et al. Detection and genotyping of Torque Teno virus (TTV) in healthy blood donors and patients infected with HBV or HCV in Qatar. J Med Virol. 2015; 87(7): 1184-91. doi: 10.1002/jmv.24146.
4. Mazzola JC, Saito PK, Yamakawa RH, et al. Prevalence of Torque Teno virus in healthy donors of Paraná State, southern Brazil. Rev Bras Hematol Hemoter. 2015; 37(5): 336-40. doi: 10.1016/j.bjhh.2015.07.005.
5. De Castro Amarante MF, Kashima S, Covas DT. TT virus (TTV) genotyping in blood donors and multiple transfused patients in Brazil. Virus Genes. 2007; 35(3): 503–9. doi: 10.1007/s11262-007-0124-x.
6. Nishizawa T, Okamoto H, Konishi K, et al. A novel DNA virus (TTV) associated with elevated transaminase levels in posttransfusion hepatitis of unknown etiology. Biochem Biophys Res Commun. 1997;241(1):92–7. doi: 10.1006/bbrc.1997.7765.
7. Okamoto H, Nishizawa T, Kato N, et al. Molecular cloning and characterization of a novel DNA virus (TTV) associated with posttransfusion hepatitis of unknown etiology. Hepatol Res. 1998; 10(1): 1-16. doi: https://doi.org/10.1016/S1386-6346(97)00123-X.
8. Gerner P, Wirth S. [Characteristics and clinical significance of new hepatotropic virus's hepatitis G virus, TT-virus and SEN-virus]. Monatsschrift Kinderheilkd. 2002; 150(1): 19-26. https://doi.org/10.1007/s112-002-8175-3.
9. Tanaka Y, Primi D, Wang RY, et al. Genomic and molecular evolutionary analysis of a newly identified infectious agent (SEN virus) and its relationship to the TT virus family. J Infect Dis. 2001; 183(3): 359-67. doi: 10.1086/318091.
10. Hino S, Miyata H. Torque Teno virus (TTV): current status. Rev Med Virol. 2007; 17(1): 45-57. doi: 10.1002/rmv.524.
11. Tyschik EA, Shcherbakova SM, Ibragimov RR, et al. Transplacental transmission of Torque Teno virus. Virol J. 2017; 14(1): 92. doi: 10.1186/s12985-017-0762-0.
12. Jahan S, Rizvi M, Azam M, et al. SEN and Torque Teno virus: Putative agents of Non-A-E viral hepatitis. J Immunol Vaccine Technol. 2015; 1(1): 104.
13. Wolff C, Diekmann A, Boomgaarden M, et al. Viremia and excretion of TT virus in immunosuppressed heart transplant recipients and in immunocompetent individuals. Transplantation. 2000; 69(3): 351-6.
14. Pirouzi A, Bahmani M, Feizabadi MM, et al. Molecular characterization of Torque Teno virus and SEN virus co-infection with HIV in patients from Southern Iran. Rev Soc Bras Med Trop. 2014; 47(3): 275-9. doi: http://dx.doi.org/10.1590/0037-8682-0073-2014.
15. Maggi F, Pistello M, Vatteroni M, et al. Dynamics of persistent TT virus infection, as determined in patients treated with alpha interferon for concomitant hepatitis C virus infection. J Virol. 2001; 75(24): 11999–2004. doi: 10.1128/JVI.75.24.11999-12004.2001.

16. Chattopadhyay S, Das BC, Gupta RK, et al. Presence of TT virus infection in chronic hepatitis patients from a hospital in New Delhi, India. Indian J Med Res. 2005; 122(1): 29-33.

17. Zandieh T, Babaahmadi B, Pourfathollah A, et al. Transfusion transmitted virus (TTV) infection in thalassemic patients. Iran J Public Health. 2005; 34(4): 24-9.

18. Al-Qahtani AA, Alabsi ES, AbuOdeh R, et al. Prevalence of anelloviruses (TTV, TTMDV, and TTMV) in healthy blood donors and in patients infected with HBV or HCV in Qatar. Virol J. 2016; 13(1): 208. doi: 10.1186/s12985-016-0664-6.

19. Ukita M, Okamoto H, Nishizawa T, et al. The entire nucleotide sequences of two distinct TT virus (TTV) isolates (TJN01 and TJN02) remotely related to the original TTV isolates. Arch Virol. 2000; 145(8): 1543–59. doi: 10.1007/s007050070075.

20. Umemura T, Alter HJ, Tanaka E, et al. Association between SEN virus infection and hepatitis C in Japan. J Infect Dis. 2001; 184(10): 1246–51. doi: 10.1086/324210.

21. Jafari S, Buxton JA, Afshar K, et al. Tattooing and risk of hepatitis B: A systematic review and meta-analysis. Can J Public Heal. 2012; 103(3): 207-12.

22. Deschesnes M, Demers S, Finès P. Prevalence and characteristics of body piercing and tattooing among high school students. Can J Public Heal. 2006; 97(4): 325-9.

23. Lemon SM, Layden TJ, Seeff L, et al. The 20th United States-Japan Joint Hepatitis Panel Meeting. Hepatology. 2000; 31(3): 800-6. doi: 10.1002/hep.510310338

24. Mushahwar IK. Recently discovered blood-borne viruses: Are they hepatitis viruses or merely endosymbionts? J Med Virol. 2000; 62(4): 399-404.

Correspondence to Dr. Arwa M. A. Al-Shuwaikh
E-mail: arwa.mujahid@colmed-alnahrain.edu.iq, arwa_alshwaikh_2004@yahoo.com

Received Sep. 9th 2018
Accepted Dec. 4th 2018

Iraqi JMS

Published by Al-Nahrain College of Medicine
P-ISSN 1681-6579
E-ISSN 2224-4719
Email: iraqijms@colmed-alnahrain.edu.iq
http://www.colmed-alnahrain.edu.iq
http://www.iraqijms.net
Iraqi JMS 2019; Vol. 17(1)

Effects of Different Doses of Gamma Rays and Ascorbic Acid Concentration on Human RBCs for Conservation Purpose

Kharman A. Faraj[1] *PhD*, **Samera H. Abdullah**[2] *MSc*, **Sameen F. Muhammad**[2] *PhD*

[1]Dept. of Physics, College of Science, University of Sulaimani, Iraq, [2]Dept. of Nursing, Technical Institute of Kirkuk, Northern Technical University, Iraq

Abstract

Background Blood preservation and the development of sterile collection sets made possible the developments in blood components preparation, storage and transfusion that we have in today's blood banks and transfusion services.

Objective To investigate the effects of gamma irradiation, ascorbic acid and the combined effect of both on the lifespan of erythrocytes through determining red blood cells hemolysis for conserving it as long as possible without any change in erythrocytes biophysical properties.

Methods The blood was drawn from 10 healthy (5 males and 5 females) volunteers. Sample has been irradiated using 137Cs source. Different concentrations of ascorbic acid were used as an anti-oxidative agent for erythrocytes in blood suspension samples. A spectrometer was used for recording the data.

Results The results showed that 25% of RBCs hemolysis occurred after irradiation with 5Gy of gamma ray during 5th week of storage time while in un-irradiated sample 33.8% of RBCs hemolysis occurred during the 5th week. 25% of RBCs hemolysis for 10 µM of ascorbic acid concentration started after 7th week while for control started after 4th week. The minimum rates of RBCs hemolysis observed in the samples which pre-treated with (7 and 10) µM concentrations of ascorbic acid then irradiated with 1 Gy.

Conclusion The results indicated that irradiation of human blood with a certain doses of gamma ray, treated with small concentration of ascorbic acid or both, the two factors together can protect the blood from hemolysis for a longer time and the minimum rate of red blood cells hemolysis was observed for 10 µM ascorbic acid concentration then irradiation to 1 Gy of gamma ray.

Keywords Gamma ray, ascorbic acid, blood storage, red blood cells, oxidative damage

Citation Faraj KA, Abdullah SH, Muhammad SF. Effects of different doses of gamma rays and ascorbic acid concentration on human RBCs for conservation purpose. Iraqi JMS. 2019; 17(1): 50-56. doi: 10.22578/IJMS.17.1.8

List of abbreviations: ATP = Adenosine triphosphate, DPG= Biophosphoglyceric, GVHD = Graft-versus-host disease, Hb = Hemoglobin, K = Potassium, Na = Sodium, OH = Hydroxyl, RBCs = Red blood cells, SOD= Superoxide dismutase

Introduction

There is a growing concern about the possible health effects associated with exposure to electromagnetic fields.

Due to the insufficient number of blood donors in the suitable time, the process of blood conservation has become a necessary and an inevitable process. The preserved red blood cells (RBCs) for clinical transfusions must meet minimum requirements to the stored blood to continue its metabolic functions and deplete the metabolites necessary to maintain RBCs viability and function.

It is so significant to recognize that RBCs undergo structural and morphological changes

associated with Adenosine Triphosphate (ATP) depletion and oxidative damage of membrane lipids upon storage; such lesions are called storage lesions that depend on storage conditions, storage length and additives used in blood productions [1]. For this, the whole blood used for transfusion can be stored in blood bags at 4 °C, and used within approximately one month to achieve its vital goal.

Mammalian RBCs do not have nucleus like other cells in the body, therefore during their circulation through capillaries they get deformed [2]. This deformation of RBCs is one of properties to maintain viability. If the deformation of cells is too large then the cells will get hemolysis easily. The cytoskeleton structures of the RBCs membranes responsible to restore its shape after deformation by the capillaries [2]. Exposure to ionizing radiation can kill some types of cells while modify others [3].

For checking the validity of stored blood has been investigated by (Marjani et al. 2007) and (Huyut et al. 2016). These authors investigated the malondialdhyde formation and antioxidant enzyme activity in stored blood [4,5]. Reactive oxygen metabolites and free radicals are generated normally in aerobic organisms. Membrane lipids are major targets for cellular damage of radical mediated lipid peroxidation. It is recognized that aerobic cells are primary protected from the free radical damage by endogenous antioxidants [6]. The effect of gamma radiation on the stored blood was investigated in several studies using wide range of doses. Moor and Ledoford (1985) who studied the effect of 40 Gy irradiation on the in-vitro storage properties of packed red cells [7], Anand et al. (1997) from zero to 50 Gy [8], and Brugnara and Churchill (1992) 2000 cGy [9]. The prevention of graft - versus – host disease (GVHD) is probably one of the major reasons for blood irradiation, and its use in susceptible patients has increased [10,11].

Many studies; Britten (1999), Davey et al. (1992), Pribush et al. (1994) and Katz et al. (1996) indicated that, as the radiation sensitivity of T lymphocyte is different widely from that of red blood cells a high enough dose should be applied to destroy almost all the T lymphocytes while causing as little as possible damage to the red cells [12-15]. Recent studies indicate that doses higher than 30 Gy, are required [16]. Since it causes only minor changes in the concentration of the essential constituents of the red cells, e.g. ATP, biophosphoglyceric (DPG) catalase, glutathione peroxides and superoxidedismutase (SOD). However, these higher doses cause potassium leakage from the red blood cells which is attributed to the damage occurred in the cell membrane. In the range of doses used (10-30) Gy, the effect of irradiation when RBCs are stored at 4 °C is to promote a balanced passive exchange of intracellular Potassium (K) for extra cellular Sodium (Na); this exchange does not affect the volume of the RBCs [9]. Radiation damage to RBCs occurs as the hydroxyl (OH) radicals can react with other cellular components in addition to the target molecule.

The aim of this study was to investigate the effects of gamma radiation, ascorbic acid (vitamin c) added in small concentrations, and the combined effect of both on the lifespan of erythrocytes through determining RBCs hemolysis for conserving it as long as possible without any change in erythrocytes biophysical properties.

Methods

A volume of 2.9 ml of blood was drawn from 10 healthy volunteers (5 males and 5 females) with ages between 30-40 years and collected in EDTA tubes.

Gamma irradiation

Blood samples were exposed to different doses of gamma radiation (1, 5, 10, 20 and 30 Gy) and stored at 4 °C. Gamma source was standard from 137Cs source (0.66) MeV (model GB-150 type B). The exposure rate of 0.34 Gy/min. has been calibrated by standard ionization chamber type (NE-2571) of volume 0.06 cc with air kerma, Calibration factor NK=41.0+0.25 mGy/min. measured by electrometer type NE2571/1 manufactured by nuclear enter press

Ltd in UK. The value of absorbance (RBCs hemolysis) was recorded for each dose and repeated weekly up to 10 weeks.

Adding ascorbic acid

0.1 ml of ascorbic acid with different concentrations (1, 3, 5, 7, and 10) µM of ascorbic acid was added to 2.9 ml of blood and stored at 4 °C. Each week 0.05 ml of blood was added to 9 ml of NaCl saline in a tube then 3 mL from the suspension was taken in a standard curette of the spectrophotometer, after shacking gently and carefully the value of absorbance at 577 nm was recorded. This process was repeated each week up to ten weeks.

Ascorbic acid with gamma irradiation

Different concentrations of ascorbic acid (1, 3, and 5 µM) were added into different blood samples and exposed to 20 Gy of gamma radiation. Also, the two (7 and 10 µM) concentrations of ascorbic acid added in to another blood samples then exposed to 1 Gy of gamma radiation, the samples stored at 4 °C and examined for 10 weeks.

The data were recorded using spectrometer model JASCO (V-530), UV/VIS in Japan. The intensity of the peak at 577nm of absorbance spectrum represented the degree of hemoglobin breakdown (degree of hemolysis) [17].

Statistical analysis

The results were presented as mean ± Standard deviation (SD) of percentage and statistical analysis were performed using students t-test (paired and unpaired two tailed) taking ($p < 0.01$) as the significance.

Results

Table (1) shows the percentage of hemolysis of RBCs in the case of control (without irradiation or adding the ascorbic acid) through 10 weeks; in this case we observed that the hemolysis increased as the storage time increased.

Table 1. The relation between absorbance values (RBC hemolysis) for control samples and storage times through 10 weeks at 4 °C

Storage time in weeks	Percentages of RBC hemolysis ±SD
1	7.0±1.49
2	13.0±1.34
3	18.5±1.49
4	25.0±1.49
5	33.8±1.55
6	39.0±1.49
7	44.9±1.17
8	50.2±1.49
9	56.3±1.20
10	63.7±1.63

The effect of different doses of gamma radiation (1, 5, 10, 20, and 30) Gy on the RBCs hemolysis of the blood samples are shown in table (2). It was found that irradiation of the blood samples increased RBCs hemolysis, which was undesirable, but with increasing the storage time the rate of the hemolysis decreased in all samples exposed to gamma radiation compared with the controls. It was observed that 25% of RBCs hemolysis occurred after irradiation with 5 Gy during the fifth week, while in un-irradiated sample 33.8% of RBCs hemolysis occurred during the fifth week, the difference was significant ($p<0.01$) between them.

Table 2. The relation between RBC hemolysis for blood samples exposed to different doses of gamma radiation and stored at 4 °C through 10 weeks

Gamma radiation dose Gy	Percentages of RBC hemolysis ±SD through 10 weeks									
	1	2	3	4	5	6	7	8	9	10
1	4.0± 1.22	8.0±1 .26	13.0± 1.56	16.0± 1.67	20.0± 1.58	23.9± 1.25	27.6± 1.42	30.0± 1.43	32.4± 1.67	34.7± 1.37
5	6.4± 1.19	9.2± 1.11	15.0± 1.21	21.2± 1.56	25.0± 1.87	29.5± 1.73	33.0± 1.63	36.3± 1.69	38.2± 1.72	41.1± 1.24
10	6.8± 1.33	10.0± 1.55	15.9± 1.48	22.5± 1.59	26.0± 1.23	31.0± 1.20	34.7± 1.31	38.0± 1.40	41.2± 1.73	43.4± 1.82
20	7.2± 1.87	12.0± 1.17	17.3± 1.28	24.0± 1.51	27.5± 1.29	32.0± 1.62	35.4± 1.77	39.0± 1.30	42.2± 1.14	45.0± 1.32
30	7.5± 1.15	13.5± 1.43	19.0± 1.23	25.0± 1.49	28.9± 1.54	34.0± 1.17	37.2± 1.40	41.8± 1.53	44.9± 1.61	47.8± 1.31

The results of the blood samples treated with different ascorbic acid concentrations (1, 3, 5, 7 and 10) µM are shown in table (3). The results showed that 25% of RBCs hemolysis started after 7th week while in untreated one RBCs hemolysis reaches 25% after 4th week only. 25% of RBCs hemolysis for 10 µM of ascorbic acid concentration started after 7th week while for control started after 4th week, this difference was significant ($p<0.01$).

Table 3. The relation between RBC hemolysis for blood samples treated with different concentrations of ascorbic acid and storage times through 10 weeks at 4 °C

concentrations of ascorbic acid µM	Percentages of RBC hemolysis ±SD through 10 weeks									
	1	2	3	4	5	6	7	8	9	10
1	1.0± 1.23	2.0± 1.34	3.0± 1.40	4.3± 1.28	5.7± 1.32	8.0± 1.65	10.0± 1.54	12.0± 1.48	14.2± 1.39	16.7± 1.46
3	1.3± 1.26	2.6± 1.87	4.2± 1.94	6.3± 1.82	8.4± 1.25	10.0± 1.67	13.0± 1.97	15.3± 1.60	18.0± 1.59	21.3± 1.33
5	1.9± 1.11	3.0± 1.19	7.0±1 .61	11.6± 1.75	14.0± 1.83	17.0± 1.54	19.4± 1.20	21.9± 1.16	23.0± 1.22	25.0± 1.43
7	4.0± 1.19	9.8± 1.41	12.0± 1.49	15.2± 1.62	17.7± 1.58	20.1± 1.67	22.5± 1.92	25.0± 1.44	27.2± 1.88	29.5± 1.73
10	5.2± 1.13	10.4± 1.56	13.7± 1.37	17.2± 1.47	20.4± 1.73	22.9± 1.94	25.0± 1.76	27.0± 1.57	29.3± 1.53	33.0± 1.82

The changes in percentages of RBCs hemolysis with respect to the storage time (through 10 weeks) were studied for blood samples pre-treated with (1,3 and 5) µM and (7 and 10) µM then irradiated with 20 Gy and 1 Gy respectively, the results are shown in tables (4 and5). In pre-treated with (1, 3 and 5) µM concentrations of ascorbic acid then irradiated to 20 Gy, we observed that 25% of RBCs hemolysis for 3µM concentration of ascorbic acid started at 5th week while for 1µM this ratio of hemolysis started between 6th and 7th weeks as shown in table (4).

Table 4. The relation between percentages of RBC hemolysis for blood samples treated with low ascorbic acid concentrations then exposed to 20 Gy dose of gamma radiation and storage times through 10 weeks at 4 °C

Blood samples treated with ascorbic acid and exposed to 20 G	Percentages of RBC hemolysis ±SD through 10 weeks									
	1	2	3	4	5	6	7	8	9	10
1	3.0± 1.23	7.9± 1.1	14.0± 1.24	17.5± 1.26	20.4± 1.38	23.8± 1.57	27.6± 1.68	30.0± 1.75	32.2± 1.89	33.2± 1.66
3	4.8± 1.23	9.6±1 .45	14.4± 1.34	20.2± 1.33	25.0± 1.67	27.5± 1.42	30.0± 1.56	33.3± 1.38	35.2± 1.74	36.8± 1.56
5	6.7± 1.21	10.0± 1.35	15.2± 1.60	21.5± 1.43	26.0± 1.49	28.9± 1.65	31.7± 1.23	34.5± 1.72	36.2± 1.56	38.4± 1.29

The minimum rates of RBCs hemolysis observed in samples which pre-treated with (7 and 10) µM concentrations of ascorbic acid then irradiated with 1 Gy as shown in table (5), in this case 25% of RBCs hemolysis started at 9th week.

Table 5. The relation between percentages of RBC hemolysis for blood samples treated with different high ascorbic acid concentrations then exposed to 1 Gy dose of gamma radiation and storage times through weeks at 4 °C

Blood samples treated with a ascorbic acid and exposed to 1 Gy	Percentages of RBC hemolysis ±SD through 10 weeks									
	1	2	3	4	5	6	7	8	9	10
7	2.3± 1.31	5.5± 1.82	7.8± 1.32	12.0± 1.59	15.5± 1.42	18.4± 1.63	20.0± 1.49	22.0± 1.24	24.0± 1.28	26.5± 1.26
10	1.8± 1.16	4.0± 1.22	6.2± 1.43	10.0± 1.38	13.0± 1.12	15.5± 1.29	17.6± 1.61	20.0± 1.52	22.0± 1.18	24.0± 1.38

Discussion

The validity of stored blood has been investigated by different studies and the current study was done to show the effects of gamma radiation, ascorbic acid added in small concentrations, and the combined effect of both on the lifespan of erythrocytes through determining RBCs hemolysis for conserving it as long as possible without any change in erythrocytes biophysical properties. As shown in table (1), the percentage of hemolysis of RBCs in the case of control increased as the storage time increased through 10 weeks due to the elevation of lipid peroxidation and hemoglobin oxidation [8] and extracellular potassium (K) concentration in red blood cells storage [18].

In irradiation of blood samples to different doses of gamma radiation (1, 5, 10, 20, and 30) Gy, it was found that RBCs hemolysis increased but with increasing the storage time the rate of the hemolysis decreased in all samples compared with the controls. During the fifth week, it was observed that 25% and 33.8% of RBCs hemolysis occurred after irradiation with 5Gy and in un-irradiated sample respectively, and the difference was significant ($p<0.01$) between them as shown in table (2). Mintz and Anderson (1993) found that the mean Potassium (k) and hemoglobin (Hb) concentration at the end of 35 days of storage for both the irradiated group (30 Gy) and the un-irradiated one were not significantly different [19].

Treated the blood samples with different ascorbic acid concentrations (1, 3, 5, 7 and 10) µM showed the role of antioxidant effect of ascorbic acid which increased with increasing the concentrations up to 10µM. Results of this study match the results that by Lenton et al (2003), who affirmed that ascorbic acid plays a vital and central role in the defense against free radicals and oxidants that are implicated in chronic diseases [20].

Different effects were obtained in pre-treated with of ascorbic acid then irradiated to gamma ray. The minimum rates of RBCs hemolysis observed in samples which pre-treat high concentrations of ascorbic acid then irradiated with 1 Gy. The results indicated that with increasing ascorbic acid concentration low dose of gamma radiation required to obtain minimum RBCs hemolysis.

The obtained results indicated that irradiation of human blood with a certain doses of gamma ray, treated with small concentration of ascorbic acid or both, the two factors together can protect RBCs of blood from hemolysis for a longer time. The minimum rate of RBCs hemolysis was observed for 10 µM ascorbic acid concentration with irradiation to 1 Gy of gamma ray. We observed when the concentration of the ascorbic acid in blood samples increased; low levels of gamma irradiation were added to obtain the minimum RBCs hemolysis.

Acknowledgments

The authors would like to thank General Kirkuk Hospital and Faculty of Pure Science at Tikrit University for their help and facilities.

Author contribution

Dr. Faraj: writing the article with resolving the data. Abdullah and Dr. Muhammad performed the practical part of the work.

Conflict of interest

None.

Funding

personal funding.

References

1. Veale MF, Healey G, Sparrow RL. Effect of additive solutions on red blood cell (RBC) membrane properties of stored RBCs prepared from whole blood held for 24 hours at room temperature. Transfusion. 2011; 51 Suppl 1: 25S-33S. doi: 10.1111/j.1537-2995.2010.02960.x.
2. Murray RK, Bender DA, Botham KM, et al. Harper's illustrated biochemistry. 28th ed. The McGraw-Hill Companies; 2009.
3. Ali FM, Elgebaly RH, Kheder MH, et al. Evaluation of the hazards associating injection of heart diseased patient with radiophrmaceuticals for imaging with gamma camera. Proceedings of seventh international symposium on change and continuity in radiation protection", Cardiff, UK, 2005.
4. Marjani A, Moradi A, Ghourcaie AB. Alterations in plasma lipid peroxidation and erythrocyte superoxide dismutase and glutathione peroxidase enzyme activities during storage of blood. Asian J Biochem. 2007; 2: 118-23. DOI: 10.3923/ajb.2007.118.123.
5. Huyut Z, Şekeroğlu MR, Balahoroğlu R, et al. The relationship of oxidation sensitivity of red blood cells

and carbonic anhydrase activity in stored human blood: effect of certain phenolic compounds. Biomed Res Int. 2016; 2016(4): 1-8. doi: 10.1155/2016/3057384.

6. Lobo V, Patil A, Phatak A, et al. Free radicals, antioxidants and functional foods: Impact on human health. Pharmacogn Rev. 2010; 4: 118-26. doi: 10.4103/0973-7847.70902.
7. Moor GL, Ledoford ME. Effect of 4000 rad irradiation on the in vitro storage properties of packed red cell. Transfusion. 1985; 25(6): 583-5.
8. Anand AJ, Dzik WH, Imam A, et al. Radiation induced red cell damage; role of reactive oxygen species. Transfusion. 1997; 37: 160-5.
9. Brugnara C, Churchill WH. Effect of Irradiation on red cell cation content and transport. Transfusion. 1992; 32: 246-8.
10. Moroff G, Leitman SF, Luban NL. Principles of blood irradiation dose validation and quality control. Transfusion. 1997; 37(10): 1084-92.
11. Jacob GB. A review on the effect of ionizing radiation on blood and blood components. Radiat Physics Chem. 1998; 53(5): 511-23.
12. Britten AJ. A method to evaluate intra-operative gamma probes for sentinel lymphatic node localization. Eur J Nucl Med. 1999; 26(2): 76-83.
13. Davey RJ, McCoy NC, Yu M, et al. The effect pre-storage radiation on post transfusion red cell survival. Transfusion. 1992; 32(6): 525-8.
14. Pribush A, Agam G, Yermiahu T, et al. Radiation damage to the erythrocyte membrane the effect of medium and cell concentration. Free Radic Res. 1994; 21(3): 135-46.
15. Katz D, Mazor D, Dvilansky A, et al. Effect of radiation on Red blood cell membrane and intra cellular oxidative defense system. Free radiology Research. 1996; 24(3): 199-204.
16. Effects of ionizing radiations on blood and blood components: a survey, IAEA, Austria, 1997.
17. Goldberg B, Stern A. The role of superoxide anion as a toxic species in the erythrocyte. Arch Biochem Biophys. 1977; 178(1): 218-25.
18. Marnett LJ. Lipid peroxidiation-DNA damage by malondialdehde. Mutat Res. 1999; 424(1-2):83-95.
19. Mintz PD, Anderson G. Effect of gamma irradiation on the in vivo recovery of stored red blood cells. Ann Clin Lab Sci. 1993; 23(3): 216-20.
20. Lenton KJ, Sané AT, Therriault H, et al. Vitamin C augments Lymphocytes glutathioue in subjects with ascorbic deficiency. Am J Clin Nutr. 2003; 77(1): 189-95. doi: 10.1093/ajcn/77.1.189.

Correspondence to Dr. Kharman A. Faraj
E-mail: kharman.faraj@univsul.edu.iq
Received Mar. 8th 2018
Accepted Aug. 9th 2018

Iraqi JMS

Published by Al-Nahrain College of Medicine
P-ISSN 1681-6579
E-ISSN 2224-4719
Email: iraqijms@colmed-alnahrain.edu.iq
http://www.colmed-alnahrain.edu.iq
http://www.iraqijms.net
Iraqi JMS 2019; Vol. 17(1)

Association of Human Herpes Virus 6A Infection in Endometrial Epithelial Cells with Miscarriage

Huda S. Dosh[1] *BSc*, **Ahmed S. Abdulamir**[2] *PhD*, **Sahar H. Abdul-Razzaq**[3] *FACMS-OG, CABOG, DGO*

[1]Dept. of Medical Laboratory Techniques, Ministry of Health Directorate of Wasit, General Al-Suwayrah Hospital
[2]Dept. of Microbiology, [3]Dept. of Gynecology, College of Medicine, Al-Nahrain University, Baghdad, Iraq

Abstract

Background The microbial infections are major cause in abortion, of which viruses appear to be the most frequently involved pathogens. Human Herpes Virus-6 (HHV-6) has been implicated in cases of poor pregnancy outcome.

Objective Detection and quantification of HHV-6 viral load along with titer of IgM and IgG in endometrial epithelial cells of women with unexplained miscarriage (cases group) compared to the decidual endometrial tissue in women with full term conception (the control group).

Methods A total of 90 samples, 45 were taken by curettage from miscarriage women at first and second trimesters of pregnancy and 45 were taken from women with full term pregnancy during caesarian operation. HHV-6 DNA was extracted from uterus lining tissue samples, and then it was detected and measured by real time qPCR; in addition, 3 ml of blood were collected from each subject for detection of HHV-6 IgM and IgG antibodies.

Results HHV-6 DNA was detected in uterus lining tissue of 20% of abortion and 22.22% of controls (P value=0.79). The median viral load of HHV-6 per 106 of uterine cells in abortion group was 15, while for control group was 13 (P=0.54). HHV-6 load increased with increased number of previous abortions, and it increased with presence of recurrent abortion history. It showed significant differences in the median of HHV-6 DNA load between recurrent abortion and non-recurrent abortion groups (P value =0.033). Positive anti-HHV-6 IgM serum antibodies in HHV-6 DNA detected and no-detected subjects were 0%, and 9.85% respectively; the positive anti-HHV-6 IgG serum antibodies in detected HHV-6 DNA were 100%, while 83% in HHV-6 DNA non-detected subjects. The combination of positive/negative results of the anti-HHV-6 IgG and IgM serum antibodies in HHV-6 DNA detected versus non-detected group showed 100% of HHV-6 DNA detected were positive for IgG and negative for IgM, versus 73% in non-detected group.

Conclusion HHV-6 might associated with unexplained miscarriage. HHV-6 infection might increase with increased number of previous abortions, and increased with presence of recurrent abortion history.

Keywords Human Herpes Virus 6A, Miscarriage

Citation Dosh HS, Abdulamir AS, Abdul-Razzaq SH. Association of Human Herpes Virus 6A infection in endometrial epithelial cells with miscarriage. Iraqi JMS. 2019; 17(1): 57-65. doi: 10.22578/IJMS.17.1.9

List of abbreviations: HHV-6 = Human herpesvirus-6, IgG = Immunoglobulin G, IgM = Immunoglobulin M

Introduction

Human herpesvirus-6 (HHV-6) is an ubiquitous beta-herpes virus commonly distributed in the general population. The primary infection usually occurs in the early years of life and remains latent in the host for the lifelong period [(1)]. HHV-6 has been recognized as the etiological agent of *Rosella infantum*. HHV-6 includes two variants, HHV-6A and HHV-6B. Recently, two major subspecies have distinguished on the basis of biological, immunological, and genetic divergence [(2)].

Both variants infect mainly T-cells. The majority of population is infected by HHV6-B by 2 years of age and HHV-6A infection frequently occurs later in life. HHV-6A and B have varied tropism [(1,3)]. Evidence suggests that HHV-6 can infect and replicate in human genital tract. HHV-6 DNA has

been detected in genital tract secretion from pregnant and non-pregnant women. Numerous studies have reported the high viral shedding in pregnant women [4-6].

Clinical manifestation of HHV-6 have not been clearly defined, especially in adult patients and the role of HHV-6 in human diseases remains to be completely explained. Numbers of studies trying to find and evaluate the role of beta-herpes viruses' infection in the development of various chronic diseases, but still there is no final answer for this question. It may be due to their ubiquitous nature and different mechanisms to interference with the host that these viruses are using [1,5,7].

Miscarriage is the spontaneous loss of pregnancy. There are two types of miscarriage before 12 weeks called early miscarriage and from 12 to 24 weeks called late miscarriage [8]. Pregnancy loss has been attributed to several factors involved in human reproduction. The causes of abortions in many cases are still unknown. However microbial infections have been characterized as a major cause in abortion, of which viruses appear to be the most frequently involved pathogens [9,10].

HHV-6 has been implicated in cases of poor pregnancy outcome due to ability of virus to disrupt endometrium epithelial cells function that lead to inhibit the creation of appropriate uterine environment for implantation and fetal growth that lead to infertility and miscarriage [11].

In 2016, HHV-6A was found in endometrial biopsies from primary unexplained infertile women, but not in control women. On the contrary, HHV-6B was not found in endometrial biopsies of both groups [12]. In another studies, the diagnosis of HHV-6A infection in pregnant women, and the detection of HHV-6A DNA in all of fetal tissue, umbilical cord blood samples of healthy newborns, and the placental tissue suggest the possible role of the virus in miscarriage at 24 weeks of gestation [7,13,14].

Several diagnostic methods used for viral detection among them quantitative real time polymerase chain reaction (real-time qPCR) in endometrial epithelial cells samples which is considered the method of choice [12].

This study aimed to investigate the association of HHV-6 with the unexplained miscarriage of women by doing the following:

1. Detection of HHV-6 in endometrial epithelial cells of women with unexplained miscarriage (cases group) compared to the decidual endometrial tissue in women with full term conception, (the control group).
2. Serological evaluation of HHV-6 in the serum of women with unexplained miscarriage compared to control group, namely the in women with full term conception.

Also aimed to evaluate the feasibility of using HHV-6 serology as a cheap and simple indicator for underlying HHV-6 infection of endometrium and assess the odd ratio of using HHV-6 serology as a possible predictor for unexplained miscarriage.

Methods

The current study is case control study conducted in the period from November 2017 to September 2018. A total of 90 subjects were involved in this study, 45 women miscarriage at first and second trimesters of pregnancy and 45 women with full term pregnancy who underwent caesarian operation in Al-Imamein Al-Kadhimein Medical City. A consent letter was signed by each volunteer, and the study was approved by the ethical committees of the Ministry of Health of Iraq and of Al-Nahrain Collage of Medicine.

Inclusion criteria

Women with age ranged from 20 to 40 years old with unexplained miscarriage till 24 weeks of pregnancy were taken as cases, while women with full-term pregnancy during the conduction of cesarean operation have more than one successful pregnancy were taken as controls.

Exclusion criteria

Women with other causes of miscarriage such as endocrine disorder (diabetes mellitus, thyroid disorder), anatomical causes acquired or congenital thrombophilia and other infection

causes miscarriage such as toxoplasmosis, cytomegalovirus infection, rubella and herpes simplex-2.

HHV-6 DNA detection and quantification

Accordingly, 90 biopsies of uterus-lining tissues in which HHV-6 is suspected to be found, were collected. The tissue lining uterus at the first trimester of pregnancy was routinely taken by curettage from 45 women with unexplained miscarriage; the endometrial tissue was identified from placental tissue by the consultant gynecologist of this study. In addition, the decidual tissue, the uterus lining tissue at full term gestation was taken from 45 women with full-term pregnancy during the conduction of cesarean operation. Tissue samples of approximately 2 grams were cut and put in Eppendorf tubes and stored at -20 ^{o}C till DNA extraction. HHV-6 DNA was detected and measured by real time qPCR. Reaction volume=25 µl (15 µl reaction mix +10 µl of extracted DNA). The thermal protocol used was specified by the kit manufacturer (Sacace/ Italy), namely HHV-6 Real-TM Quant.

Serology of HHV-6 IgG and IgM antibodies

In addition, 3 ml of blood were collected from each subject and put in a plane tube and then were subjected to centrifugation for serum separation. Serum samples were subjected for Enzyme Linked Immune Sorbent Assay (ELISA) technique for detection of HHV-6 IgM and IgG. The HHV-6 IgM and IgG ELISA kit (VIDIA/Czechoslovakia) for the semi-quantitative measurement of IgM and IgG antibodies titer to HHV-6 in human serum. The strips are coated with native HHV-6 antigen. If the specimens contain IgM or IgG antibodies to HHV-6, it will bind to the immobilized antigens to form immobilized immune complexes. The immune complexes are recognized by animal anti- human IgM or IgG antibodies with enzyme. The enzymatic reaction was revealed with chromogen substrate to produce a yellow color indicating the amount of HH-6 IgM and IgG antibodies present in the specimens.

Statistical analysis

For the nominal qualitative data, Chi square and Fisher exact tests were used for association measurement. For quantitative data mean or median difference measuring tests were used. For parametric data, student t-test was used, while for non-parametric data Mann-Whitney test was used. Odds ratio is used to study the to compare the relative odds of occurrence of the outcome. In addition, P value was used to test the null hypothesis and determine the significance of testing. P value less than 0.05 was indicative for a significant result.

Results

The mean of age of abortion group was 29.17 and of control group was 28.22 range from 20 to 40 years old. This study showed that uterus lining tissue of 9 out of 45 abortion (20%) and 10 out of 45 control group (22.22%) harbored HHV-6 in by using Real Time PCR. However, the rate of HHV-6 detection was not significantly different (P value=0.79) between abortion and control groups (Table 1).

Table 1. Detection rate of HHV-6 in uterus lining tissue between abortion and controls groups

	Detected	Non-detected	Total
Abortion	9	36	45
	20.0%	80.0%	100%
Control	10	35	45
	22.22%	77.78%	100%
Chi square p value=0.79			

This study showed a higher frequency of recurrent abortions in HHV-6 DNA detected group than HHV-6 DNA non-detected group but it did not reach the statistical significance level (P value=0.14). It was 15.79% from HHV-6 DNA detected group versus only 5.63% of HHV-6 DNA non-detected group with recurrent abortions (Table 2).

Table 2. The frequency of recurrent abortions between HHV-6 DNA detected and HHV-6 DNA non-detected in both abortion and control groups

Recurrent Abortions	Yes	No	Total
HHV-6 DNA Detected	3	16	19
	15.79%	84.21%	100%
HHV-6 DNA Non-detected	4	67	71
	5.63%	94.37%	100%
Chi square p value=0.14			

In the abortion group only, this study also showed a higher frequency of recurrent abortions in HHV-6 DNA detected group than HHV-6 DNA non-detected group but it did not reach the statistical significance level this study showed non-significant differences (P value=0.1). It showed 33.33% of HHV-6 DNA detected with recurrent abortions, and only 11.11% of HHV-6 DNA non-detected group with recurrent abortions (Table 3).

Table 3. The frequency of recurrent abortions between HHV-6 DNA detected and HHV-6 DNA non-detected in abortion group

Recurrent Abortions	Yes	No	Total
HHV-6 DNA Detected	3	6	9
	33.33%	66.67%	100%
HHV-6 DNA Non-detected	4	32	36
	11.11%	88.89%	100%
Chi square p value=0.1			

On the other hand, the comparison of HHV-6 DNA load in HHV6-DNA positive only subjects in both abortion and control groups in regard to presence or absence of recurrent abortion history showed significant differences in the median of HHV-6 DNA between recurrent abortion and no recurrent abortion groups (P value =0.033). The median of HHV-6 DNA load in women with recurrent abortion was 36 copy no./10^6 cells, and 13 copy no./10^6 cells in non-recurrent abortion (Table 4).

Table 4. Comparison of HHV-6 DNA load in uterus lining tissue regard to recurrent abortion history

Recurrent Abortions	Yes	No
Median (Copy no./10^6 cells)	36	13
Standard Deviation	20.43	1946.9
Mann Whitney, P=0.033 (Significant)		

The positive/negative results of the anti-HHV-6 IgM serum antibodies in both abortion and control groups subjects with detected HHV-6 DNA versus non-detected HHV-6 DNA showed (0%) of HHV-6 DNA detected were with positive IgM index versus up to (10%) in HHV-6 DNA non- detected group but it did not reach the statistical significant (P value=0.33). The odds ratio 4.53 refers that the odds of PCR to detect HHV-6 DNA in the lining tissue of uterus is 4.5 when IgM-HHV-6 is negative (Table 5).

Table 5. The positive/negative results of the anti-HHV-6 IgM serum antibodies in both abortion and control groups subjects with detected HHV-6 DNA versus non-detected HHV-6 DNA

	Negative IgM Index	Positive IgM Index	Total
HHV-6 DNA Detected	19	0	19
	100%	0%	100%
HHV-6 DNA Non-detected	64	7	71
	90.14%	9.85%	100%
Fisher exact, P= 0.33, Odds ratio=4.53, P=0.32			

The positive/negative results of the anti-HHV-6 IgG serum antibodies in both abortion and control group subjects between detected HHV-6 DNA versus non-detected HHV-6 DNA groups showed (100%) of HHV-6 DNA detected subjects were positive, while (83%) of HHV-6 DNA non-detected were positive with borderline statistical significance (P value=0.057). The odds ratio 8.2 that mean the odds for PCR to detect HHV-6 is 8 when IgG-HHV-6 is positive (Table 6).

The combination of positive/negative results of the anti-HHV-6 IgG and IgM serum antibodies in both abortion and control group subjects with detected HHV-6 DNA versus non-detected showed significant association between serology of HHV-6 antibodies and the presence of HHV-6 DNA in uterus tissue (Fisher exact, P value=0.038). It was shown 100% of HHV-6 DNA detected subjects were positive for IgG and negative for IgM versus 73% of non-detected subjects (Figure 1).

Table 6. The positive/negative results of the anti-HHV-6 IgG serum antibodies in both abortion and control groups subjects with detected HHV-6 DNA versus non-detected HHV-6 DNA

	IgG Index Positive	IgG Index Negative	Total
HHV-6 DNA Detected	19	0	19
	100%	0%	100%
HHV-6 DNA Non-detected	59	12	71
	83.1%	16.9%	100%
Fisher exact, P= 0.057, Odds ratio=8.2, P=0.15			

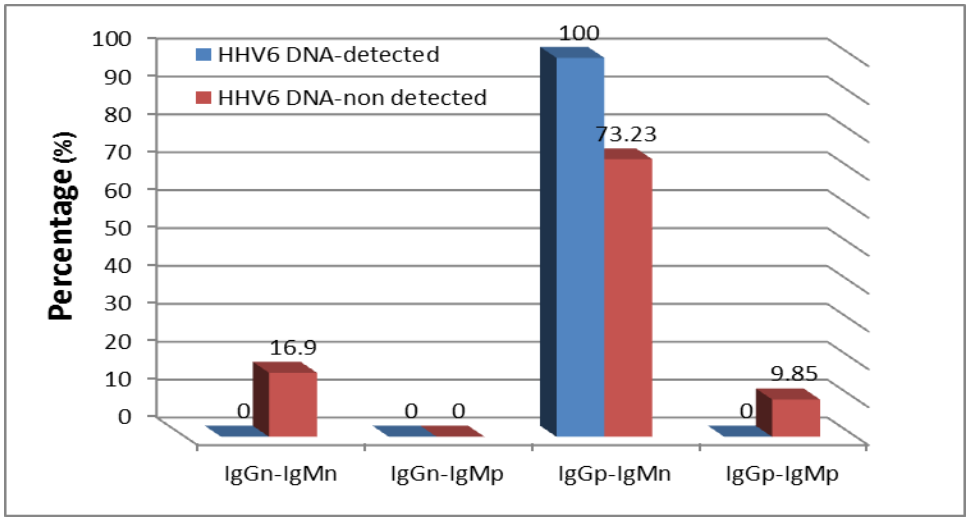

Figure 1. The combination of positive/negative results of the anti-HHV-6 IgG and IgM serum antibodies in both abortion and control group subjects with detected HHV-6 DNA versus non-detected HHV-6 DNA, a: IgGn-IgMn: both serum IgM and IgG are negative, b: IgGn-IgMp: serum IgG is negative and IgM is positive , c: IgGp-IgMn: serum IgG is positive and IgM is negative, d: IgGp-IgMp: both serum IgM and IgG are positive

Discussion

Miscarriage and infertility are prevalent around the world and in many cases, the causes are unknown. HHV-6 (particular HHV-6A) is an etiological agent or a risk factor in a portion of these cases [7,11,12].

In this study, HHV-6 DNA positivity was detected in 20% of miscarriage endometrial tissue while the uterus lining tissue of control group showed HHV-6 DNA in 22.22%. There was no significant difference in the detection of the virus between both groups. Previous reports showed that HHV-6 DNA has been detected in genital tract secretions from pregnant and non-pregnant women [4,5]; HHV-6 DNA and antigens have been identified in biopsies of archived cervical samples [15,16], and these suggest that the female genital tract may be the secondary site for HHV-6 persistence.

The effect of virus on the pregnancy outcome related with other factors such as hormonal effect [12], immunological statues of individual [12,17,18] that lead to reactivate of virus, and inhibit the creation of appropriate uterine environment for implantation and fetal growth that lead to miscarriage [11].

However, the history of previous abortions and recurrent abortions showed non-significant effect on HHV-6 DNA detection rate; it may be due to low samples size; this disagrees with the

results of previous study that showed recurrent abortion due to maternal infections transmissible in utero at various stage of gestation can be caused by a wide array of organisms including HHV-6 [19].

The viral load of HHV-6/10^6 cells of uterus lining tissue in positively detected cases was approximately 14.5 HHV-6 per 10^6; the low viral load could be attributed to low samples size because one of abortion cases showed viral load equal 336100 virion/10^6 cells. In addition, the viral load of HHV-6/10^6 cells of uterus lining tissue showed non-significant differences between abortion and control groups. This disagrees with the results of previous research carried out recently in Italy on females with primary unexplained infertility in which viral load in endometrial tissue was shown to be 4 copies of HHV-6A per one diploid cell [12]. This stark difference in level of HHV-6 load in our study compared to that previous study may be attributed to less association of virus with miscarriage than female infertility. It could also be due to variations in environmental factors, estrogen levels, and immunological status between the population of the two studies effecting viral behavior.

The viral load significantly increased with the presence of recurrent abortion status (P value =0.033), this supports the notion that HHV-6 may be associated with unexplained recurrent abortion; and this agrees with the findings of a couple of previous studies that showed recurrent abortion due to maternal infections transmissible in utero at various stage of gestation can be caused by a wide array of organisms include HHV-6 [19,20], the clinical importance of this finding warrants further investigation.

Enzyme linked immune sorbent assay test for measurement HHV-6 IgM showed only 6.67% positive from abortion group, and 8.89% of control group was positive with non-significant differences; immunosuppression during pregnancy could be a possible reason for low percentage in both groups [21].

This result agrees with results of previous studies on women who developed pityriasis rosea, which associated with the reactivation of HHV-6 and 7 during first trimester of pregnancy leading to high rate of abortion that showed HHV-6 IgM–negative for all samples [22,23]. This disagrees with the results of (Ando et al, 1992) who showed increased anti-HHV-6 IgM titer in abortion group [24]; this is may be due to geographical differences as well as to variation in the type of infection, primary or reactivation of latent infection [25,26].

All HHV-6 detected samples in abortion and control group was negative for anti HHV-6 IgM with odds ratio 4.53, which means the odds are 4.53 for PCR to detect HHV-6 in uterus lining tissue when IgM-HHV-6 is negative; it may be due to immunosuppression status during pregnancy where more immunosuppression leads to more chances of HHV-6 reactivation and lower titer or absence of HHV-6 IgM [21], and reactivation of latent infection due to various factors including hormonal effect [12] that more related with elevation of IgG antibodies [26] than IgM antibodies that related with primary infection [25]. This result agrees with the result of previous studies on women with pityriasis rosea, who were associated with the reactivation of HHV-6 and 7 during first trimester of pregnancy leading to high rate of abortion; they showed that HHV-6 IgM–negative for all women with positive PCR for HHV-6 [22,23].

The results of ELISA test detection anti HHV-6-IgG antibody supporting this idea that showed all HHV-6 detected samples in abortion and control group was positive for anti HHV-6-IgG with odds ratio 8.2 that mean PCR turns out positive for HHV-6 when IgG-HHV-6 is positive by ELISA.

Also, 100% of HHV-6 DNA detected samples were positive for IgG and negative for IgM serum anti-HHV-6 antibodies with significant differences. This finding supports the above explanation that HHV-6 reactivate in uterus with immune suppression where IgM is missing and IgG is elevated.

The results of ELISA test for HHV-6 IgG showed 91.11% positive from abortion group, and 82.22% of control group was positive with non-significant differences between both groups. It showed high value of IgG index may be due to same reason of above (reactivation of latent infection due to various factors including

hormonal effect [12] that related with elevation of IgG antibodies [26]).

More studies are needed to confirm the results of this study, further research to confirm whether HHV-6, in particular HH-6A, is an etiological agent or risk factor associated with unexplained miscarriage, and using of accurate treatment may make possible to carry healthy babies for more women.

This study concluded HHV-6 might be associated with unexplained miscarriage. The rate of HHV-6 infection might increase with higher number of previous abortions, and with the presence of recurrent abortion history. Moreover, serum anti-HHV-6 IgM and IgG antibodies by ELISA might be a useful diagnostic method for detection of HHV-6 infection in uterus lining tissue. The rate of HHV-6 infection in uterus lining tissue was higher when serum IgG positive and IgM negative.

Acknowledgments

To the staff members of Gynecology department in Al-Imamein Al-Kadhimein Medical City for their helping in samples collection.

Author contribution

All authors contributed to this manuscript. Dr. Abdulamir: design, interpreted and arranged this manuscript. Dosh: performed all the laboratory work, implementation and progress of this study. Dr. Abdul-Razzaq: help in clinical aspect and samples collection.

Conflict of interest

There is conflict of interest.

Funding

There is no funding.

References

1. Sultanova A. Significance of Beta-herpesviruses (HHV-6, HHV-7) infection under the conditions of immune disorders. Doctoral Thesis 2014. Virology, Rigas Stradina University, Latvia.
2. Ablashi D, Agut H, Alvarez LR, et al. Classification of HHV-6A and HHV6-B as distinct viruses. Arch Virol. 2014; 159(5): 863-70. doi: 10.1007/s00705-013-1902-5.
3. Ahlqvist J, Fotheringham J, Akhyani N, et al. Differential tropism of human herpesvirus-6 (HHV-6) variants and induction of latency by HHV-6A in oligodendrocytes. J Neuro virol. 2005; 11: 384-94. doi: 10.1080/13550280591002379.
4. Ohashi M, Yoshikawa T, Ihira M, et al. Reactivation of human herpesvirus-6 and 7 in pregnant women. J Med Virol. 2002; 67(3): 354-8.
5. Baillargeon J, Piper J, Leach CT. Epidemiology of human herpesvirus-6 (HHV-6) infection in pregnant and non-pregnant women. J Clin Virol. 2000; 16(3): 149-57.
6. Leach CT, Newton ER, Mc Parlin S, et al. Human herpesvirus-6 infection of the female genital tract. J Infect Dis. 1994; 169(1): 1281-3.
7. Revest M, Minjolle S, Veyer D, et al. Detection of HHV-6 in over a thousand samples: new types of infection revealed by an analysis of positive results. J Clin Virol. 2011; 51(1): 20-4. doi: 10.1016/j.jcv.2011.02.001.
8. Giakoumelou S, Wheelhouse N, Cuschieri K, et al. The role of infection in miscarriage. Hum Reprod Update. 2016; 22(1): 116-33. doi: 10.1093/humupd/dmv041.
9. Rock JA, Zacur HA. The clinical management of repeated early pregnancy wastage. Fertil Steril. 1983; 39(2): 123-40.
10. Khameneh, ZR, Hanifian H, Barzegari R, et al. Human parvovirus B19 in Iranian pregnant women: a serologic survey. Indian J Pathol Microbiol. 2014; 57(3): 442-4. doi: 10.4103/0377-4929.138748.
11. Eliassen E, Marci R, Luca DD, et al. The use of heparin in infertility and recurrent pregnancy loss: Are its antiviral properties at play? J Medical Hypotheses. 2017; 102: 41-7. doi: 10.1016/j.mehy.2017.03.009.
12. Marci R, Gentili V, Bortolotti D, et al. Presence of HHV-6A in endometrial epithelial cells from women with primary unexplained infertility. PLoS One 2016; 11(7): e0158304. doi: 10.1371/journal.pone.0158304.
13. Adams O, Krempe C, Kogler G, et al. Congenital infections with human herpesvirus-6. J Infect Dis. 1998; 178(2): 544-6.
14. Aubin, JT, Poirel L, Agut H, et al. Intrauterine transmission of human herpesvirus -6. Lancet. 1992; 340(8817): 482-3.
15. Wang H, Chen M, Berneman ZN, et al. Detection of human herpesvirus-6 in paraffin-embedded tissue of cervical cancer by polymerase chain reaction. J Virol Methods. 1994; 47: 297-305. doi: 10.1016/0166-0934(94)90026-4.
16. Yadav M, Arivananthan M, Kumar S. HHV-6 antigen and viral DNA detected in cervical cells from archived tissue using histochemical staining and hybridization. Clin Diagn Virol. 1996; 7:23–33.
17. Seshadri S, Sunkara SK. Natural killer cells in female infertility and recurrent miscarriage: a systemic review and meta-analysis. Hum Reprod Update. 2014; 20(3): 429-38. doi: 10.1093/humupd/ dmt056.
18. Ozkan ZS, Deveci D, Kumbak B, et al. What is the impact of Th1/Th2 ratio, SOCS3, IL17, and IL35 levels in unexplained infertility? J Reprod Immunol. 2014; 103: 53-8. doi: 10.1016/j.jri.2013.11.002.
19. Nigro G, Mazzocco M, Mattia E, et al. Role of infections in recurrent spontaneous abortion. J Matern Fetal

Neonatal Med. 2011; 24(8): 983-9. doi: 10.3109/14767058.2010.547963.
20. Haider M, Rizvi M, Khan N, et al. Serological study of herpes virus infection in female patients with bad obstetric history. Biol Med. 2011 3; 2: 284-90.
21. Dahl H, Fjaertoft G, Norsted T, et al. Reactivation of human herpesvirus-6 during pregnancy. J Infec Dis. 1999; 180: 2035-8. doi: 10.1086/315115.
22. Drago F, Broccolo F, Zaccaria E, et al. Pregnancy outcome in patients with pityriasis rosea. J Am Acad Dermatol. 2008; 58(5 suppl 1): 78-83. doi: 10.1016/j.jaad.2007.05.030.
23. Drago F, Broccolo F, Javor S, et al. Evidence of human herpsvirus-6 and -7 reactivation in miscarrying women with pityriasis rosea. J Am Acad Dermatol. 2014; 71(1): 198-9. doi: 10.1016/j.jaad.2014.02.023.
24. Ando Y, Kaufman K, Ekuni Y, et al. HHV-6 infection during pregnancy and spontaneous abortion. Lancet. 1992; 340(8830): 1289.
25. Fox JD, Ward P, Briggs M, et al. Production of IgM antibody to HHV-6 in reactivation and primary infection. Epidemiol Infect. 1990; 104(2): 289-96.
26. Ihira M, Yoshikawa T, Suzuki K, et al. Correlation between human herpesvirus 6 and 7 infections after living related liver transplantation. Microbiol Immunol 2001; 45(3): 225-32. doi: https://doi.org/10.1111/j.1348-0421.2001.tb02611.x.

Correspondence to Huda S. Dosh
E-mail: hudadosh1992@yahoo.com
Received Oct. 21st 2018
Accepted Dec. 23rd 2018

Iraqi JMS

Published by Al-Nahrain College of Medicine
P-ISSN 1681-6579
E-ISSN 2224-4719
Email: iraqijms@colmed-alnahrain.edu.iq
http://www.colmed-alnahrain.edu.iq
http://www.iraqijms.net
Iraqi JMS 2019; Vol. 17(1)

Correlation Between Cardiac and Hepatic T2* MRI and Serum Ferritin Level in Patients with Transfusion dependent β-Thalassemia Major

Ahmed K. Yaseen[1] *MBChB*, **Sawsan S. Abbas**[2] *CABP, Associate MRCPCh*, **Ahmed M.A. Abdulhadi** [1] *FICP*

[1]Dept. of Pediatrics, Al-Imamein Al-Kadhimein Medical City, Baghdad, Iraq, [2]Dept. of Pediatrics, College of Medicine, Al-Nahrain University, Baghdad, Iraq

Abstract

Background β-Thalassemia occurs in higher frequency in the Mediterranean area and the Middle East. Classically serum ferritin and liver biopsy have been needed to monitor patient's response to chelation therapy. Magnetic resonance imaging (MRI) has been proved effective in detecting and quantifying iron in the heart and liver.

Objective To assess the value of MRI T2* procedure in assessment of hepatic (LIC) serum ferritin level and MRI T2* of liver and myocardium in patients with BTM.

Methods A cross -sectional study was conducted at Al-Imamein Al-Kadhimein Medical City in Baghdad, from September 2016 to September 2017, 45 patients with BTM were collected from the Thalassemia center in Ibn AL-Baldy Hospital. Hepatic and myocardial T2* MRI results were taken from the file records of the patients from the same center.The results of hepatitis B and C and serum ferritin were taken from the file records.

Results The total number of patients was 60, 15 patients were excluded because of heart failure and hepatitis B and C, the mean age was (19.95±6.85 years), male: female ratio equal to 1:1. Fifty-one patients (85%) were on Deferasirox and 19 patients (31.76%) were splenectomized. Serum ferritin ranged between (1009-10600 ng/l). Liver T2* MRI ranged from (0.5-17 ms) with a mean of (3.66±3.13 ms). Mild-moderate severity found in the majority, 18 cases (40%), 13 cases (28.9%) respectively, with strong inverse correlation between liver MRI and serum ferritin level. Myocardial T2* MRI ranged from (1.88-33.2 ms) with a mean of (13.16±7.81 ms). Moderate-severe degree of severity in the majority, 12 cases (26.67%), 19 cases (42.22%) respectively, with significant inverse correlation between serum ferritin and myocardial MRI. There is no positive correlation between myocardial T2* MRI nor liver T2* MRI with mean age of the patients.

Conclusion The study shows that MRI is an accurate and non-invasive method to assess iron overload in liver and heart in β-thalassemia patients with regular transfusion.

Keywords β-Thalassemia major, blood transfusion, serum ferritin, cardiac MRI, hepatic MRI

Citation Yaseen AK, Abbas SS, Abdulhadi AMA. Correlation between cardiac and hepatic T2* MRI and serum ferritin level in patients with transfusion dependent β-thalassemia major. Iraqi JMS. 2019; 17(1): 66-73. doi: 10.22578/IJMS.17.1.10

List of abbreviations: BTM = β- thalassemia major, FOV = Field of view, Hb = Hemoglobin, LIC = hepatic iron concentration, MIC = Myocardial iron concentration, MRI = Magnetic resonance imaging, R2 = Relaxation rate, SIR = Signal intensity ratio, T2*: Transverse relaxation rate two star

Introduction

β-Thalassemia occurs in higher frequency in the Mediterranean area and the Middle East [(1)]. Infants with severe beta

thalassemia major (BTM) are well at birth. Symptoms emerge during the second six months of life when fetal hemoglobin (HbF) is replaced by adult hemoglobin (HbA) [2]. In transfused patients with BTM, cardiac hemosiderosis is the most feared complication. Without early institution of iron chelation therapy, they develop a sterile pericarditis, ventricular arrhythmias, end-stage restrictive cardiomyopathy, heart failure, and death [3].

Hepatomegaly is prominent early in the disease, even in the absence of transfusion, the accelerated rate of erythropoiesis enhances dietary iron absorption from the gut, resulting in a chronic state of iron overload, with liver fibrosis and, potentially, end-stage liver disease [4]. Serum ferritin levels in those with BTM may be quite elevated, reflecting the presence of iron overload [5].

Magnetic resonance imaging (MRI) methods for assessing tissue iron can be separated into two groups: signal intensity ratio (SIR) methods and relaxometry methods (R2) [6]. R2 of the liver demonstrates a significant positive correlation with serum ferritin and hepatic iron concentration (LIC) determined from liver biopsy material [7].

Myocardial iron has been evaluated by MRI using SIR and relaxometry techniques. MRI studies have demonstrated discordant results regarding the relationship of myocardial iron with hepatic iron and serum ferritin [8].

MRI studies have shown that cardiac siderosis increases with age, but that siderosis progresses more slowly in the heart than in the liver [9].

This study aimed to assess the value of MRI T2* procedure in assessment of hepatic (LIC) and myocardial iron concentration (MIC). Also, to assess the correlation between serum ferritin and T2* MRI of liver as well as the correlation of serum ferritin and T2* MRI of myocardium.

Methods

A cross-sectional study was conducted at AL- Al-Imamein Al-Kadhimein Medical City in Baghdad, from September 2016 to September 2017. Cases were taken from Ibn Al-Balady hospital – thalassemia center including all the patients who had been diagnosed as BTM with specific inclusion criteria at the previous time period, they were older than 5 years and transfusion dependent. The total number of cases was 60 patients. Each patient was inquired for his name, sex, age, chelating agents used and whether splenectomy was done. All the included patients had to fulfill the following criteria: BTM patient of more than 5 years old, and were receiving regular transfusion of packed red blood cells 10-15 ml per Kg body weight at 2-4 weeks interval (in order to maintain their hemoglobin level above 10 mg\dl), without any incidence of hepatitis B or C or any congenital or acquired liver diseases and who didn't develop heart failure. Screening for hepatitis B and C were taken from the file records of the patients.

For each patient included in the study, a T2* MRI study (for assessment of iron overload in the liver and the heart) had to be documented, the results were taken from the file records of the patients, for the patients who didn't have the required MRI study, researcher had arranged an MRI appointment for them in the Radiology Department of Al-Imamein Al-Kadhimein Medical City, furthermore, serum ferritin study was conducted monthly for each of them. Measurements were carried out by an enzyme-linked immune florescent assay, (BiomeriEux closed system Minividas apparatus, French). The results were taken from the file records of the patients. Each case was sent for ECHO study to exclude any patients with evidence of heart failure.

The exclusion criteria were any patients younger than 5 years, and patients with associated hepatitis B or C, to avoid any other parenchymal changes that can alter our results. Ethical committee approval had been taken to perform MRI study for the included patients.

MRI of liver

MRI studies performed for the elected BTM patients with a 1.5 T Avanto (magneto) system (Siemens medical System). Three transverse images were recorded using a body matrix coil,

visualizing the right liver lobe, and posterior vertebral muscles in the same slice.

MRI of heart

MRI studies performed for the elected BTM patients with a 1.5 T Avanto (magneto) system (Siemens medical System). Three mid-ventricular short axis slices were imaged with a slice thickness of 10 mm and a slice gap of 0.6 mm. using body matrix coil, gradient echo sequence (T2*) with 8 echo times (3.6-16 ms) was used to obtain the images. The field of view was 400 mm with the Field of view (FOV) was 75%, with prospective ECG triggering. The images were acquired at the mid-diastolic phase whereas the heart motion was minimal.

Normal values of MRI T2* of Liver and Heart to measure iron overload (Table 1).

Table 1. Normal values of MRI T2* of Liver and Heart to measure iron overload [8]

	Normal/ms	Mild/ms	Moderate/ms	Severe/ms
T2* Heart	>20	14-20	10-14	<10
T2* Liver	>6.3	2.8-6-3	1.4-2.7	<1.4

Statistical analysis

Data were analyzed by IBM SPSS statistics version 24; spearman test had been used to correlate S. ferritin level, liver T2* in ms, cardiac T2*value in ms. Chi square test and paired sample T-Test with Bootstrap to assess P. value, a value of > 0.05 is considered significant. Descriptive variables were presented as numbers and percentage, continuous variables were presented as mean and standard deviation.

Results

Sixty patients were included in this cross sectional study, their mean age was (19.95 ± 6.85) years distributed in numbers (Table 2), 31 cases (52%) were females and 29 (52%) were males, male:female ratio 1:1. All are BTM, on regular blood transfusion and on regular iron chelating agent for more than 18 months, 15 of the patients were excluded from the study because 11 was hepatitis C positive and 4 had with heart failure. MRI was done for 45 patients only.

Table 2. Age distribution of the patients

Age (year)	No.	%
<10	2	3
10-14	11	18
15-19	21	35
20-23	13	22
>24	13	22
Total	60	100

Fourteen patients had undergone splenectomy (23.33%) and fifty-one patients (85%) were on Deferasirox (Exjade), only five of the sixty patients (8.3%) were on Deferoxamine (Desferal) and four patients (6.7%) stopped the treatment as shown in (Table 3).

Table 3. Type of chelating agents

Type of chelating therapy	No.	%
Deferasirox	51	85
Deferoxamine	5	8.3
Non-compliant	4	6.7

The serum ferritin range between (1009-10600) ng/l with mean (3737.16±2759.56) ng/l. Correlation of liver T2* MRI with serum ferritin and mean age shown in (Table 4), the majority had mild to moderate degree of severity.

Table 4. Correlation of liver T2* MRI with serum ferritin and mean age

Liver T2* MRI	No. (%)	Age (year)	MRI liver (ms)	S. Ferritin (ng/l)
Normal	5 (11.1)	24.8±4.88	10.48±3.8	2351.0±1945.63
Mild	18 (40.0)	22.94±7.13	4.41±1.11	2238.28±1994.29
Moderate	13 (28.9)	17.0±6.1	1.86±0.36	3898.77±1783.64
Severe	9 (20.0)	16.89±3.72	0.97±0.34	7271.56±2532.85
Total	45 (100)	20.22±6.72	3.66±3.13	3737.16±2759.56
r. value				-0.528
P. value				<0.001

There was strong inverse correlation between liver MRI and serum ferritin level (r = -0.528) with significant P value of <0.001. as it is shown in (Figure 1).

Correlation of myocardial T2* MRI with serum ferritin and mean age is shown in (Table 5), the majority had moderate to severe degree of severity.

There was strong inverse correlation between myocardial MRI and S. ferritin (r = -0.468) with significant P value of 0.001 (Figure-2).

The mean myocardial T2* MRI was (13.16±7.81), While the mean liver T2* MRI was (10.48±3.8) ms, with a discordant result regarding the relationship between myocardial iron content and hepatic iron content (Figure 3).

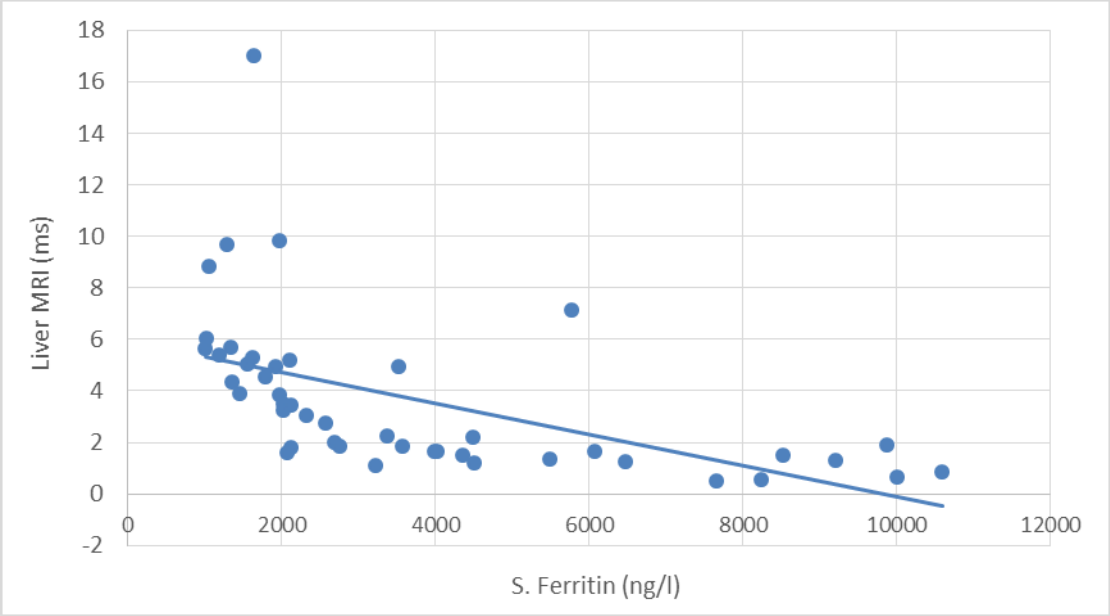

Figure 1. Relationship between liver T2* MRI and serum ferritin

Table 5. Correlation of myocardial T2* MRI with serum ferritin and mean age

Myocardial T2* MRI	No. (%)	Age (year)	MRI liver (ms)	S. Ferritin (ng/l)
Normal	10 (22.2)	21.7±6.38	25.05±4.36	2882.3±2843.73
Mild	4 (8.89)	22.5±6.95	16.62±1.05	2149.0±428.15
Moderate	12 (26.68)	19.83±8.19	12.95±1.07	2380.75±1334.9
Severe	19 (42.23)	19.21±6.13	6.31±2.18	5378.11±2901.13
Total	45 (100)	20.22±6.72	13.16±7.81	3737.16±2759.5
r. value				-0.468
P. value				0.001

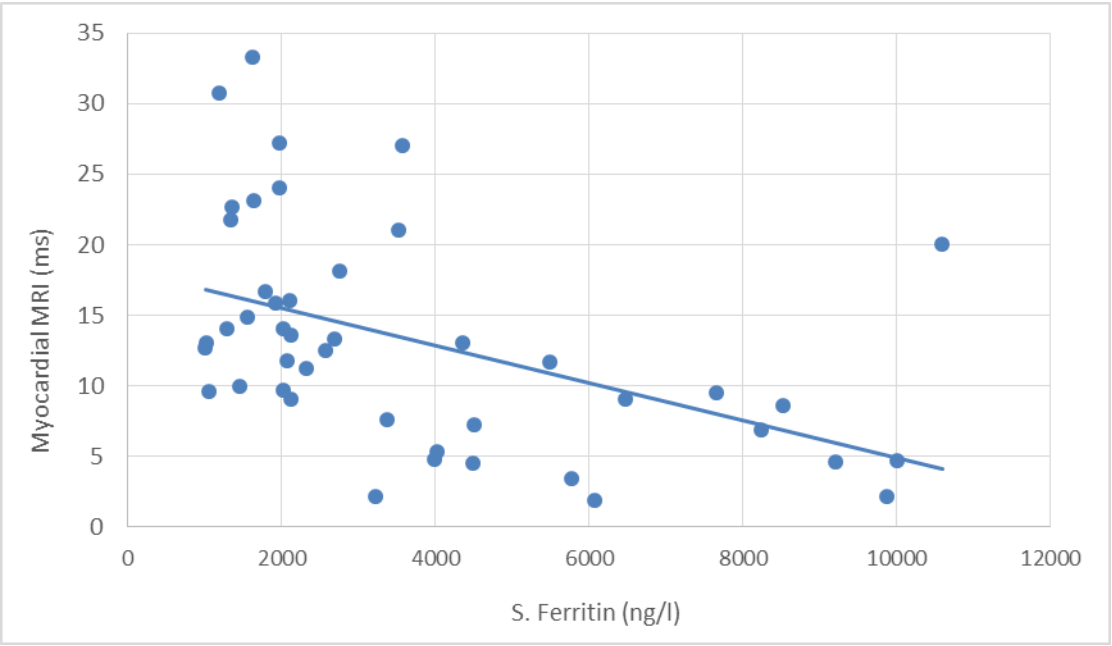

Figure 2. Relationship between myocardial T2* MRI and serum ferritin

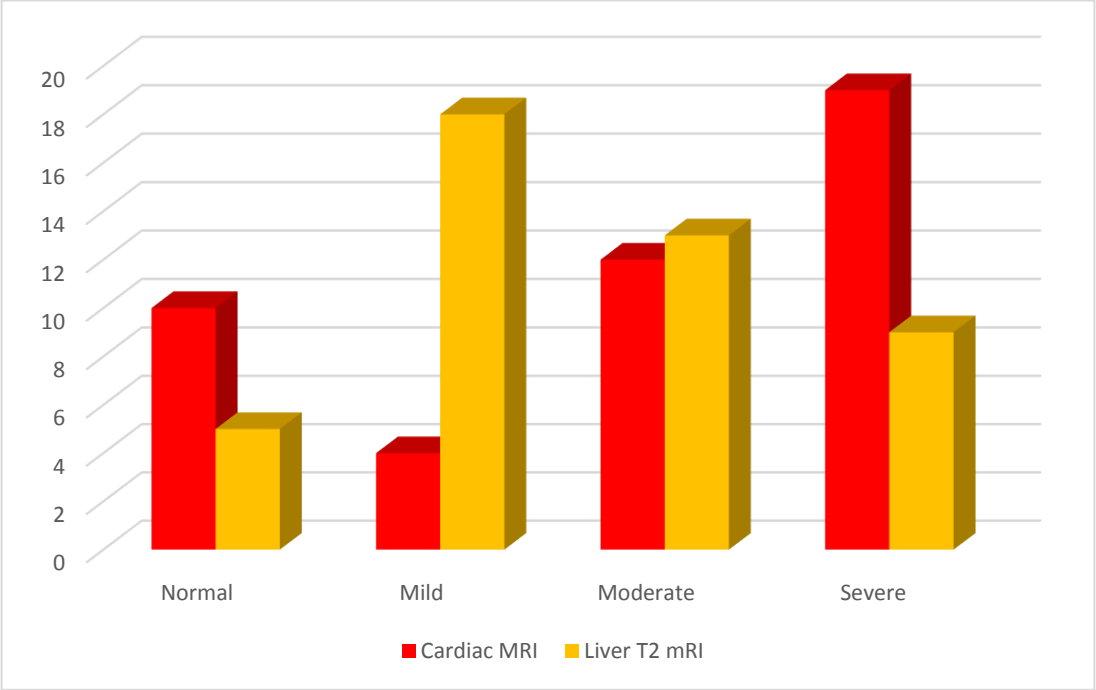

Figure 3. Difference between liver and myocardial T2* MRI in the study group

Discussion

Regarding the distribution of severity of iron overload in hepatic and cardiac T2 MRI finding, 14 cases (31%) were of normal to mild severity in cardiac MRI where 31 cases (69%) were of moderate to severe degree in cardiac MRI, on other hand; 23 (51%) cases were of mild to moderate severity and the rest 22 (49%) cases were of moderate to severe degree, which is the same outcome of Noetzli et al. study in Tehran, which shows that there is no correlation between cardiac and hepatic T2 MRI in relation to the severity of the disease [(11)].

Correlating hepatic MRI finding with the severity of the disease, a relatively small number of cases (9 cases; 20%) were with severe degree of hepatic MRI finding, which may be due to the fact that the chelating agents acting more efficiently on the liver with easy excretion of iron overload from the liver than that of the heart.

In the present study, analyzing of correlation between hepatic T2 MRI finding and the serum ferritin, the study shows that there is significant correlation between them with a P value of <0.001, which is the same finding in Majd et al. study in Iran [(10)], Eghbali et al. study [(12)], and Zamani et al. study in Iran [(13)]. Such results can be accepted when understanding that 70% of iron stores in the body are deposited in the liver.

Regarding the correlation between cardiac T2 MRI finding and serum ferritin level, the study shows that there is strong correlation between them with a P value equal to 0.001, which is also significant in Majd et al. study in Iran [(10)] and Kirk et al. study in UK [(14)]. Such finding can be explained due to iron overload toxicity in the heart that occurs in disease process; which is associated with many cardiac complications such as heart failure and arrhythmias which are the main causes of death in patients with BTM. This finding is in contrast to Azarkeivan et al. study in Iran, which shows there is no significant correlation between serum ferritin level and cardiac T2 MRI, which is explained by documentation of paradoxical low serum ferritin level in certain patients with heavy iron overload [(15)].

Differences in iron kinetics and variations in chelation schemes may be responsible for the lack of correlation of the MRI-determined myocardial iron with that of the liver. More active elimination of iron from the hepatocytes than from the myocytes may play a role in the absence of correlation in patients receiving chelation therapy [(8)].

Splenectomy was done for 15 out of 45 cases (33%) cases, while in Vichinsky et al. study, 45% of 407 cases had been splenectomized [(16)], while in Fahmy et al. study, 12 cases (17%) of the seventy cases had been splenectomized [(17)].

This study concluded that there is strong correlation between serum ferritin and liver and heart MRI findings, so we can depend on serum ferritin in centers which lack MRI technique to assess the iron overload in heart and liver.

MRI is accurate and non-invasive method to assess iron over load in the vital organ (heart and liver), in β - thalassemia patients who are on regular transfusion.

This study recommends:

1. A larger study to find the correlation between serum ferritin and iron overload in heart, liver, pituitary gland and pancreas.
2. Using MRI as a yearly standard follow up in β-thalassemia patients with regular transfusion who are at high risk with iron overload so we can early detect the complications of the organs that suffer from iron overload.

Acknowledgments

To all children with β-thalassemia who fight for their survival.

Author contribution

All authors contributed to this manuscript. They coordinated study recruitment, implementation and progress of this study and helped with data interpretation and manuscript organization and editing.

Conflict of interest

The authors have no conflict of interest.

Funding

Self- funding.

References

1. Vichinsky EP. Changing patterns of thalassemia worldwide. Ann N Y Acad Sci. 2005; 1054: 18-24.
2. Bird EM, Parameswaran U, William T, et al. Transfusion-transmitted severe Plasmodium malaria

in a splenectomized patient with beta-thalassaemia major in Sabah, Malaysia: a case report. Malar J. 2016; 15(1): 357. doi: 10.1186/s12936-016-1398-z.
3. Aessopos A, Berdoukas V. Cardiac function and iron chelation in thalassemia major and intermedia: a review of the underlying pathophysiology and approach to chelation management. Mediterr J Hematol Infect Dis. 2009; 1(1): e2009002. doi: 10.4084/MJHID.2009.002.
4. Tanno T, Bhanu N V, Oneal PA, et al. High levels of GDF15(Growth differentiation factor) in thalassemia suppress expression of the iron regulatory protein hepcidin. Nat Med. 2007; 13(9): 1096-101. doi: 10.1038/nm1629.
5. Modell B, Darlison M. Global, epidemiology of haemoglobin disorders and derived service indicators. Bull World Health Organ. 2008; 86(6): 480-7.
6. Argyropoulou MI, Astrakas L. MRI evaluation of tissue iron burden in patients with beta-thalassaemia major. Pediatr Radiol. 2007; 37(12): 1191-200. doi: 10.1007/s00247-007-0567-1
7. Alexopoulou E, Stripeli F, Baras P, et al. R2 relaxometry with MRI for the quantification of tissue iron overload in β-thalassemic patients. J Magn Reson Imaging. 2006; 23(2): 163-70. doi: 10.1002/jmri.20489
8. Pennell DJ. T2* magnetic resonance and myocardial iron in thalassemia. Ann N Y Acad Sci. 2005; 1054: 373-8. doi: 10.1196/annals.1345.045.
9. Perifanis V, Christoforidis A, Vlachaki E, et al. Comparison of effects of different long-term iron-chelation regimens on myocardial and hepatic iron concentrations assessed with T2* magnetic resonance imaging in patients with β-thalassemia major. Int J Hematol. 2007; 86(5): 385-9. doi: 10.1532/IJH97.E0734.
10. Majd Z, Haghpanah S, Ajami GH, et al. Serum ferritin levels correlation with heart and liver MRI and LIC in patients with transfusion-dependent thalassemia. Iran Red Crescent Med J. 2015; 17(4): e24959. doi: 10.5812/ircmj.17(4)2015.24959.
11. Noetzli LJ, Carson SM, Nord AS et al. Longitudinal analysis of heart and liver iron in thalassemia major. Blood. 2008; 112(7): 2973-8. doi: 10.1182/blood-2008-04-148767.
12. Eghbali A, Taherahmadi H, Shahbazi M et al. Association between serum ferritin level, cardiac and hepatic T2-star MRI in patients with β-thalassemia major. Iran J Ped Hematol Oncol. 2014; 4(1): 17-21.
13. Zamani F, Razmjou S, Akhlaghpoor S, et al. T2* magnetic resonance imaging of the liver in thalassemic patients in Iran. World J Gastroenterol. 2011; 17(4): 522-5. doi: 10.3748/wjg.v17.i4.522.
14. Kirk P, Roughton M, Porter JB, et al. Cardiac T2* magnetic resonance for prediction of cardiac complications in thalassemia major. Circulation. 2009; 120(20): 1961-8. doi: 10.1161/CIRCULATIONAHA.109.874487.
15. Azarkeivan A, Hashemieh M, Akhlaghpoor S, et al. Relation between serum ferritin and liver and heart MRI T2* in beta thalassemia major patients. East Mediterr Health J. 2013; 19(8): 727-32.
16. Vichinsky E, Neumayr L, Trimble S, et al. Transfusion complications in thalassemia patients: a report from the Centers for Disease Control and Prevention (CME). Transfusion. 2014; 54(4): 972-81; quiz 971. doi: 10.1111/trf.12348.
17. Fahmy HS, Khatir NH, El-Shahat HM, et al. Reassessing the value of MRI T2* in evaluation of hepatic and myocardial iron concentration: an institutional study. Egypt J Radiol Nuclear Med. 2015; 46(4), 1085-90. doi: https://doi.org/10.1016/j.ejrnm.2015.06.008.

Correspondence to Dr. Sawsan S. Abbas
E-mail: sawsansati@yahoo.com
saw-195@colmed-alnahrain.edu.iq
Received Oct. 22nd 2018
Accepted Jan. 9th 2019

Iraqi JMS

Published by Al-Nahrain College of Medicine
P-ISSN 1681-6579
E-ISSN 2224-4719
Email: iraqijms@colmed-alnahrain.edu.iq
http://www.colmed-alnahrain.edu.iq
http://www.iraqijms.net
Iraqi JMS 2019; Vol. 17(1)

Multiplex RT-PCR Based Detection of Human Bocavirus and Other Respiratory Viruses in Infants and Young Children with Lower Respiratory Tract Infection

Ziad S. Rasheed[1] *BSc*, **Arwa M. A. Al-Shuwaikh**[2] *PhD*, **Khaled R. Issa**[3] *FICSMS*

[1]Al-Shaheed Al-Sadr General Hospital, Ministry of Health, [2]Dept. of Microbiology, College of Medicine, Al-Nahrain University, Baghdad, Iraq, [3]Dept. of Pediatrics, Ibn Al-Baladi Hospital, Baghdad, Iraq

Abstract

Background Human Bocavirus (hBoV) has recently been identified as a causative agent of acute respiratory infection (ARI) in infant and young children. However, there is little information about its frequency and importance as a cause of lower respiratory tract infection (LRTI) in Iraq.

Objective To assess the frequency of hBoV and co-infection rate in infants and young children with LRTI.

Methods Nasopharyngeal/throat swabs were collected during the peak winter months from 100 hospitalized infants and young children less than 5 years of age with LRTI in Baghdad, Iraq. Five viruses were investigated by Multiplex Real-Time Polymerase Chain Reaction (RT-PCR).

Results It was found that 71 (71%) had a viral infection either single or mixed. Six out of 100 samples (6%) were hBoV positive, 10 (10%) were parainfluenza virus positive, 12 (12%) tested positive for adenovirus, 58 (58%) were rhinovirus positive and none of the samples gave positive results for coronavirus. Sixteen samples showed mixed infection with rhinovirus, eight of these (8%) with parainfluenza virus, 6 (6%) with adenovirus, and 2 (2%) with hBoV. There were no significant differences between the viral infected and non-infected patients with respect to age, gender, crowding status, gestational age, diagnosis at presenting and hospital stay (the average stay of viral-infected patients in the hospital was three days). In contrast, exposure to smoking was significantly more associated with viral-infected children than virus-free children (80% vs. 44.83%, P<0.001). In addition, viral-infected children showed significantly less proportion for need for intensive care unit than virus-free children (15.5% vs. 79.31%, P<0.001). Breastfeeding was found to have significant adverse association with viral infection (P<0.001). About one-third of viral-infected children had a medical history involving a disease other than respiratory infection (P<0.001). Finally, viral-infected children were reported to have longer period of illness before hospital admission (6.32±5.49 days) than virus-free children (6.32±5.49 days vs. 3.38±2.37 days, P<0.01).

Conclusion More than two third of children with respiratory tract infection have been viral infection either single or mixed. Multiplex RT-PCR has the potential for clinical use in the rapid and differential detection of viral infection.

Keywords Viral respiratory tract infection, Children, Multiplex Real Time PCR

Citation 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

List of abbreviations: hBoV = Human bocavirus; hPiv = Human parainfluenza; hRv = Human rhinovirus; hAdv = Human adenovirus; hCov = Human coronavirus, LRTI = Lower respiratory tract infection

Introduction

Respiratory infections are the leading cause of morbidity and mortality in children worldwide. The World Health Organization (WHO) ranks respiratory tract infections as the second leading cause of death in children aged less than five years of age, predominantly in developing countries [1,2]. Human bocavirus (hBoV) is a ssDNA virus that first discovered in 2005. It has been classified as a member of Parvoviridae family and associated

with respiratory illness and gastroenteritis in children [3]. Currently, four genotypes have been described (hBoV 1 to 4). The hBoV1–2 has been associated with respiratory tract infections, while hBoV2–4 associated with gastrointestinal disease [1]. There has been controversy regarding hBoV as a sole causal pathogen due to the high observation of co-infection with other viruses and the inability to fulfill Koch postulates given the challenges of viral culture [4]. In addition, individuals with low viral load are more likely to be co-infected with other pathogens compared to those with high viral load (57% vs. 38.9%). The rate of co-infections in subjects with respiratory infections and hBoV-positivity ranges from 8.3% to 100% [5].

Other respiratory viruses, human parainfluenza viruses (hPiv 1 to 4) are ssRNA viruses belongs to Paramyxoviridae family. It has been associated with a broad spectrum of respiratory tract disease including common cold, croup, bronchitis, bronchiolitis and pneumonia and certain serotype are frequently associated with certain illness [6,7]. Human adenoviruses (hAdv) are dsDNA virus, which belongs to Adenoviridae family and classified as seven species (hAdv A to G), acute respiratory infections (ARI) are mainly caused by species B, C and E worldwide. Children with hAdv pneumonia may be misdiagnosed and inadequately treated [8]. Human rhinoviruses (hRv) are ssRNA viruses, which belongs to Picornaviridae family. They are divided into three species, (hRv A to C) and approximately 150 distinct genotypes identified within those species. Although once thought to cause only the common cold, it is now known that hRv are associated with Lower respiratory tract infection (LRTI) including bronchiolitis and pneumonia and can exacerbate asthma in children and adult [9,10]. Finally, Human coronaviruses (hCov) are ssRNA viruses that most commonly associated with mild respiratory illnesses, but can cause severe illness, such as severe acute respiratory syndrome (SARS) coronavirus [11]. To date, six hCov have been identified of which four (OC43, E229, NL63, and HKUI) are globally circulate in the human population [12]. The role of the most common respiratory viruses has been well described, while the roles of other less known, viruses such as hBoV remains largely unknown especially in developing countries [13,14].

This study attempted to determine the frequency of HBoV in infant and young children with lower respiratory tract infection, as well as its potential to cause infection as a sole etiological agent or as co-infection with other viral pathogens and to give real insight to prevalence of viral infections in a sample of Iraqi children.

Methods

Specimen collection:

One hundred nasopharyngeal/throat swabs samples were collected during November, 2017 to March, 2018 from hospitalized infants and young children with LRTI at Ibn Al-Baladi Teaching Hospital, Medical City Hospital and Al-Imamein Al-Kadhimein Medical City in Baghdad, Iraq. Children age range was 1 month to 60 months, they were enrolled in this study after an informed consent was obtained from parents and approved from Institutional Review Boards of College of Medicine, Al-Nahrain University (approval no. M.M.M./22 in 27/12/2017).

Nasopharyngeal/throat swabs were taken using flocked swab regular (catalog number 80346C, Copan Diagnostic, Italy) and flocked swab nasopharyngeal (catalog number 80503CS, Copan Diagnostic, Italy) and then combined into a single tube of viral universal transport medium (3 ml UTM, Copan Diagnostic, Italy). Specimens were transported in cold packs to the laboratory within 8 hours of collection and preserved frozen at (−70 °C) until tested. Questionnaire form was used to collect information of clinical symptoms before admission to the hospital and demographic information by parental interview. Medical files were reviewed for selected clinical data of the patients, only patients with LRTI were included. The crowding index was calculated according to the American crowding index [15].

RNA and DNA extraction

RNA/DNA were extracted from frozen specimen aliquots according to manufacturer's instructions using Ribo-Sorb RNA/DNA Extraction kit (Sacace Biotec, Italy). Internal Control (IC) was used in the isolation procedure which serves as an amplification control for each individually processed specimen and to identify possible reaction inhibition. The concentration and purity of RNA/DNA extracts were measured by a nanodrop (BioDrop µLITE, BioDrop Co., UK). The isolated RNA/DNA was stored frozen at (−70 °C) until analysis.

Multiplex RT-PCR

RNA was reverse transcribed to cDNA, which used as a template for Multiples RT-PCR. Reactions mix were prepared according to the manufacturer's instructions using ARVI Screen Real-TM kit (Sacace, Itaily). Real-time PCR was performed with the Magnetic Induction Cycler (Mic, Australia) using primers and probe sequences that amplified a fragment encompassing specific region to each virus, allowing detection of five viruses hBov, hPiv (1-4), hAdv (B, C, E), hRv and hCov (OC43, E229, NL63, and HKUI). Positive and negative controls were included to verify the validation of the reaction. Instrument setting according to manufacture instruction for roter type instruments. The real-time thermal condition included holding step at 1 cycle at 95 °C for 15 min (as an initial denaturation), cycling step including 10 cycles of 95 °C for 10 sec, 54 °C for 25 sec and 72 °C for 25 sec, and 35 cycles of 95 °C for 10 sec, 54 °C for 25 sec and 72 °C for 25 sec with fluorescence data collected during the 54 °C annealing/extension step. Fluorescence is detected is in FAM/Green, JOE/Yellow/HEX/Cy3 and ROX/Orange/TexasRed fluorescent channels. The results were interpreted by the software of Real Time PCR instrument by the crossing (or not crossing) of the fluorescence curve with the threshold line. The sensitivity of detection with this method is approaching 100 copies/reaction in respiratory specimens according to manufacturer.

Statistical analysis

Data were analyzed using SPSS version 22.0 (Chicago, IL, USA). Descriptive statistic mean ± S.D. and frequency were calculated, Comparisons of demographic and clinical data between groups were made using Chi-square ($\chi 2$) test for categorical variables and student t-test for mean differences. A P value <0.05 was taken as threshold of statistical significance.

Results

Patients and sample inclusion

This study included 100 infants and young children with LRTI. The age range of study population was (1 to 60) months. Sixty percent of the them were male while female represented 40%. Most children (82%) were mature; however, 24% of them needed an intensive care unit for sometimes. Over half (58%) did not have breastfeeding compared with only 22% having bottle feeding and 20% with mixed feeding. In addition, 70% were exposed to smokers. Fifty-two of children were found to live in severely crowded conditions. Approximately two-third of children were present with pneumonia with or without bronchitis. The majority of children (82%) had a hospital stay for less than 3 days, and about half of them had a medical history especially jaundice (14%) and pneumonia (6%) as shown in table (1).

Table 1. Perinatal and clinical characteristics of 100 infant and young children included in this study

Variables		No. (%)
Age groups (months)	1-6	60 (60%)
	7-12	26 (26%)
	13-60	14 (14%)
Gender	Female	40 (40%)
	Male	60 (60%)
Exposure to smoking	Exposed	70 (70%)
	Not exposed	30 (30%)
Crowding index	Severely crowded	52 (52%)
	Crowded	26 (26%)
	Not crowded	22 (22%)
Gestational age	Premature	18 (18%)
	Mature	82 (82%)
History of Neonatal ICU	Yes	24 (24%)
	No	76 (76%)
Breastfeeding	Yes	22 (22%)
	No	58 (58%)
	Mix	20 (20%)
No. days prior to admission	≤ 3 days	47 (47%)
	4-14 days	45 (45%)
	> 14 days	8 (8%)
Diagnosis	Pneumonia	62 (62%)
	Bronchitis	24 (24%)
	Bronchiolitis	10 (10%)
	Asthma	4 (4%)
Length of hospital stay	1-3 days	82 (82%)
	> 3 days	18 (18%)
Medical history	None	52 (52%)
	Pneumonia	6 (6%)
	Seizure	2 (2%)
	Sepsis	4 (4%)
	Cardiac septal defect	2 (2%)
	Meningitis	2 (2%)
	Cough	2 (2%)
	Cystitis	2 (2%)
	Hernia	8 (8%)
	Jaundice	14 (14%)
	Mixed	6 (6%)

Viral infection and co-infection

In total 100 patients, 71 (71%) were found to have a viral infection either single or mixed. Primarily, the study targeted five viruses; however, only four were detected. These were hBov, hPiv, hAdv and hRv which represented 6%, 10%, 12% and 58%, respectively (Figure 1). On the other hand, none of the samples gave

positive results for hCov. All mixed infections were found to be a combination of two viruses one of them hRv. Eight of these (8%) with hPiv, 6(6%) with hAdv, and 2 (2%) with hBov (Figure 2).

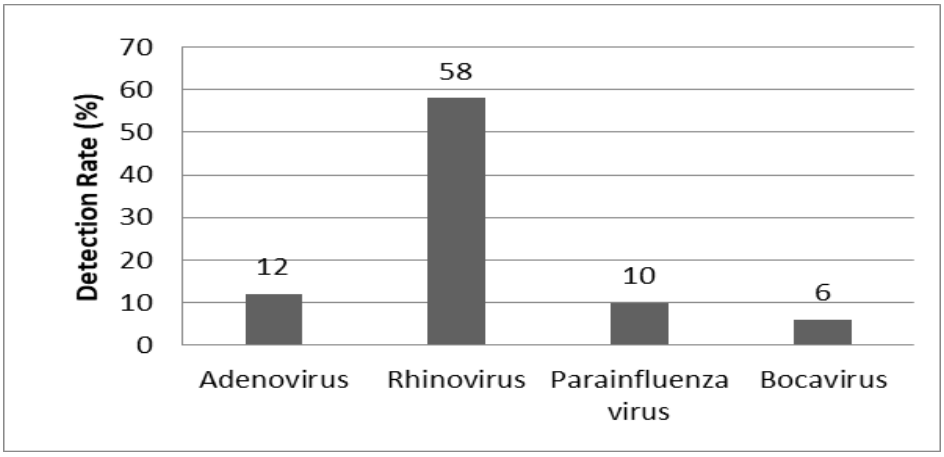

Figure 1. The prevalence of respiratory viruses during the studied period

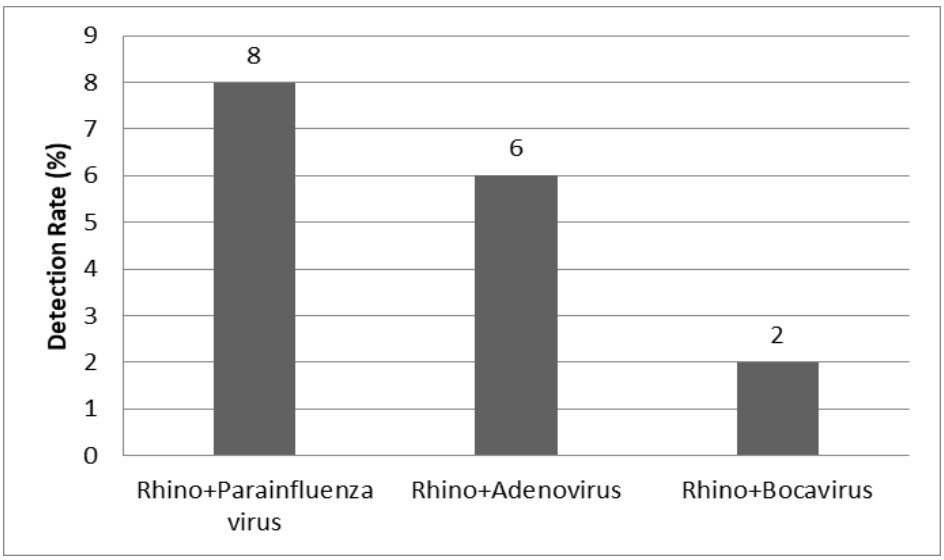

Figure 2. The prevalence of co-infection during the studied period

Demographic and clinical characteristics

Overall, 71 children were found to have viral (one or two) infection versus 29 children without such infection (may have other viruses or other microoganisms). Each of age of the child, gender, crowding status, gestational age, diagnosis at presenting and hospital stay did not have a significant association with viral infection. On the other hand, five characteristics associated in some way significantly with the viral infection as shown in table (2).

More than 80% of viral-infected children were reported to be exposed for smoking versus 44.8% of virus-free children (P<0.001). In contrast, viral-infected children showed significantly less proportion for need for intensive care unit (ICU) than virus-free children (15.5% vs. 79.31%, P<0.001). Breastfeeding was

found to have significant adverse association with viral infection, since a high proportion of virus-free children (41.38%) had breast feeding (P=0.004).

About one-third of viral-infected children had had a medical history involving a disease other than respiratory infection such as jaundice and sepsis compared with only 10.34% in virus-free children (P=0.003). Finally, viral-infected children were reported to have longer period of illness before hospital admission (6.32±5.49 days) than virus-free children (3.38±2.37 days) (P=0.02). Also, viral-infected children were more likely to stay an extra day in the hospital (3 vs. 2 days, 0.082), although it not reached statistical significance (Table 2).

Table 2. Association of demographic and clinical characteristic with general viral infection

Variables	Viral detected 71	No Virus detected 29	P-value
Age per months (mean±SD)	9.49±8.86	14.34±12.2	0.411
Gender, No. (%)			
Male	44(61.97%)	16(%)	0.53
Female	27(38%)	13(%)	
Exposure to smoking, No. (%)			
No	14 (19.72%)	16 (55.17%)	<0.001
Yes	57 (80.28%)	13 (44.83%)	
Crowding, No. (%)			
Not crowded	13 (18.31%)	9 (31%)	0.173
Crowded	58 (81.69%)	20 (69%)	
Gestational age, No. (%)			
Mature	57 (80.28%)	25 (86.2%)	0.457
Premature	14 (19.72%)	4 (13.79%)	
History of neonatal ICU, No. (%)			
No	60 (84.5%)	6 (20.69%)	<0.001
Yes	11 (15.5%)	23 (79.31%)	
Breastfeeding, No. (%)			
No	45 (63.38%)	13 (44.83%)	0.089
Yes	10 (14.08%)	12 (41.38%)	0.004
Mixed	16 (22.54%)	4 (13.79%)	0.308
Diagnosis, No. (%)			
Pneumonia	45(63.38%)	17(58.62%)	0.657
Bronchitis and bronchiolitis	31(43.66%)	11(37.93%)	0.597
Wheezing and asthma	12(16.9%)	6(20.69%)	0.658
Medical history, No. (%)			
No	37 (52.11%)	25 (86.21%)	0.001
Respiratory infection	7 (9.86%)	1 (3.45%)	0.248
Others	27 (30.03%)	3 (10.34%)	0.003
No. days prior to admission (mean±SD)	6.32±5.49	3.38±2.37	0.020
Hospital stay per days (mean±SD)	3.37±2.73	2.35±2.09	0.082

Discussion

In this study, Multiplex RT-PCR allows detection of five viruses at the same time. However, some respiratory viruses were escaped the detection because they not included within the ARVI Screen Real-TM kit. The current study showed that the frequency of hBoV, hPiv, hAdv, hRv and hCov were 6%, 10%, 12%, 58% and 0%, respectively. This result in consistent with other studies reported that the prevalence of human bocavirus infection in children aged three years and younger is (2.3-5.7%), which suggests that hBoV is an uncommon cause of respiratory illness [16-18].

Previous study found that a high rate of hBov co-infections with other viral respiratory pathogens, such as hRv and hAdv. Co-infections have been found in up to 83% of respiratory samples [5]. In this study, only 2 out of 6 hBov-infected children were co-infected with hRv, so it is difficult to confirm the role of hBov as a sole etiological agent due to a small sample size. Jula et al. in 2013 suggested that other respiratory viruses might reactivate hBov from latency, as has been seen with several other DNA viruses, or that they alter the intranasopharyngeal environment and release persisting hBov [19]. In contrast, Moesker et al. in 2015 found that (14%) of cases with severe ARI admitted to ICU showed single hBov-infected which provides strong support that hBov can cause severe ARI in children in the absence of other viral or bacterial co-infections [1]. In addition, some studies indicated that a single recent hBoV infection is associated with a higher viral load than in combination with other viruses [1,20-22].

A study done in Mexico showed that (81.6%) of young children were positive for viral infected, which support current study in the total percentage of viral infection but slightly differ in the percentage of each virus such as hBov was found in (0.4%), hPiv in (5.5%), hAdv in (2.2%) and hRv in (16.6%) [23]. Another study conducted in Korea for detection of 11 respiratory viruses showed that viral infection was identified in (60.6%) of patients, which hPiv-3 was detected in (6.2%) while hPiv-1 and hCov-NL63 in (1.6%) [24]. This difference in prevalence of specific virus infection may be due to the difference in the time period for sample collection, size of samples, diverse geographical climate, activity of the virus pandemic in the community, testing techniques used and the viral pattern studied [25].

Human Adv infections in current study were comparable to that documented in Jordan (11.5%) of hospitalized children less than two years of age and within the range (0.8-27.3%) reported in other countries [26]. In addition, Miller et al. in 2009 detected hRv in (33%) of hospitalized children with respiratory symptoms and/or fever [10], while Kaplan et al. (2008) identified hRv only in (11%) of hospitalized young children in Jordan [27]. The viruses most frequently co-detected with hRv in ARI samples were hAdv, hPiv, and respiratory syncytial virus (RSV), (12.6%, 7.2% and 5.3%, respectively) [5]. Current study showed that 6 out of 12 Adv-infected children were co-infected with hRv. It was reported that the presence of hRv increases the risk for disease severity in acute bronchiolitis by approximately 5-fold [28].

Our data showed a significant difference in clinical characteristics between viral-infected and viral-free children in regards to exposure to smoking in households (80.28% vs. 44.83%, respectively) ($P<0.001$) (Table 2). It was hypothesized that cigarette smoke exposure increases epithelial susceptibility to some viral infection by increasing the abundance of their receptor [29]. Cigarette smoke is a major risk factor increasing the morbidity and mortality rates of viral infection and activating latent infection [26]. In addition, a significant difference between viral-infected and viral-free children with respect to breastfeeding was found. Breastfeeding showed to have a protective effect; children who were breastfed for less than one month had 7 times less risk for being hospitalized for acute bronchiolitis in the first three months of life [30]. The sensitized T cells or antigen on macrophages in colostrums or milk may transfer to infants and stimulation immune response. Also, breast milk suppresses IgE response which may be important in the pathogenesis of bronchiolitis [31].

Viral-infected children were found to have less history of requirement to intensive care unit admission and mechanical ventilation and

longer period of illness before hospital admission. This could explain by the fact that some patients with prior hospitalization seeking medical assistance earlier in their illness or, more probably, to the decision to refer patients to hospital being influenced by a history of hospitalization, another factors such: underlying medical condition, home environment and maternal care can play a role [32].

In conclusion, this study has demonstrated the frequency of five respiratory viruses throughout four winter months therefore we may have underestimated the true frequency of some detected viruses due to seasonal variations. Further study with larger sample size and out spreads over multiple seasons is required to understand the epidemiology of respiratory viruses. This study might encourage viruses consideration in respiratory infection diagnosis in children to avoid inappropriate antibiotic therapies.

Acknowledgments

The authors thank the resident physicians at Ibn Al-Baladi Teaching Hospital, Medical City Hospital and Al-Imamein Al-Kadhimein Medical City for their help in samples collection. This work was supported by L'Oreal-UNESCO for Women in Science Levant and Egypt.

Author contribution

Dr. Al-Shuwaikh and Rasheed are co-first authors and equally contributed to this paper. Dr. Al-Shuwaikh designed and wrote this manuscript. Rasheed performed all the laboratory work and statistical analysis. Dr. Issa was involved in the patient's management and helped in specimen's collection. All the authors have read and approved the final version of this manuscript.

Conflict of interest

The authors declare no conflicts of interests for this article.

Funding

Dr. Arwa M. A. Al-Shuwaikh is supported by L'Oreal-UNESCO for Women in Science Levant and Egypt 2017.

References

1. Moesker FM, van Kampen JJA, van der Eijk AA, et al. Human bocavirus infection as a cause of severe acute respiratory tract infection in children. Clin Microbiol Infect. 2015; 21(10): 964.e1–964.e8. doi: https://doi.org/10.1016/j.cmi.2015.06.014
2. Kaplan NM, Dove W, Abu-Zeid AF, et al. Human Bocavirus Infection among Children, Jordan. Emerging Infectious Diseases. 2006; 12(9). 1418-20. DOI: 10.3201/eid1209.060417.
3. Gu K, Van Caeseele P, Dust K, et al. Atypical pneumonia due to human bocavirus in an immunocompromised patient. CMAJ. 2017; 189(19): E697-E699. doi: 10.1503/cmaj.161134.
4. Broccolo F, Falcone V, Esposito S, et al. Human bocaviruses: possible etiologic role in respiratory infection. J Clin Virol. 2015; 72: 75-81. doi: 10.1016/j.jcv.2015.09.008.
5. Guido M, Tumolo MR, Verri T, et al. Human bocavirus: Current knowledge and future challenges. World J Gastroenterol. 2016; 22(39): 8684-97. doi: 10.3748/wjg.v22.i39.8684.
6. Schomacker H, Schaap-Nutt A, Collins PL, et al. Pathogenesis of acute respiratory illness caused by human parainfluenza viruses. Curr Opin Virol. 2012; 2(3): 294-9. doi: 10.1016/j.coviro.2012.02.001.
7. Yea C, Cheung R, Collins C, et al. The complete sequence of a human parainfluenzavirus 4 genome. Viruses. 2009; 1(1): 26-41. doi: 10.3390/v1010026.
8. Qiu F-Z, Shen X-X, Zhao M-C, et al. A triplex quantitative real-time PCR assay for differential detection of human adenovirus serotypes 2, 3 and 7. Virol J. 2018; 15(1): 81. doi: 10.1186/s12985-018-0983-x.
9. Howard LM, Johnson M, Gil AI, et al. Molecular Epidemiology of Rhinovirus Detections in Young Children. Open Forum Infect Dis. 2016; 3(1): ofw001. doi: 10.1093/ofid/ofw001.
10. Miller EK, Najwa K-B, Williams JV, et al. Human rhinovirus C associated with wheezing in hospitalised children in the Middle East. J Clin Virol. 2009; 46(1): 85-9. doi: 10.1016/j.jcv.2009.06.007.
11. Khuri-Bulos N, Payne DC, Lu X, et al. Middle East respiratory syndrome coronavirus not detected in children hospitalized with acute respiratory illness in Amman, Jordan. Clin Microbiol Infect. 2014; 20(7): 678-82. doi: 10.1111/1469-0691.12438.
12. van der Hoek L. Human coronaviruses: what do they cause? Antivir Ther. 2007; 12(4 Pt B): 651-8.
13. Ali SA, Williams JW, Chen Q, et al. Human metapneumovirus in hospitalized children in Amman, Jordan. J Med Virol. 2010; 82(6): 1012-6. doi: 10.1002/jmv.21768.
14. Endo R, Ishiguro N, Kikuta H, et al. Seroepidemiology of human Bocavirus in Hokkaido Prefecture, Japan. J Clin Microbiol. 2007; 45(10): 3218-23. doi: 10.1128/JCM.02140-06.

15. Goodyear RK, Fabian A, Hay J. Finding the crowding index that works best for New Zealand (Statistics New Zealand Working Paper No 11–04). 2011. Available from www.stats.govt.nz.

16. Principi N, Piralla A, Zampiero A, et al. Bocavirus infection in otherwise healthy children with respiratory disease. PLoS One. 2015; 10(8): e0135640. doi: 10.1371/journal.pone.0135640.

17. Chow BD, Huang YT, Esper FP. Evidence of human bocavirus circulating in children and adults, Cleveland, Ohio. J Clin Virol. 2008; 43(3): 302-6. doi: 10.1016/j.jcv.2008.07.009.

18. Bastien N, Chui N, Robinson JL, et al. Detection of human bocavirus in Canadian children in a 1-year study. J Clin Microbiol. 2007; 45(2): 610-3. doi: 10.1128/JCM.01044-06.

19. Jula A, Waris M, Kantola K, et al. Primary and secondary human bocavirus 1 infections in a family, Finland. Emerg Infect Dis. 2013; 19(8): 1328-31. doi: 10.3201/eid.1908.130074.

20. Ricart S, Garcia-Garcia JJ, Anton A, et al. Analysis of human metapneumovirus and human bocavirus viral load. Pediatr Infect Dis J. 2013; 32(9): 1032-4. doi: 10.1097/INF.0b013e3182932f4f.

21. Zhao B, Yu X, Wang C, et al. High human bocavirus viral load is associated with disease severity in children under five years of age. PLoS One. 2013; 8(4): e62318. doi: 10.1371/journal.pone.0062318.

22. Uršič T, Jevšnik M, Zigon N, et al. Human bocavirus and other respiratory viral infections in a 2-year cohort of hospitalized children. J Med Virol. 2012; 84(1): 99-108. doi: 10.1002/jmv.22217.

23. Wong-Chew RM, Garcia-Leon ML, Noyola DE, et al. Respiratory viruses detected in Mexican children younger than 5 years old with community-acquired pneumonia: a national multicenter study. Int J Infect Dis. 2017; 62: 32-8. doi: 10.1016/j.ijid.2017.06.020.

24. Choi EH, Lee HJ, Kim SJ, et al. The Association of Newly Identified Respiratory Viruses with Lower Respiratory Tract Infections in Korean Children, 2000–2005. Clin Infect Dis. 2006; 43(5): 585-92. doi: 10.1086/506350.

25. Zhan Y, Yang Z, Chen R, et al. Respiratory virus is a real pathogen in immunocompetent community-acquired pneumonia: comparing to influenza like illness and volunteer controls. BMC Pulm Med. 2014; 14: 144. doi: 10.1186/1471-2466-14-144.

26. Al-Toum R, Bdour S, Ayyash H. Adenovirus infections in Jordanian hospitalized pediatric patients: prevalence and clinical features. Jordan Med J. 2009; 43 (3): 171-9.

27. Kaplan NM, Dove W, Abd-Eldayem SA, et al. Molecular epidemiology and disease severity of respiratory syncytial virus in relation to other potential pathogens in children hospitalized with acute respiratory infection in Jordan. J Med Virol. 2008; 80(1): 168-74. doi: 10.1002/jmv.21067.

28. Papadopoulos NG, Moustaki M, Tsolia M, et al. Association of rhinovirus infection with increased disease severity in acute bronchiolitis. Am J Respir Crit Care Med. 2002; 165(9): 1285-9. doi: 10.1164/rccm.200112-118BC.

29. Sharma P, Kolawole AO, Core SB, et al. Sidestream smoke exposure increases the susceptibility of airway epithelia to adenoviral infection. PLoS One. 2012; 7(11): e49930. doi: 10.1371/journal.pone.0049930.

30. Albernaz EP, Menezes AMB, Cesar JA, et al. [Risk factors associated with hospitalization for bronchiolitis in the post-neonatal period]. Rev Saude Publica. 2003; 37(4): 485-93.

31. Kumar SG, Majumdar A, Kumar V, et al. Prevalence of acute respiratory infection among under-five children in urban and rural areas of puducherry, India. J Nat Sci Biol Med. 2015; 6(1): 3-6. http://dx.doi.org/10.4103/0976-9668.149069.

32. Meyer-Rath G, Brennan AT, Fox MP, et al. Rates and cost of hospitalisation before and after initiation of antiretroviral therapy in urban and rural settings in South Africa. J Acquir Immune Defic Syndr. 2013; 62(3): 322-8. doi: 10.1097/QAI.0b013e31827e8785.

Correspondence to
Dr. Arwa M. A. Al-Shuwaikh
E-mail:
arwa.mujahid@colmed-alnahrain.edu.iq
arwa_alshwaikh_2004@yahoo.com

Received Nov. 7th 2018
Accepted Jan. 7th 2019

Iraqi JMS

Published by Al-Nahrain College of Medicine
P-ISSN 1681-6579
E-ISSN 2224-4719
Email: iraqijms@colmed-alnahrain.edu.iq
http://www.colmed-alnahrain.edu.iq
http://www.iraqijms.net
Iraqi JMS 2019; Vol. 17(1)

Immunohistochemical Expression of Monocarboxylate Transporter 1&4 in Tanycyte–like Cells of the Sulcus Medianus Organum

[1]Haider F. Jawad *BVM&S*, **Muthanna A. Al-Kaabi[2]** *PhD*, **Anam R. Al-Salihi** *PhD*

[1]Dept. of Human Anatomy, College of Health and Medical Technology, Uruk University, Baghdad, Iraq, [2]Dept. of Human Anatomy, College of Medicine, Al-Nahrain University, Baghdad, Iraq, College of Health and Biomedicine, Victoria University, Melbourne, Victoria, Australia

Abstract

Background Circumventricular organs (CVOs) are specialized structures border the brain ventricles and lack the blood-brain barrier. These CVOs are lined by specialized ependymal cells (ECs) called tanycyte. The sulcus medianus organum (SMO) locates at the floor of the 4th ventricle at the rostral part of the sulcus medianus (SM).

Objective To explore the expression of the monocarboxylate transporter 1 and monocarboxylate transporter 4 (MCT1 & MCT4) in the tanycytes of the median eminence (ME) and tanycyte-like cells of the SMO to add a functional evidence for describing the SMO as another CVOs and to start a roadmap for the citing the SMO specifically as a sensory or a secretory CVO.

Methods Ten adult male rats (Rattus norvegicus albinus), aged 3-6 months with 300±50 g, were used to study the histological characteristics of ECs in ME and SMO with Hematoxylin & Eosin and to explore the immunohistochemical expression of MCT1 & MCT4 in ME and SMO.

Results The ependymal cells were arranged in in 2-3 layers in the depth of SMO region and single layer in the ME region as seen with H&E stains. Immunohistochemical expression of MCT1 & MCT4 using Aperioscope image analysis in tanycytes of the ME is higher than that in tanycyte-like cells of the SMO with significant differences between the two regions as proved by t-test.

Conclusion the SMO has different structural and functional properties compared to ME suggesting that the SMO may be a sensory CVO.

Keywords Circumventricular organs, tanycytes, sulcus medianus organum, median eminence

Citation Jawad HF, Al-Kaabi MA, Al-Salihi AR. Immunohistochemical expression of monocarboxylate transporter 1&4 in tanycyte–like cells of the sulcus medianus organum. Iraqi JMS. 2019; 17(1): 83-99. doi: 10.22578/IJMS.17.1.12

List of abbreviations: AP = Area postrema, BBB = Blood-brain barrier, CSF = Cerebrospinal fluid, CVOs = Circumventricular organs, ECs = Ependymal cells, MCTs = Monocarboxylate transporters, ME = Median eminence, NH = Neurohypophysis, OVLT = Organum vasculosum of the lamina terminalis, PBS = Phosphate buffer saline, SCO = Subcommissural organ, SEM = Scanning electron microscope, SFO = Subfornical organ, SM = Sulcus medianus, SMO = Sulcus medianus organum, TEM = Transmission electron microscope

Introduction

Circumventricular organs (CVOs) are specialized structures in the brain that lack the blood-brain barrier (BBB) and border the ventricular cavities [(1)]. The basic vascular, ependymal and neural association of these structures contrasts that found in other cerebral tissue [(2)].

There are seven CVOs are situated in different locations in the rat brain: subfronical organ (SFO), organum vasculosum of lamina terminalis (OVLT), pineal gland (Pi), subcommissural organ (SCO), median eminence (ME), neurohypohysis (NH) and area

postrema (AP); all found in the wall of the 3rd ventricle except AP locates in the wall of the 4th ventricle [2]. The CVOs differ from other structures of the brain because they are highly vascularized and have unusual vascular arrangement and several capillary loops extend to the surface of ventricle [3].

Johnson and gross [4] divided CVOs into two groups sensory (SFO, OVLT and AP) and secretory (NH, ME and Pi). Sensory CVOs have the capacity to detect plasma molecules and afterward pass that information to different locations of the brain [1]. Secretory CVOs secret hormones and glycoproteins into peripheral vascular system using feedback from brain environment and external stimuli [5]. The BBB is absent in all CVOs with exception SCO of CVO, lack the BBB allows direct exchange of substance between the blood and nervous tissue of these organs [6].

Ventricular surface of CVOs formed by specialized ependymal cells (ECs) are different in appearance from cuboidal ECs covering other ventricular surfaces covering other ventricular surfaces. CVOs ECs can be elongated or columnar and they are non-ciliated or have few cilia on their luminal surface [7]. ECs of CVOs have tight junctions between them in contrast other ventricular ECs [8].

Sulcus medianus organum (SMO) first described as CVO in the region of median sulcus of 4th ventricle by Collins [9] in the rabbit brain. The SMOs located at rostral part of the Sulcus medianus where the epithelium is pseudo stratified and has long projections that terminate near blood vessel. Microvilli covers the apical surface of these cells; just a few cilial bunches are seen. Pinocytotic vesicles are between these microvilli when examined under transmission electron microscope (TEM). However, the structural and functional characterization of the SMO is still under study.

Tanycytes are specific type of ECs situated in the lower part of the ventricular walls and the floor of the 3rd ventricle and have an elongated morphology and are not ciliated [10]. The name tanycyte is derived from the Greek word "tanus" which means "elongated" [12].

In human cellular differentiation of tanycytes begins in day 19 of embryonic development while that of rat begins in day 18 [13]. Here tanycyte are produced in the last two days of pregnancy and the first few days postnatally [14].

Tanycytes that are located close to the dorsomedial and ventromedial hypothalamic nuclei are called alpha-1 and alpha-2 while tanycytes which are near the arcuate nuclei and median eminence are called beta-1 and beta-2 tanycytes [15]. Beta-1 and beta-2 tanycytes are located in the ventral part of the 3rd ventricle while alpha-1 and alpha-2 tanycytes are present dorsal to beta-tanycytes [16].

Monocarboxylate transporters (MCTs) are a group of transporters responsible on diffusion of monocarboxylates like ketone bodies, lactate and pyruvate [17].

According to sequence homologies, MCTs classify into 14 members recognized as MCT-9, MCT11-14 and T-type amino-acid transporter-1(TAT1) [18]. These monocarboxylates have main roles in metabolism of carbohydrates, fat, and amino acid where MCTs transport them across plasma membrane of cells [19].

There are other members of MCTs belonging to solute carrier family SLC5 that play important role in transportation of monocarboxylates across endothelium of the gut and kidney [20]. MCT1-4 transport more and less common endogenous monocarboxylates (pyruvate, lactate, and ketone bodies are more common) and (acetate, propionate and butrate are less common) [21].

Monocarboxylate transporter 1 (MCT1) first demonstration was in transportation of L-lactate and pyruvate to red blood cells in human [22]. MCT1 is detected in the cerebral cortex, hippocampus and cerebellum of young and adult rats from day 15 of post-natal life especially in parenchymal cells [23]. MCT1 is found in the cell body, processes and plasma membrane of astrocytes [24]. MCT1 also present

in the endothelial cells of blood vessels and ECs that line the ventricles of the brain [25].

The MCT1 was identified in alpha-tanycytes particularly on ventricular cell membrane and on end feet processes reaching blood vessels endothelium. Similarly, the apical membranes of beta-1 tanycytes and astrocytes show high levels of MCT1 [26]. Ketone bodies and lactate require MCT1 to enter the cells of muscle [27].

Monocarboxylate transporter 4 (MCT4) is wide spread particularly in tissues that depend on glycolysis like white fibers of skeletal muscle, white blood cells, and astrocytes [28]. MCT4 is responsible for lactic acid transportation from the fibers of glycolytic muscle [29]. The MCT4 has low affinity to the substrates and inhibitors in contrast MCT1 [30].

This pilot study aims to quantify the immunohistochemical expression of MCT1 and MCT4 in tanycyte-like cells of the SMO in comparison to their expression in the ME in order to add functional evidence for describing the SMO as another CVO and to start roadmap for citing the SMO specifically as a sensory or a secretory CVO.

Methods

Animals and tissue preparation

A sample of 10 adult male rats (Rattus norvigicus albinus) where chosen from the Animal House of Biotechnology Research Center, Al-Nahrain University during the academic year 2017-2018 on basis of being apparently healthy and active. The animals aged 3-6 months with 300±50 g body weight. They feed with standard pellet diet.

Animals were euthanized with chloroform-soaked cotton in an air tight chamber for 5 minutes. Then, skull dissection was done to deliver the brain into 4% paraformaldehyde (the fixative prepared by 4gm paraformaldehyde powder , 5 ml concentrate PBS (pH=7.2), distilled water (95 ml) for 18 hours in preparation for dissection into two parts under dissecting microscope one part contained the ME and the other part included the cerebellum, 4th ventricle and SMO.

Standard processing procedures were performed with Analar® materials and 5 µm thickness section were cut from the regions of the SMO and ME with a Richert-Jung® Biocut microtome.

Hematoxylin and Eosin (H.&E.) staining

According to Suvarna et al. [31] Harries modified hematoxylin (Riedel- de Hean®) and Eosin-Y (Fisher Scientific®) were prepared.

Immunohistochemical staining

Antigen Retrieval

After dewaxing and rehydration, the slides were put in glass jar filled with antigen retrieval solution (sodium citrate buffer) that was prepared according to Shi et al. [32] as follows:

Tri-sodium citrate (USA Fisher scientific®)
2.94 gm
Distilled water
1000 ml

They were mixed well using a magnetic stirrer (Fisher scientific®) then 0.5 ml of tween 20 was added. Sodium citrate solution pH was adjusted to 6 by adding few drops of 1N HCL. The slides were heated in an autoclave (120 °C 1.2 Bar) for 3 minutes and then left to cool down for 20 minutes. The slides were then washed with PBS before immunohistochemical staining.

Immunohistochemical procedure

Biorbyte® staining kit and primary antibodies (Anti-MCT1, Anti-MCT4) were used in immunohistochemical staining of tissue sections. Immunohistochemical staining procedure steps were (hydrogen peroxide, blocking reagent, apply primary anti-bodies (Anti-MCT1 and Anti-MCT4) were incubated for 2 hours, antibody amplifier, HRP polymer, DAB and finally slides were stained with counter stain (hematoxylin) and mounted using water soluble mounting medium (Dako®).

Controls

Negative internal controls were prepared from ME and SMO sections while positive internal controls were prepared from ME sections. External negative and positive controls were

prepared by kidney sections. Negative controls were prepared by the same immunohistochemical procedure except that replacing the primary antibody with PBS. Positive controls were prepared by the same immunohistochemical procedure of primary antibodies (Ani-MCT1 and Anti-MCT4).

Sections Examination

Sections stained with H&E and those labeled immunohistochemical were examined using light microscope (Richert Chung®) under the powers 40X, 100X, and 400X.

Analysis with Aperio ImageScope Software

Images of immunohistochemical labeling were analyzed with aperio ImageScope software (version 12.3). This program assigned four different colors (red, orange, yellow and blue) one for each reaction intensity or "positivity". Red color = strong positive, orange color = positive, yellow color = weak positive and blue color = negative or no reaction.

Ethical Statement

All applicable international, national and institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of institution or practice at which the studies were conducted.

Results

General Morphology with H & E staining

Sulcus Medianus Organum (SMO)

The SMO was marked as a region in the rostral part of the 4th ventricle floor delineated by a median longitudinal cleft. The SMO was lined by cuboidal or low columnar multilayer ECs (figures 1-2).

Median Eminence

The ME region was located in the ventral part of the 3rd ventricle. Ependymal cells (tanycytes) lined the walls and floor of the 3rd ventricle. These ECs appeared a single layer and cuboidal in shape (Figures 3-4).

Immunohistochemical Labeling

Anti-MCT1 and Anti-MCT4 in SMO

Labeling with anti-MCT1 and anti-MCT4 in the floor of the 4th ventricle was seen at the SMO region. The immunohistochemical reaction of MCT1 and MCT4 labeling appeared brown in color under light microscope (Figures 5-6).

Anti-MCT1 and Anti-MCT4 in ME

The specialized ECs or tanycytes of the ME region at the 3rd ventricle showed dark brown color labeling with anti-MCT1 and anti-MCT4 markers along its specialized ECs lining or its tanycyte-like cells when examined under light microscope (Figures 7-8).

Internal Negative Control of the SMO and ME

Sections from the SMO and ME regions prepared as internal negative control for the immunohistochemical labeling showed no brown color at ECs layer (Figure 9).

External Positive and Negative controls

Kidney sections were used as external positive and negative controls for MCT1 and MCT4 markers (Figures 10-11).

Aperio ImageScope Software analaysis

Immunohistochemical reactions images analyzed with Aperio Image scope software in the SMO and ME regions for MCT1 and MCT4 markers labeling (Figure 12).

Statistical Analysis

Statistical analysis of MCT1 labeling positive pixel algorithm showed higher mean value in the ME (0.441023) than in the SMO (0.226748) and t-Test revealed a significant difference between two regions ($p<0.05$) (Table 1).

Statistical analysis of MCT4 labeling positive pixel algorithm showed higher mean value in the ME (0.237688) than in the SMO (0.168917) and t-test revealed a significant difference between two regions ($p<0.05$) (Table2).

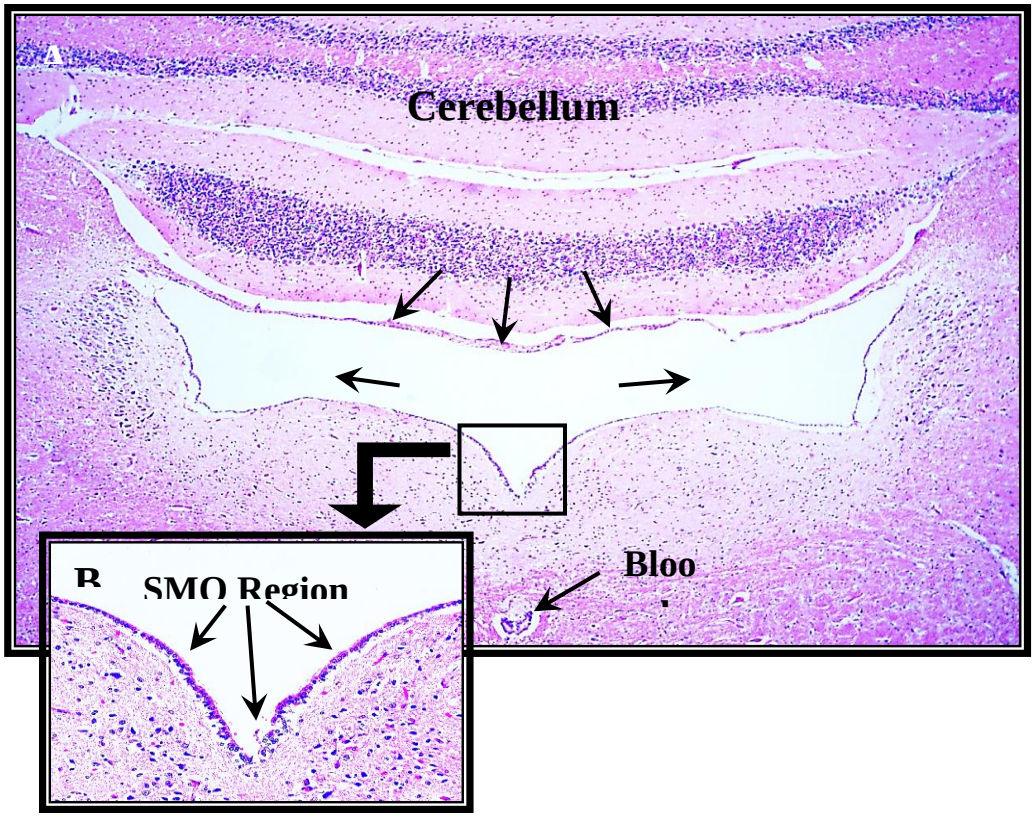

Figure 1. (A) Coronal section through the 4th ventricle. SMO region is seen at the floor of the 4th ventricle (inset). Tela choridae is seen in the roof of the 4th ventricle. (B) SMO region at the floor of 4th ventricle lined by cuboidal or low columnar multilayer ECs. H & E stain. (A) 40X (B) 200X

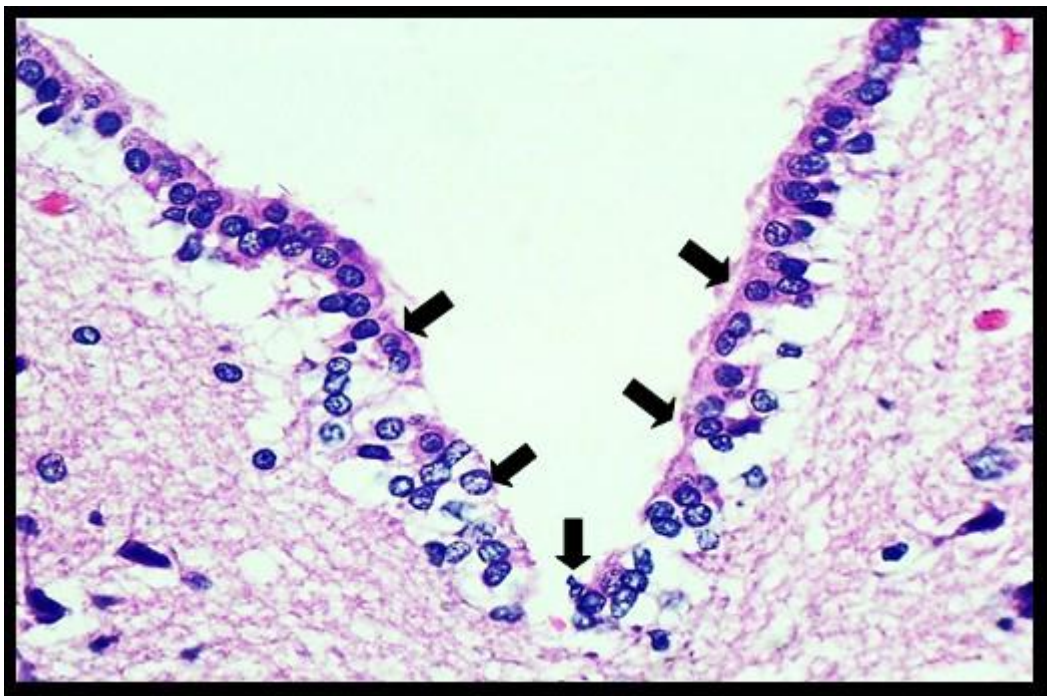

Figure 2. SMO region at the floor of 4th ventricle lined by multilayer ECs cuboidal or low columnar in shape (block arrows). H & E stain. 400X

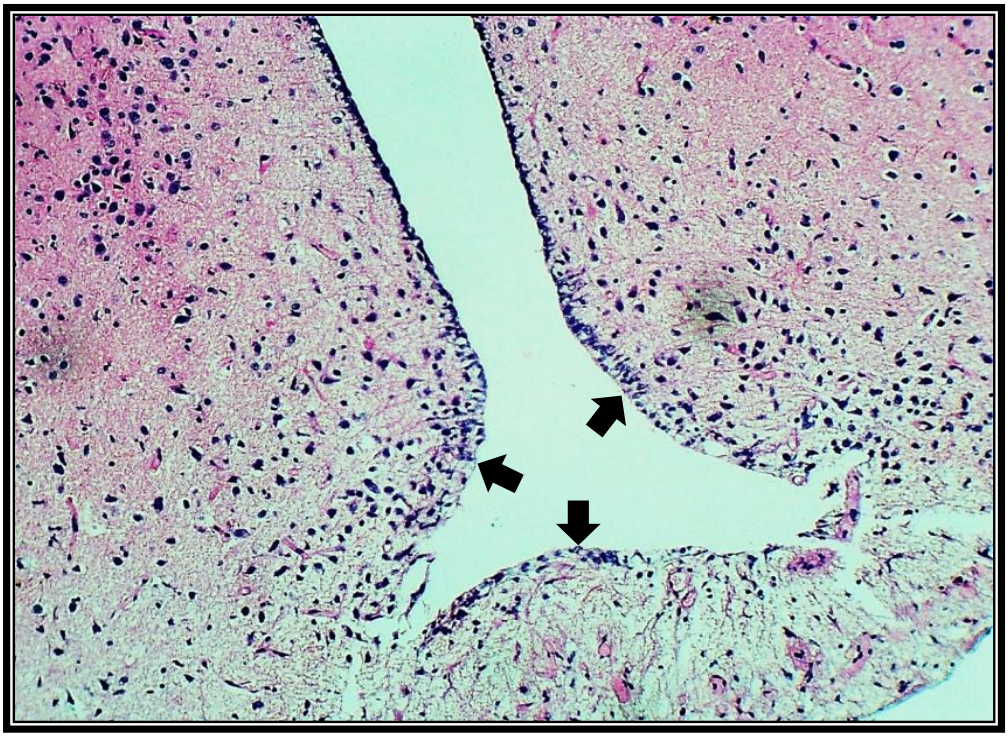

Figure 3. ME region is located at the floor of the 3rd ventricle and lined by specialized ECs called tanycytes (black arrows). H & E stain. 100X. ARH: arcuate hypothalamic nucleus. ME: median eminence

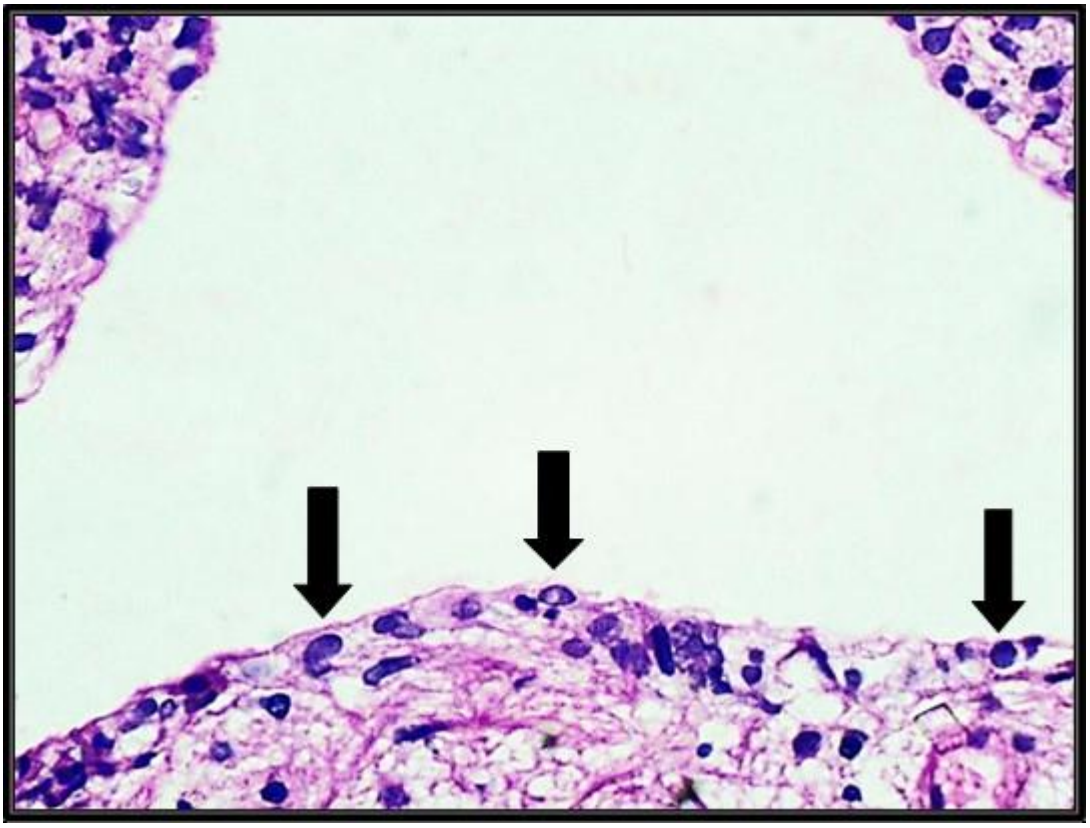

Figure 4. The floor of the 3rd ventricle is formed by ME region which is lined by non-ciliated and cuboidal specialized ECs (tanycytes). H & E stain. (400X)

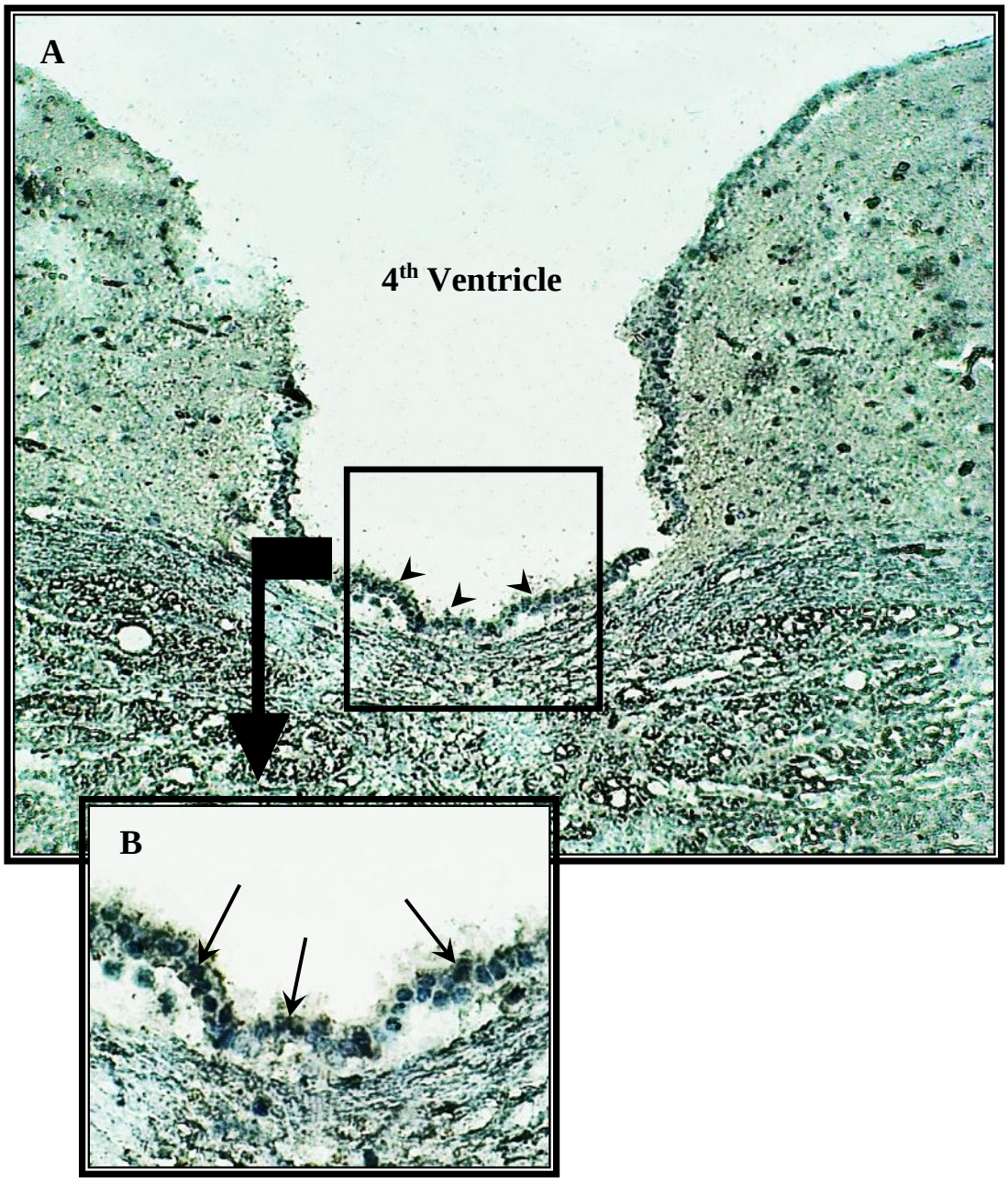

Figure 5. (A) SMO region is lined by multilayer of specialized ECs or tanycyte-like cells labeled with MCT1 marker and counterstained with hematoxylin (arrow heads) (100x). (B) Higher magnification of the inset highlighting MCT1 labeling as brown color along ECs (arrows). (A) 100X (B) 400X

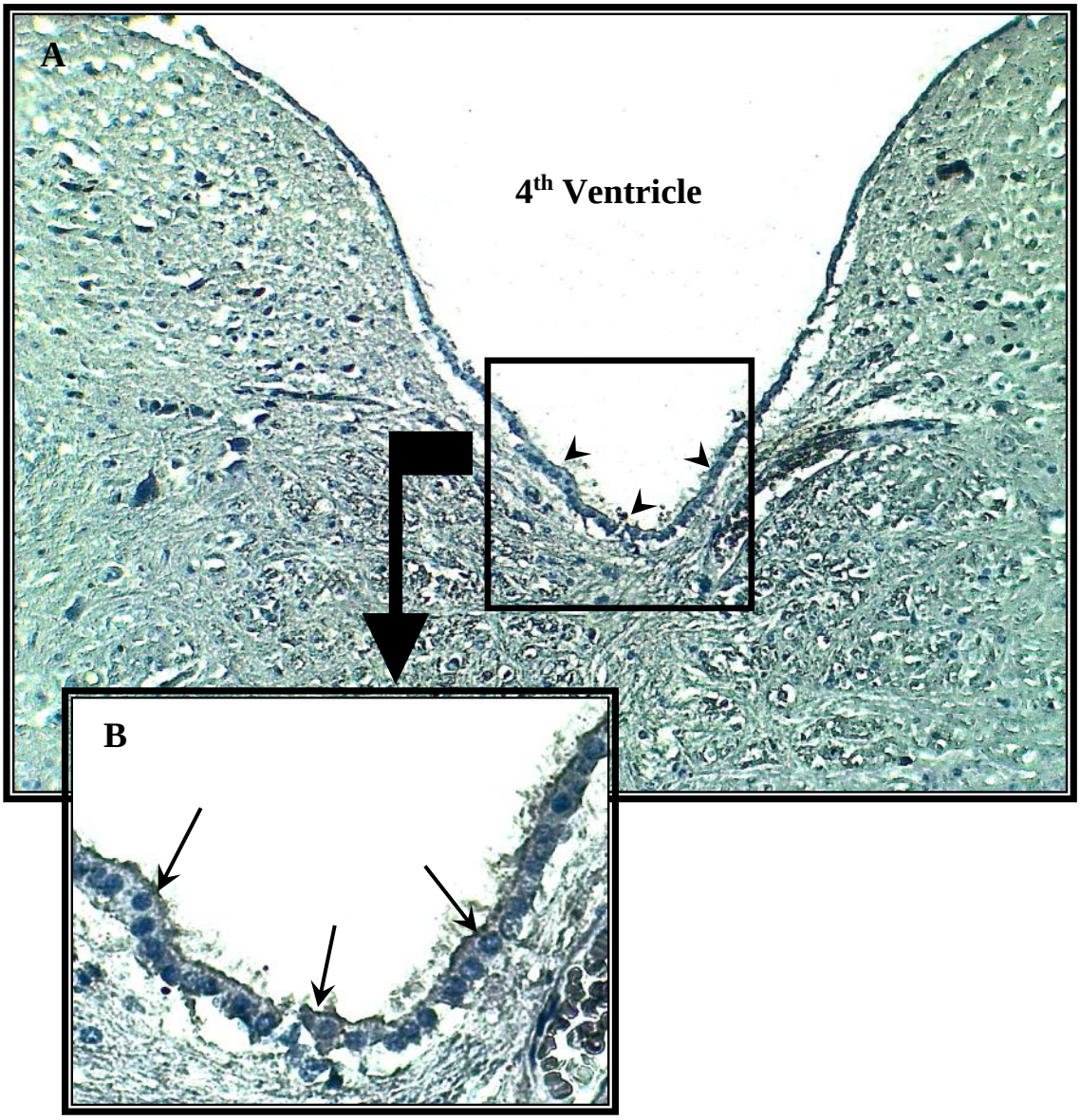

Figure 6. (A) Specialized ECs or tanycyte-like cells of the SMO region labeled with MCT4 marker and counter stained with hematoxylin (arrow heads). (B) Higher magnification if the inset emphasizing MCT4 labeling as brown color along ECs (arrows). (A) 100X (B) 400X

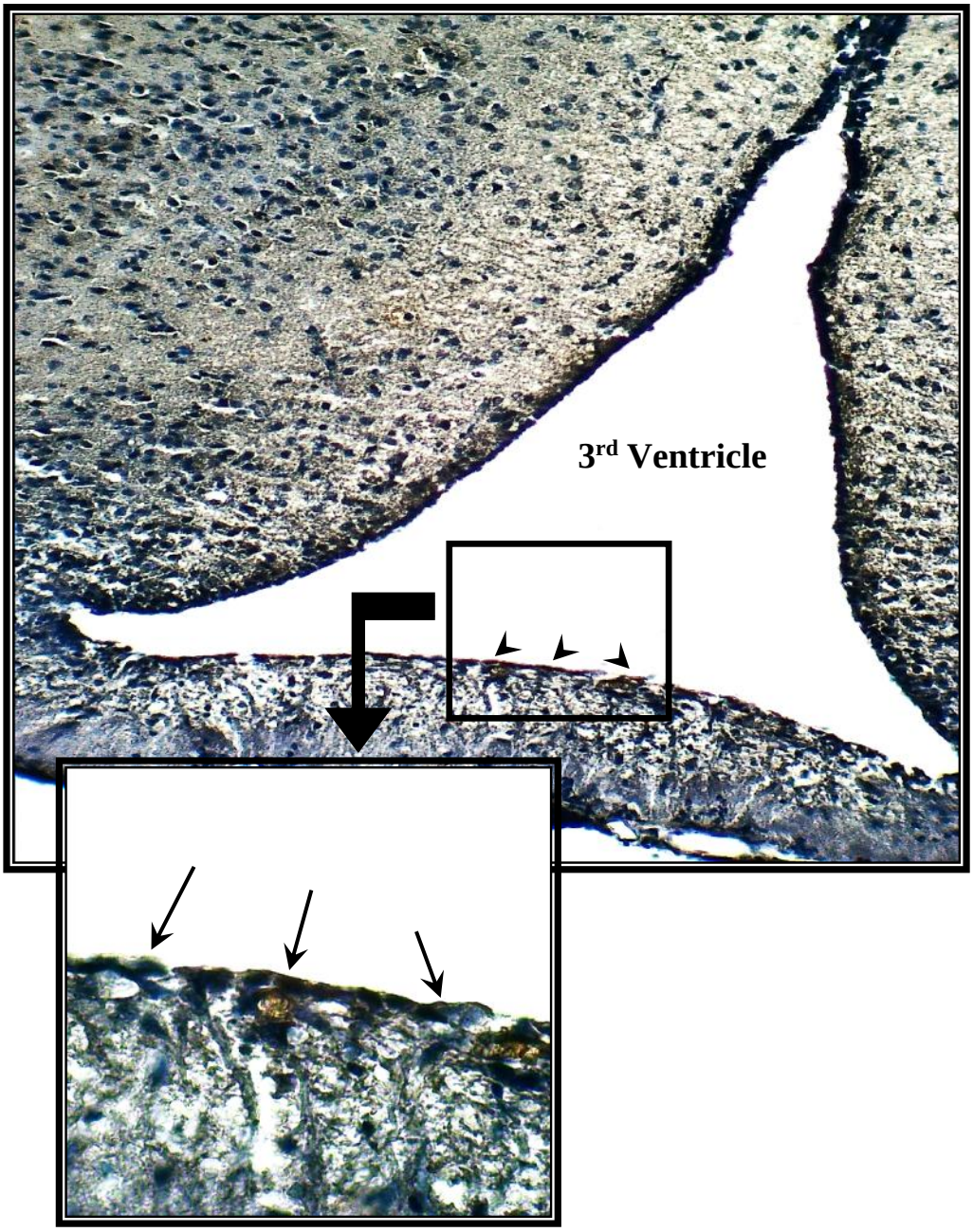

Figure 7. (A) ME region is lined by specialized ECs or tanycyte labeled with MCT1 marker and counterstained with hematoxylin (arrow heads) (B) Higher magnification of the inset highlighting MCT1 labeling as dark brown color along ECs (arrows). (A) 100X (B) 400X

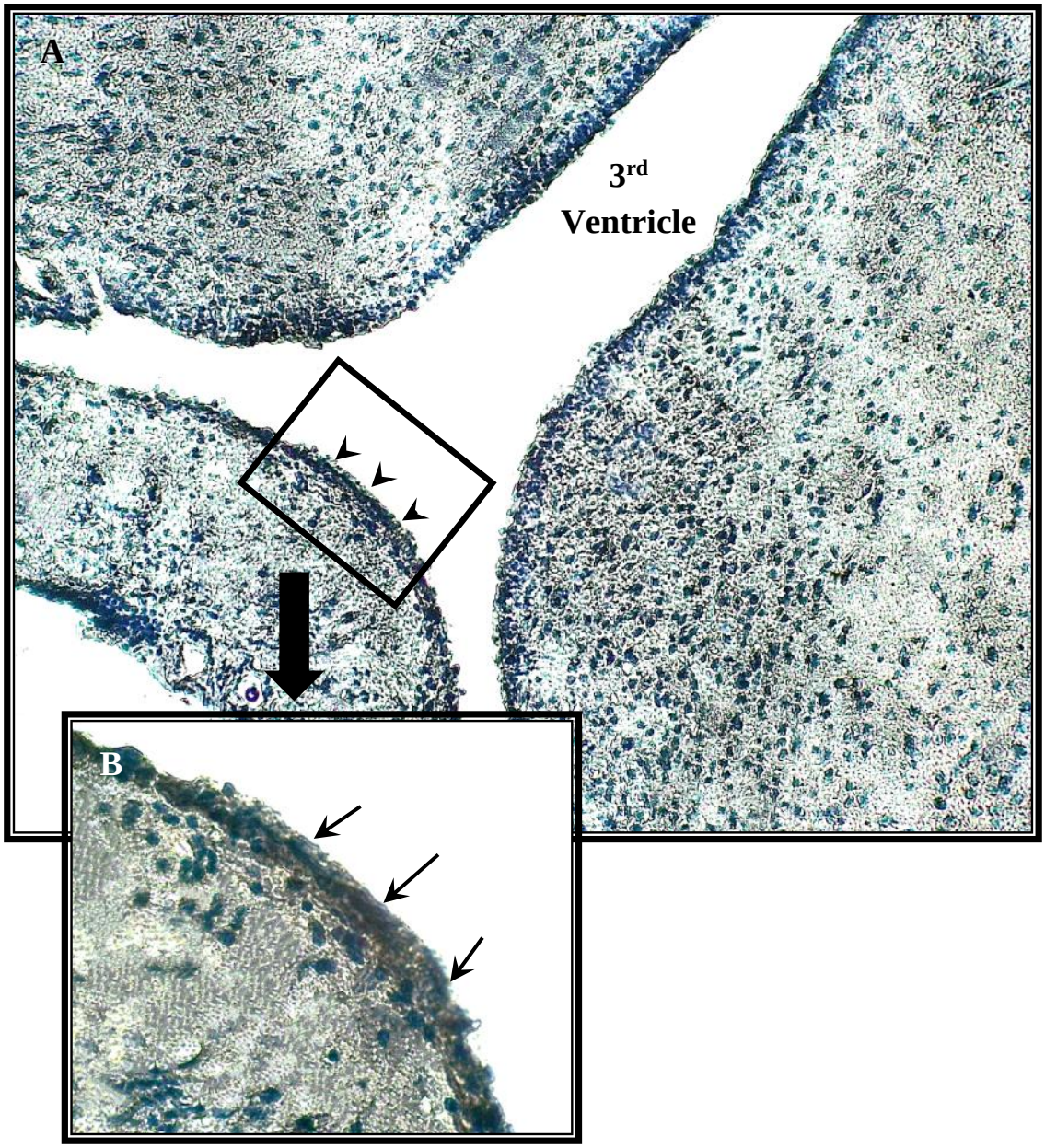

Figure 8. (A) Coronal section showing the 3rd ventricle of the brain and ME region lined by specialized ECs or tanycyte labeled with MCT4 marker and counterstained with hematoxylin (arrow heads) (B) Higher magnification if the inset emphasizing MCT4 labeling as dark brown color along ECs (arrows). (A) 100X (B) 400X

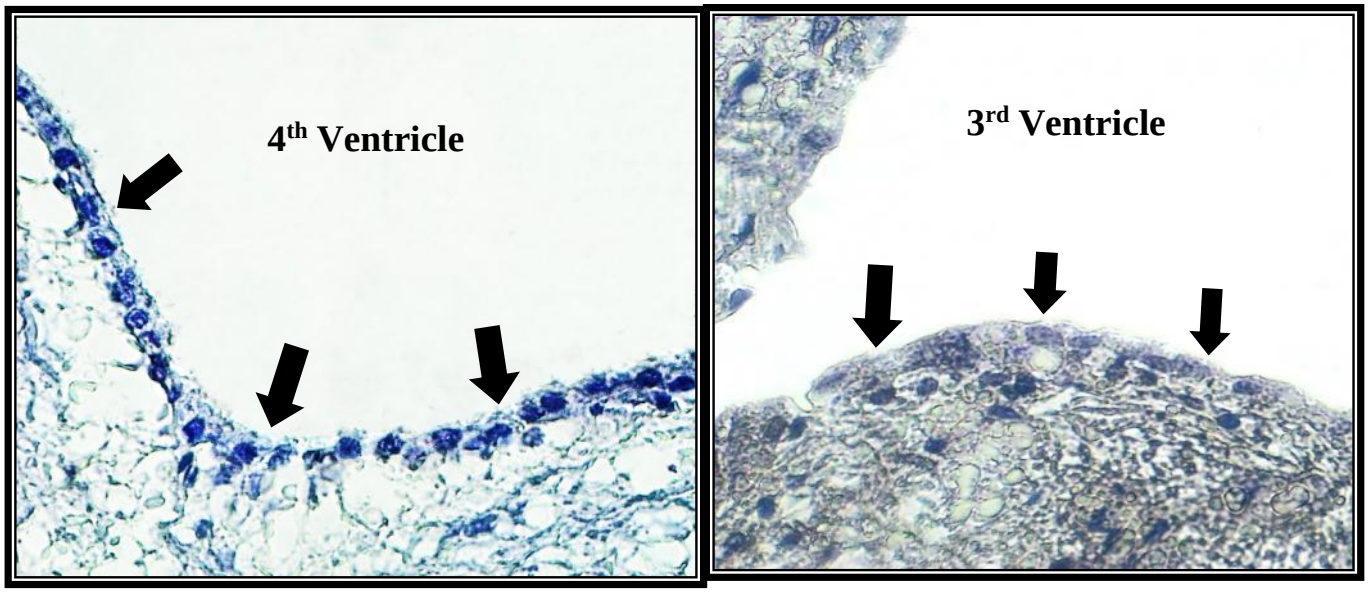

Figure 9. Internal negative control of the A-SMO B-ME regions for MCT1 and MCT4 labeling, no brown color is seen along the ECs of both regions (black arrows). Counter stain (hamatoxylin). 400X

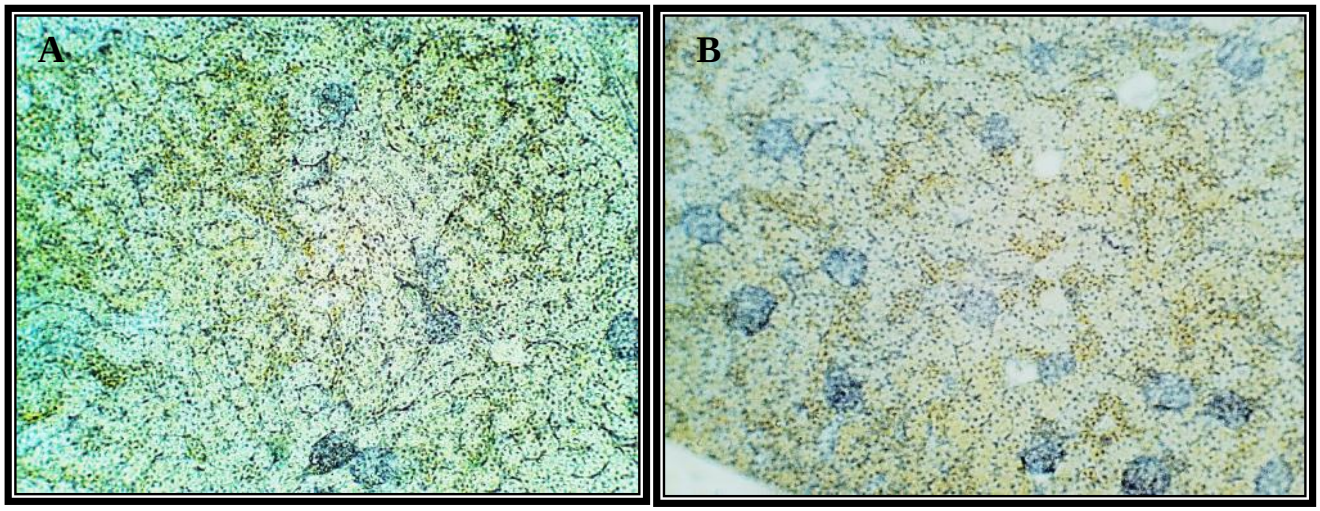

Figure 10. Sections of rat kidney labeled with (A) anti- MCT1 (B) anti-MCT4 antibodies as a positive control where the brown color marks the presence of label

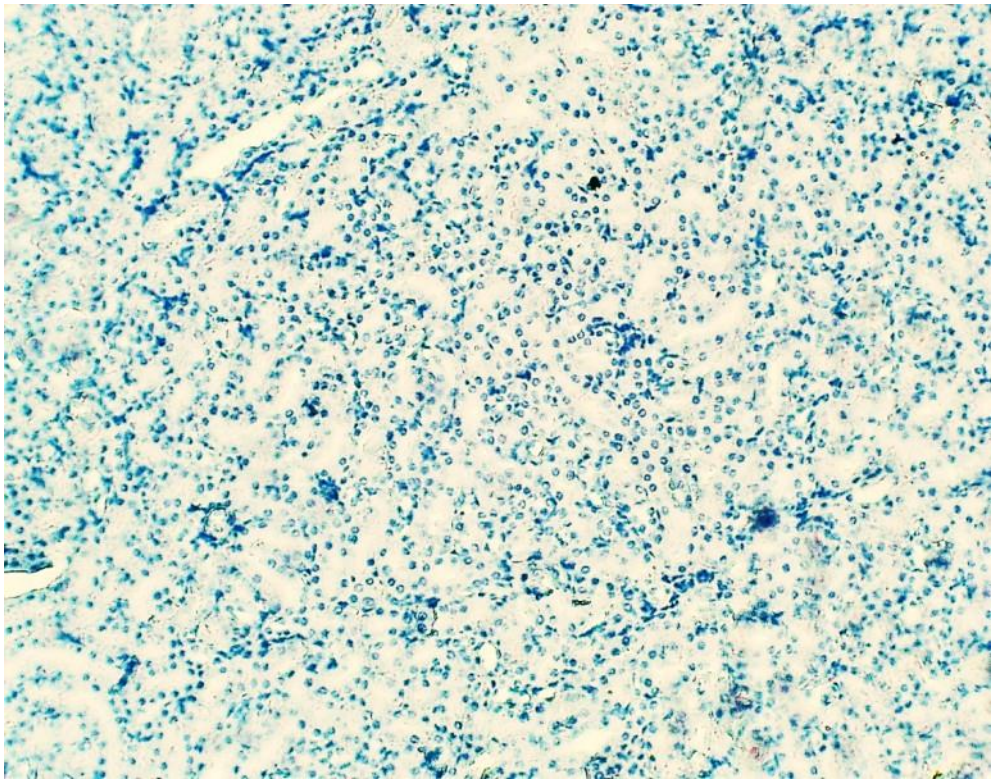

Figure 11. Section of rat kidney as an external negative control for MCT1 and MCT4 labeling, no brown color is seen at renal tubules. Nuclei appear blue in color since they stain with hematoxylin as a counterstain. 400X

Discussion

General Morphology with H & E stain

Sulcus Medianus Organum (SMO)

Rare studies have been done to describe the ECs of the SMO region using H&E stains under light microscope. SMO region with H&E staining appears as median longitudinal cleft in the floor of the 4th ventricle lined by single layer of ECs while in its deepest part it is lined by more than one layer of ECs [33].

In this study we reported that the SMO region was located in the rostral part of the of the median sulcus region at the floor of the 4th ventricle and when we stained with H&E we noted that SMO region lined by multilayer of ECs at the deepest part low columnar or cuboidal in shape (Figures 1-2).

Median Eminence (ME)

The ME forms the lower part of the brain extending caudal to the optic chiasm up to the hypophyseal stalk [35]. The ventricular surface of ME is formed by a single layer of ECs [33]. Horstmann [11] first used the word "tanycyte" when he described the ECs lining the infundibular recess of the 3rd ventricle because of their shape. These ECs have bipolar elongated shape with proximal pole near to the ventricular surface and distal pole close to the portal vessels.

Peroxidase-antiperoxidase methods revealed the labeling of tanycytes by Tanycyte-like the tanycytes [36]. Tanycytes in the CVOs were investigated by examining vemintin positive ECs lining the walls of the ventricles [37]. Al-Kaabi et al. [33] observed that vimentin-labeled ECs of the 3rd ventricle have basal processes extending toward the deeper capillaries.

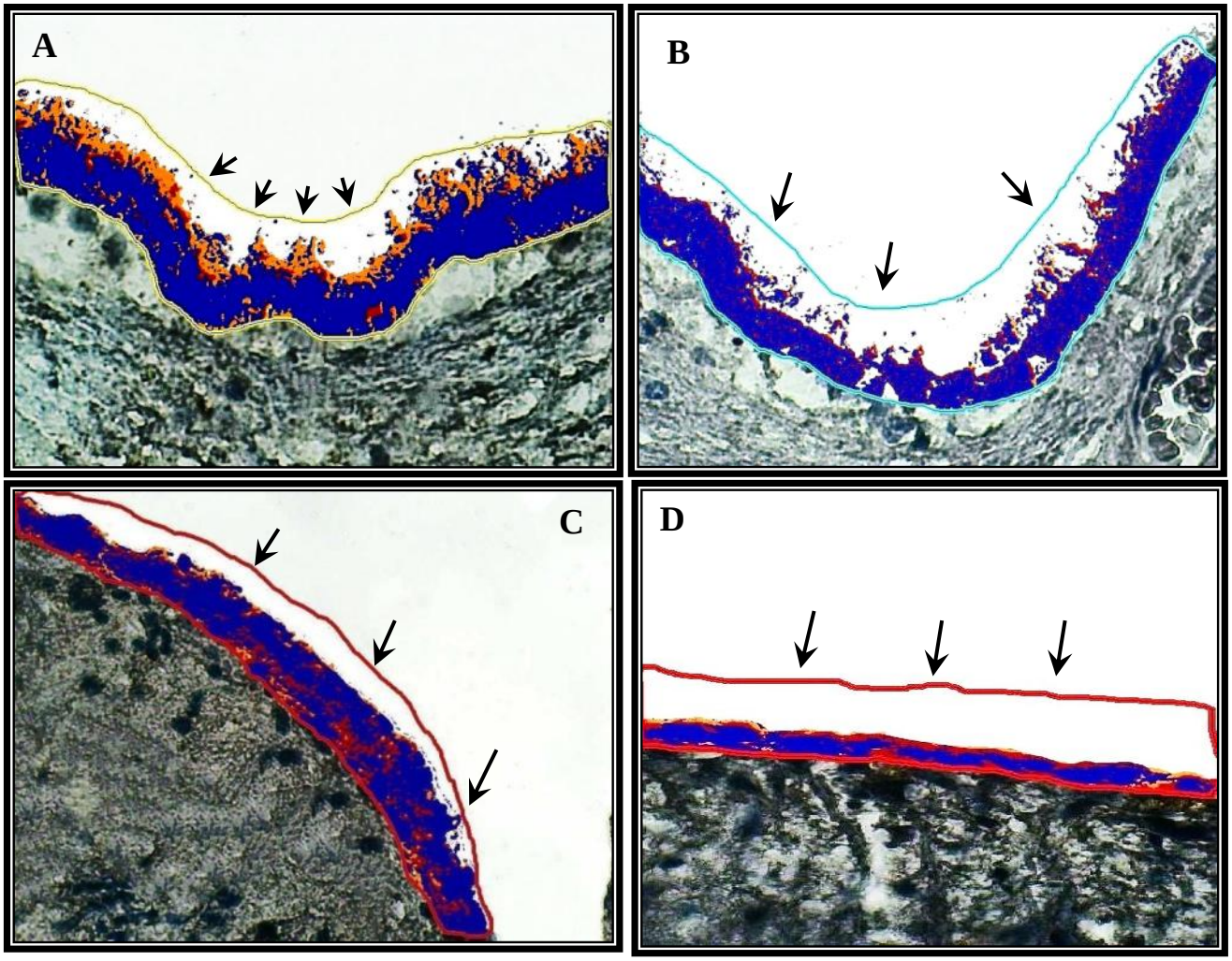

Figure 12. Immunohistochemical reaction images analyzed by AperioImageScope software and area of selections (arrows) are processed for signal intensity. (A) SMO labeled with MCT1 marker (B) SMO labeled with MCT4 marker (C) ME labeled with MCT4 marker (D) ME labeled with MCT1 marker

In this study the ME sections were stained with H&E stains and examined under light microscope to show the ECs (tanycytes) lining the walls and the floor of the 3rd ventricle. These ECs appeared cuboidal in shape and they had processes seen as fine fibers extending to the surrounding parenchyma (Figures 3-4). Later studies classified tanycytes into four groups Bata-1 and Beta-2 occupy the floor and the ventral part of the 3rd ventricle while alpha-1 and alpha-2 tanycytes locate dorsal to beta tanycytes [16].

Immunohistochemical labeling of MCT1 and MCT4

Monocarboxylate transportes (MCTs) are other important plasma membrane transporters that catalyze the proton-linked transport of monocarboxylate such as lactate, and pyruvate which have role in the cell metabolism [40]. MCTs have been demonstrated in various tissues including brain tissues [19]. Monocarboxylates provide a divergent source of energy for the brain [41]. Jackson et al. [42] reported by northern blot analysis the expression on MCT1 mRNA in the rat brain and Gerhart et al. [25] confirmed this expression of MCT1. Price et al. [43] explored the MCT4

expression in the rat during a search of the EST database for novel members of the MCT family. MCT4 expression was in skeletal muscle fibers, astrocytes, ECs of the brain, chondrocytes and white blood cells. Merezhinskaya and Fishbein [41] noted that MCT1 located in the blood vessels of the brain regulates both influx and efflux of lactate cross blood-brain barrier. Cortés-Campos et al. [26] demonstrated that tanycytes express MCT1 and MCT4 and both involved in influx and efflux of lactate, and also revealed that MCT1 express on the apical membrane and and cellular processes of the tanycytes while the MCT4 in the apical membrane. Takano et al. [44] revealed that the ECs, endothelial cells and the astrocytes express both MCT1 and lower MCT4. Chiry et al. [45] clearly demonstrated MCT1 in astrocytes and their end feet adjoining capillary while the restricted expression of MCT4 in astrocytes in certain brain regions are shown. Gerhart et al. [25] revealed during QT-PCR analysis that MCT1 is more highly expressed than the MCT4 in the hypothalamic tanycytes during detection these transporters in these cells. In this study, we revealed the expression of the MCT1 and MCT4 in the tanycytes of the ME (ordinary secretory CVO) and tanycytes-like cells of the SMO. We found that the expression of MCT1 and MCT4 in the ME higher than in the SMO. Also, we revealed that the expression of MCT1 is more expression than MCT4 in the tanycytes of the ME (same result previous studies) and also, we found that the MCT1 expression of tanycytes-like cells of the SMO is higher thanMCT4 expression. There have been no previous studies on the expression of MCT1 and MCT4 in SMO region and this study considers the first study to talk about this subject.

Functional consideration of tanycytes

Tanycytes of the ME form a barrier between the CSF and ME neuropil [46]. In addition, tanycytes of the ME in the adult brain have capacity to serve as neuronal progenitors [47]. The most probable function of tanycytes is the transportation of substances from the CSF to their terminals [48].

Tanycytes appear to regulate GnRH release into the portal blood, in this manner regulating release of LH and FSH from pituitary gland [49]. Tanycytes of the ME have an ability of absorbing substances in the CSF and transport them through their basal processes to the perivascular space [50]. Tanycytes have surface projections that bulge into the 3rd ventricle; there are variations in the number and size of such bulges under different endocrine states. These observations have led many authors to suggest them as a proof of a tanycytes secretory activity [12]. For example, tanycytes are stimulated by estrogen to secrete transforming growth factor-α (TGF-α) and prostaglandin (PG) which in turn stimulate GnRH discharge [49]. Immunoreactive 5a-reducctase enzyme is secreted from tanycytes that plays a role in the conversion of progesterone to dihydroprogesterone and testosterone to dihydroteserone [51]. MCT1 and MCT4 transport the monocarboxylates that are essential in the production of energy in the brain especially lactate [52]. The MCT1 is available in tanycytes that line the floor and walls of the 3rd ventricle [26]. The MCT1 is present in the apical membrane of tanycytes and, in addition to MCT4, is expressed in the hypothalamus. These transporters are useful in releasing the lactate from glucose in tanycytes [53]. However, MCT1 had higher expression than MCT4 in the hypothalamic tanycytes during detection of these transporters using Q-RT-PCR analysis [25].

The immune histochemical labeling of MCT1 and MCT4 markers in this study confirmed the expression of these markers in tanycytes of the ME and tanycyte-like cells of the SMO albeit at different levels in favor of the ME. On the other hand, both regions showed higher levels of MCT1 expression in comparison to that of MCT4 with statistical significance which may reflect differences in roles played by these transporters in the traffic of substances through tanycytes and tanycyte-like cells. This difference in marker expression may reflect certain properties of two regions as CVOs with specific functions for each one.

Is the SMO a sensory CVO?

It is known that MCT1 and MCT4 are responsible for transportation of monocarboxylates (lactate and pyruvate) that are important in the production of energy by cells [53]. In this study, the histochemical expression levels of MCT1 and MCT4 both in the ECs of the ME at the floor of the 3rd ventricle and in the ECs of the SMO at the floor of the 4th ventricle were found to be higher in the ME compared to those in the SMO. Such high levels of these transporters in tanycytes of the ME region indicated that these cells are more active and need more energy to perform their functions. The ME is described as a secretory CVO since its tanycytes have secretory activities and contain different types of enzymes as noted by Flament-Durand and Brion [12] where the processes of synthesis and action of such enzymes require excess amounts of energy [54].

Cells that have a secretory function are characterized by well-developed Golgi apparatus, rough endoplasmic reticulum, and numerous mitochondria [55]. Tanycytes of the ME contain numerous cisternae of rough endoplsmic reticulum found in the apical surfaces and cell bodies. There are also well-developed Golgi complexes with numerous elongated cisternae in addition to several mitochondria [41]. The ECs of the SCO are considered as secretory cells which secrete high amounts of glycoproteins. These ECs contain well-developed rough endoplasmic reticulum with several cisternae that are dilated and filled with filamentous material. Numerous cisternae of Golgi complexes are found with large numbers of mitochondria [16].

AP, SFO and OVLT are sensory CVO have the same features of Golgi apparatus, endoplasmic reticulum and mitochondria (moderate number of mitochondria, rare endoplasmic reticulum and some Golgi apparatus [56,57]. Similarly, ECs of SMO contain Golgi apparatus in the perinuclear region, a number of mitochondria on the apical surfaces and basal processes, and strand-shape short-lengthed rough endoplasmic reticulum [9]. In such context, the features of Golgi apparatus, endoplasmic reticulum and mitochondria of the SMO resemble those in the ECs and tanycytes of the sensory CVOs rather than the secretory CVOs. Therefore, the histochemical findings in this study regarding the level of expression of the functional markers MCT1 and MCT4 may support a previous suggestion made by Collins [9] that ECs or tanycyte-like cells of the SMO act as a chemosensor receiving and modulating the information which are carried in the CSF to the important brainstem nuclei located deep to it, and bridging a gap between the AP and the more rostrally located CVOs, thus putting forward the hypothesis that the SMO is a another sensory CVOs in the floor of the 4th ventricle.

Acknowledgments

Thanks to Al-Nahrain University, College of Medicine, and Department of Human Anatomy to provide the facilities and laboratories for the completion of this work.

Author contribution

Jawad: collection, assembly and interpretation of data, manuscript writing; Dr. Alkaabi: conception and design, interpretation of data, manuscript writing; Dr. Al-Salihi: interpretation of data. All authors have approved the final article.

Conflict of interest

Authors declare no conflict of interests.

Funding

No external funding sources.

References

1. Fry M, Ferguson AV. The sensory circumventricular organs: brain targets for circulating signals controlling ingestive behavior. Physiol Behav. 2007; 91(4): 413-23. doi: 10.1016/j.physbeh.2007.04.003
2. Paxinos G. The rat nervous system. 4th ed. Academic press; 2014.
3. Duvernoy HM, Risold PY. The circumventricular organs: an atlas of comparative anatomy and vascularization. Brain Res Rev. 2007; 56(1): 119-47. doi: 10.1016/j.brainresrev.2007.06.002.
4. Johnson AK, Gross PM. Sensory circumventricular organs and brain homeostatic pathways. FASEB J. 1993; 7(8): 678-86. doi: 10.1096/fasebj.7.8.8500693.
5. Cottrell GT, Ferguson AV. Sensory circumventricular organs: central roles in integrated autonomic regulation. Regul Pept. 2004; 117(1): 11-23.

6. Willis CL, Garwood CJ, Ray DE. A size selective vascular barrier in the rat area postrema formed by perivascular macrophages and the extracellular matrix. Neuroscience. 2007; 150(2): 498-509. DOI: 10.1016/j.neuroscience.2007.09.023.

7. McKinley MJ, McAllen RM, Davern PJ, et al. The sensory circumventricular organs of the mammalian brain: subfornical organ, OVLT and area postrema. Springer Science & Business Media; 2003.

8. Petrov T, Howarth AG, Krukoff TL, et al. Distribution of the tight junction-associated protein ZO-1 in circumventricular organs of the CNS. Brain Res Mol Brain Res. 1994; 21(3-4): 235-46.

9. Collins PA. The sulcus medianus organ in the fourth cerebral ventricle of the rabbit: a previously undescribed circumventricular organ. J Anat. 1989; 162: 185-93.

10. Altman J, Bayer SA. Development of the diencephalon in the rat. II. Correlation of the embryonic development of the hypothalamus with the time of origin of its neurons. J Comp Neurol. 1978; 182(4 Pt 2): 973-93.

11. Horstmann E. [The fiber glia of selacean brain]. Z Zellforsch Mikrosk Anat. 1954; 39(6): 588-617.

12. Flament-Durand J, Brion JP. Tanycytes: morphology and functions: a review. Int Rev Cytol. 1985; 96: 121-55.

13. Rützel H, Schiebler TH. Prenatal and early postnatal development of the glial cells in the median eminence of the rat. Cell Tissue Res. 1980; 211(1): 117-37.

14. Bruni JE, Clattenburg RE, Millar E. Tanycyte ependymal cells in the third ventricle of young and adult rats: a Golgi study. Anat Anz. 1983; 153(1): 53-68.

15. Bolborea M, Dale N. Hypothalamic tanycytes: potential roles in the control of feeding and energy balance. Trends Neurosci. 2013; 36(2): 91-100. doi: 10.1016/j.tins.2012.12.008.

16. Rodríguez EM, Blázquez JL, Pastor FE, et al. Hypothalamic tanycytes: a key component of brain–endocrine interaction. Int Rev Cytol. 2005; 247: 89-164. doi: 10.1016/S0074-7696(05)47003-5.

17. Garcia CK, Brown MS, Pathak RK, et al. cDNA cloning of MCT2, a second monocarboxylate transporter expressed in different cells than MCT1. J Biol Chem. 1995; 270(4): 1843-9. doi: 10.1074/jbc.270.4.1843.

18. Halestrap AP, Meredith D. The SLC16 gene family—from monocarboxylate transporters (MCTs) to aromatic amino acid transporters and beyond. Pflugers Arch. 2004; 447(5): 619-28. doi: 10.1007/s00424-003-1067-2.

19. Poole RC, Halestrap AP. Transport of lactate and other monocarboxylates across mammalian plasma membranes. Am J Physiol. 1993; 264(4 Pt 1): C761-82. doi: 10.1152/ajpcell.1993.264.4.C761.

20. Ganapathy V, Thangaraju M, Gopal E, et al. Sodium-coupled monocarboxylate transporters in normal tissues and in cancer. AAPS J. 2008; 10(1): 193-9. doi: 10.1208/s12248-008-9022-y.

21. Tamai I, Takanaga H, Maeda H, et al. Participation of a proton-cotransporter, MCT1, in the intestinal transport of monocarboxylic acids. Biochem Biophys Res Commun. 1995; 214(2): 482-9. doi: 10.1006/bbrc.1995.2312.

22. Halestrap AP, Denton RM. Specific inhibition of pyruvate transport in rat liver mitochondria and human erythrocytes by α-cyano-4-hydroxycinnamate. Biochem J. 1974; 138(2): 313-6. doi: 10.1042/bj1380313.

23. Pellerin L, Pellegri G, Martin JL, et al. Expression of monocarboxylate transporter mRNAs in mouse brain: support for a distinct role of lactate as an energy substrate for the neonatal vs. adult brain. Proc Natl Acad Sci U S A. 1998; 95(7): 3990-5. doi: 10.1073/pnas.95.7.3990.

24. Pierre K, Pellerin L, Debernardi R, et al. Cell-specific localization of monocarboxylate transporters, MCT1 and MCT2, in the adult mouse brain revealed by double immunohistochemical labeling and confocal microscopy. Neuroscience. 2000; 100(3): 617-27.

25. Gerhart DZ, Enerson BE, Zhdankina OY, et al. Expression of monocarboxylate transporter MCT1 by brain endothelium and glia in adult and suckling rats. Am J Physiol. 1997; 273(1 Pt 1): E207-13. doi: 10.1152/ajpendo.1997.273.1.E207.

26. Cortés-Campos C, Elizondo R, Llanos P, et al. MCT expression and lactate influx/efflux in tanycytes involved in glia-neuron metabolic interaction. PLoS One. 2011; 6(1): e16411. doi: 10.1371/journal.pone.0016411.

27. Juel C, Halestrap AP. Lactate transport in skeletal muscle—role and regulation of the monocarboxylate transporter. J Physiol. 1999; 517(Pt 3): 633-42. doi: 10.1111/j.1469-7793.1999.0633s.x.

28. Bonen A. The expression of lactate transporters (MCT1 and MCT4) in heart and muscle. Eur J Appl Physiol. 2001; 86(1): 6-11. doi: 10.1007/s004210100516.

29. Dimmer KS, Friedrich B, Lang F, et al. The low-affinity monocarboxylate transporter MCT4 is adapted to the export of lactate in highly glycolytic cells. Biochem J. 2000; 350 Pt 1: 219-27.

30. Halestrap AP, Wilson MC. The monocarboxylate transporter family—role and regulation. IUBMB Life. 2012; 64(2): 109-19. doi: 10.1002/iub.572.

31. Suvarna KS, Layton C, Bancroft JD. Bancroft's theory and practice of histological techniques. 8th ed. E-Book. Elsevier Health Sciences; 2018.

32. Shi SR, Chaiwun B, Young L, et al. Antigen retrieval technique utilizing citrate buffer or urea solution for immunohistochemical demonstration of androgen receptor in formalin-fixed paraffin sections. J Histochem Cytochem. 1993; 41(11): 1599-604. doi: 10.1177/41.11.7691930.

33. Al-Kaabi M, Hussam F, Al-Marsoummi S, et al. Expression of ZO1, vimentin, pan-cadherin and AGTR1 in tanycyte-like cells of the sulcus medianus organum. Biochem Biophys Res Commun. 2018; 502(2): 243-9. doi: 10.1016/j.bbrc.2018.05.151.

34. Alvarez-Morujo AJ, Toranzo D, Blázquez JL, et al. The ependymal surface of the fourth ventricle of the rat: a combined scanning and transmission electron microscopic study. Histol Histopathol. 1992; 7(2): 259-66.

35. Meister B, Ceccatelli S, Hökfelt T, et al. Neurotransmitters, neuropeptides and binding sites in the rat mediobasal hypothalamus: effects of monosodium glutamate (MSG) lesions. Exp Brain Res. 1989; 76(2): 343-68.

36. Guerra M, Blázquez JL, Peruzzo B, et al. Cell organization of the rat pars tuberalis. Evidence for open communication between pars tuberalis cells, cerebrospinal fluid and tanycytes. Cell Tissue Res. 2010; 339(2): 359-81. doi: 10.1007/s00441-009-0885-8.

37. Langlet F, Mullier A, Bouret SG, et al. Tanycyte-like cells form a blood–cerebrospinal fluid barrier in the circumventricular organs of the mouse brain. J Comp Neurol. 2013; 521(15): 3389-405. doi: 10.1002/cne.23355.

38. Cummings JP, Felten DL. A raphe dendrite bundle in the rabbit medulla. J Comp Neurol. 1979; 183(1): 1-23. doi: 10.1002/cne.901830102.

39. Collins P, Fairman S. Repair of the ependyma in hydrocephalic brains. Neuropathol Appl Neurobiol. 1990; 16(1): 45-56.

40. Dhananjay P, Ramya Krishna V, Aswani Dutt V, et al. Ocular transporters and receptors. Woodhead Publishing; 2013. p. 37-84.

41. Merezhinskaya N, Fishbein WN. Monocarboxylate transporters: past, present, and future. Histol Histopathol. 2009; 24(2): 243-64. doi: 10.14670/HH-24.243.

42. Jackson VN, Price NT, Carpenter L, et al. Cloning of the monocarboxylate transporter isoform MCT2 from rat testis provides evidence that expression in tissues is species-specific and may involve post-transcriptional regulation. Biochem J. 1997; 324 (Pt 2): 447-53. doi: 10.1042/bj3240447.

43. Price NT, Jackson VN, Halestrap AP. Cloning and sequencing of four new mammalian monocarboxylate transporter (MCT) homologues confirms the existence of a transporter family with an ancient past. Biochem J. 1998; 329 (Pt 2):321-8. doi: 10.1042/bj3290321.

44. Takano T, Tian GF, Peng W, et al. Astrocyte-mediated control of cerebral blood flow. Nat Neurosci. 2006 Feb;9(2):260-7. doi: 10.1038/nn1623.

45. Chiry O, Pellerin L, Monnet-Tschudi F, et al. Expression of the monocarboxylate transporter MCT1 in the adult human brain cortex. Brain Res. 2006; 1070(1): 65-70. doi: 10.1016/j.brainres.2005.11.064.

46. Brightman MW, Prescott L, Reese ST. Intercellular junctions of special ependyma. In: Knigge KM, Scott DE, Kobayashi H, et al (eds). Brain-endocrine interaction II - The ventricular system. Basel: Karger; 1975. p. 146-65.

47. Scott DE, Paull WK. Scanning electron microscopy of the mammalian cerebral-ventricular system. Micron (1969). 1983; 14(2): 165-86. doi: https://doi.org/10.1016/0047-7206(83)90018-3.

48. Cardona-Gomez GP, DonCarlos L, García-Segura LM. Insulin-like growth factor I receptors and estrogen receptors colocalize in female rat brain. Neuroscience. 2000; 99(4): 751-60.

49. Ojeda SR, Lomniczi A, Sandau US. Glial–gonadotrophin hormone (GnRH) neurone interactions in the median eminence and the control of GnRH secretion. J Neuroendocrinol. 2008; 20(6): 732-42. doi: 10.1111/j.1365-2826.2008.01712.x.

50. Broadwell RD, Balin BJ, Charlton HM, et al. Angioarchitecture of the CNS, pituitary gland, and intracerebral grafts revealed with peroxidase cytochemistry. J Comp Neurol. 1987; 260(1): 47-62. doi: 10.1002/cne.902600105.

51. Pelletier G, Luu-The V, Labrie F. Immunocytochemical localization of 5α-reductase in rat brain. Mol Cell Neurosci. 1994; 5(5): 394-9. doi: 10.1006/mcne.1994.1049.

52. Gladden LB. Lactate metabolism: a new paradigm for the third millennium. J Physiol. 2004; 558(Pt 1): 5-30. doi: 10.1113/jphysiol.2003.058701.

53. Elizondo-Vega R, Cortes-Campos C, Barahona MJ, et al. The role of tanycytes in hypothalamic glucosensing. J Cell Mol Med. 2015; 19(7): 1471–1482. doi: 10.1111/jcmm.12590.

54. Alberts B, Johnson A, Lewis J, et al. Molecular biology of the cell. 4th ed. New York: Garland Science; 2002.

55. Nerkar AA, Gadegone MM. Ultrastructural features of the Fallopian tube epithelium of bat, Taphozous longimanus (Hardwicke). J Basic Appl Zoology. 2013; 66(3): 148-53. doi: https://doi.org/10.1016/j.jobaz.2013.07.006.

56. Lindberg LA, Sukura A, Talanti S. Morphology of the ependymal cells of the bovine area postrema. Anat Histol Embryol. 1991; 20(2): 97-100.

57. Gomez RE, Cannata MA. Surface morphology of the subfornical organ: Effects of low and high sodium chloride diet. Experientia. 1984; 40(5): 492-4.

Correspondence to Dr. Muthanna A. Al-Kaabi
E-mail:
muthanna.alkaabi@colmedalnahrain.edu.iq

Received Sep. 12st 2018
Accepted Nov. 22nd 2018

المجلد السابع عشر، العدد الاول، 1440 هـ، 2019م
DOI: 10.22578/IJMS.17.1.

المجلة العراقية للعلوم الطبية

عنوان المراسلات إلى المجلة العراقية للعلوم الطبية، صندوق بريد 70044 بغداد، العراق. تلفون (7717516090 964+).

رقم الإيداع في دار الكتب والوثائق ببغداد 709 لسنة 2000

Iraqi Journal of Medical Sciences

IRAQI JMS

المجلة العراقية للعلوم الطبية

Volume 17, Number 1 , 2019
January - March

P- ISSN 1681-6579
E- ISSN 2224-4719

Contents

مطبعة الفرح - بغداد ٠٧٧١٣٣٥٦٩٩