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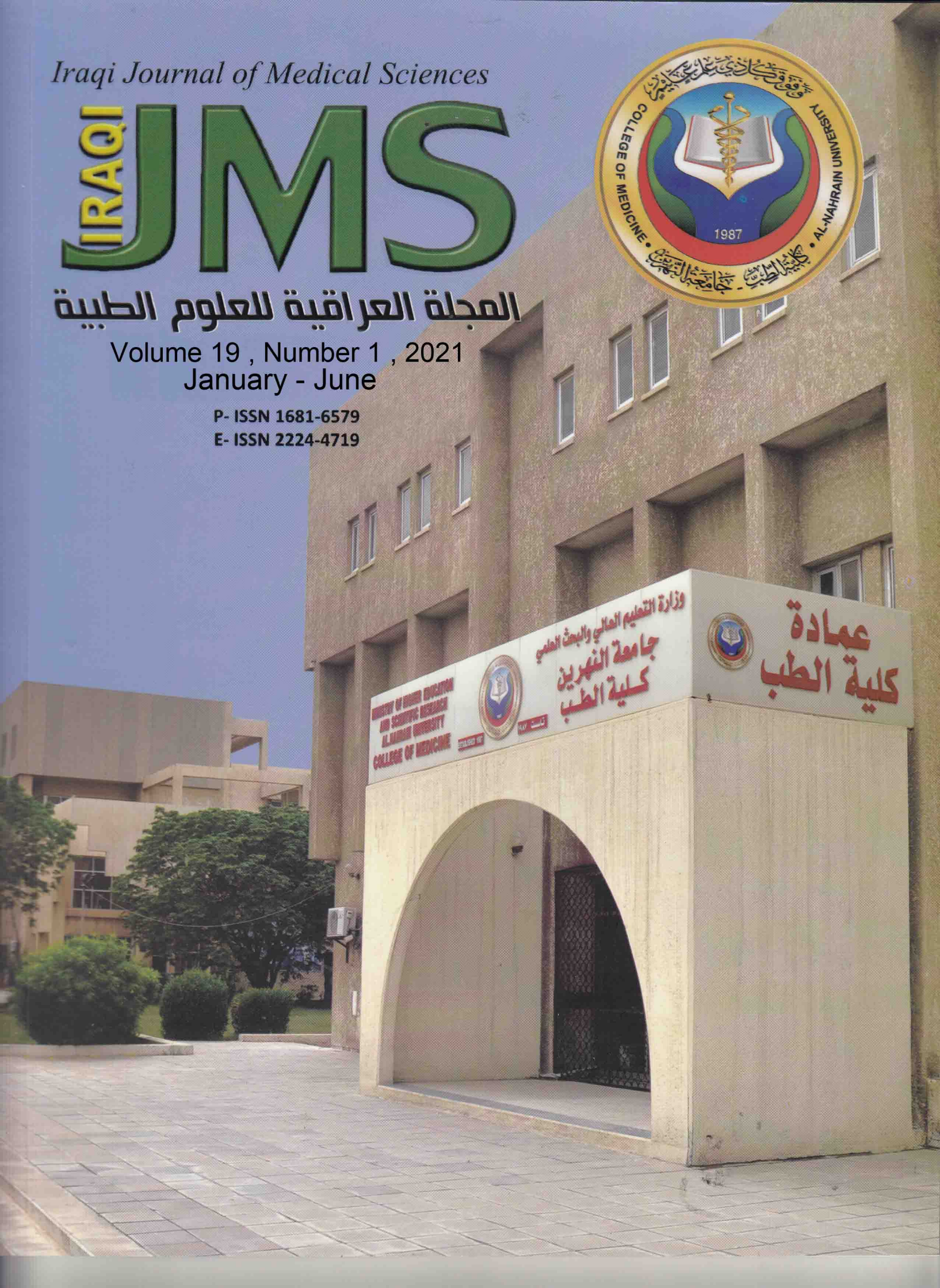

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SARS-CoV-2 and Biosafety in Laboratory

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Abstract

The newly discovered coronavirus (Severe acute respiratory syndrome coronavirus-2, SARS-CoV-2) is the causative agent of the ongoing pandemic. Broad arrangement has been done to minimize virus spreading among population and to control the worldwide outbreak. Expanded biosafety measure specifically with respect to the work require using SARS-CoV-2 in laboratory (lab.) and a special consideration should be taken to protect researcher and lab. worker during handling of specimens. Therefore, the aim of this review is to help the scientists, researchers, lab. staff and biosafety specialists to respond to the current coronavirus disease (COVID-19) through discussion of effective biosafety practices that can prevent laboratory acquired infections and to lessen the spread of infection into community and environment.

Keywords : Coronavirus, COVID-19, biosafety, research, laboratories

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List of abbreviations: BSC = Biological safety cabinet, BSL = Biosafety level, COVID-19 = Coronavirus disease 2019, HEPA = High-efficiency particulate air, Lab. = Laboratory, PPE = Personal protective equipment, SARS = Severe acute respiratory syndrome

1- Introduction

Coronavirus is one of the respiratory viruses that infect human. Outbreaks of coronaviruses have been previously reported; in 2003, the severe acute respiratory syndrome (SARS) and in 2012, the Middle East respiratory syndrome (MERS) were considered as a serious public health threat. In 2019, a novel coronavirus was emerged in Wuhan, China that is now named SARS-CoV-2 that cause coronavirus disease-19 (COVID-19) [(1)]. World health organization (WHO) announced that COVID-19 is an international health emergency on Jan. 30, 2020, the natural history of this disease is still not fully known, however, infected person was shown to be able to transmit infection before they become symptomatic, and days after recovery [(2)]. An increasing number of cases is recorded every day, override 38 million cases worldwide were recorded till the time of writing this manuscript [(3)]. Researchers and laboratories accepting specimens from patients under investigation for SARS-CoV-2 must be aware about proper handling of these specimens poses a risk of exposure or infection, which could seriously affect the staff and the community. SARS-CoV-2 has been classified as a hazard group 3 human pathogen by the Centre for Biosecurity [(4)].

2- Types of transmission and precautions

Understanding the SARS-CoV-2 mode of transmission can help to select the most suitable personal protective equipment (PPE) and disinfection procedures. SARS-CoV-2 transmission occurs mainly via droplets

(aerosol) and also contaminated surfaces (fomites). In addition, the level of exposure i.e., the amount of virus that acquire by healthcare worker has an influence on the severity of disease. Transmission through direct aerosol spread from a patient to the health worker via talking, coughing or sneezing, however, a safe distance of approximately 2 meter is recommended to reduce the virus spreading [5]. van Doremalen et al. (2020) found that SARS-CoV-2 can be detected in aerosols for up to 3 h with a decrease in viral particles concentration from $10^{3.5}$ to $10^{2.7}$ TCID50 /L of air [6].

Also, SARS-CoV-2 indirect transmission has been reported via environmental, therefore contaminated surfaces can rising the risk of contact transmission and nosocomial infection [7]. van Doremalen et al. (2020) found that SARS-CoV-2 was more stable on plastic and stainless steel than on copper and cardboard and can detected up to 72 h on these surfaces, however, the virus concentration was decreased from $10^{3.7}$ to $10^{0.6}$ TCID50/ml of medium on plastic and stainless steel after 72 and 48 h, respectively. While, no viable SARS-CoV-2 was detectedon copper and cardboard after 4 and 24 hr, respectively [6].

3- Risk Assessment

There are different hazards may encounter in lab. including sharps, chemical, biological and radiation. A Biohazard is a biological agents or substance that can cause human or animal disease and may present a hazard to the health of an individual working with the agent. Biohazard include microorganisms such as bacteria, viruses, parasites and fungi. Biohazard also include samples from humans or animals that an individual will work with either for diagnosis or research. The infection control measures including both administrative and environmental control measures. The administrative strategies are creating policies, plan for emergency and provide instruction and trainings, while the environmental strategies include providing good ventilation, building up isolation rooms with negative pressure, and creating frameworks for sterilization, disinfection and biohazard waste disposal [8].

Several organizations developed guidelines that discuss methods for handling and processing SARS-CoV-2 suspected specimen [4, 9,10]. The biosafety level should be determined depending on the risk assessment and the hazard of pathogen, detection method or experimental technique used by authorized personnel [11]. Risk assessment is the detection of hazards and risks that could adversely affect individuals, and/or the environment, their likelihood and consequences. These assessments help to define the risks and include steps, protocols and controls to minimize the effect on work activities [12]. The results of this process may be expressed in a quantitative or qualitative fashion. Risk assessment is a part of a broader risk management strategy to help reduce any potential risk-related consequences and it need to be carried out before work/activity starts. All biological agents require risk assessment and approval before work can proceed. Work would be blocked if the suitable facilities not provide adequate protection to staff, students and environment [12,13].

4- Building facilities

Facilities for undergoing research for SARS-CoV-2 should strictly implement the appropriate biosafety practices inside the lab. Both biosafety level-2 (BSL-2) and biosafety level-3 (BSL-3) are the required lab. set-up when handling SARS-CoV-2. All inactivated specimens should be performed in a validated biological safety cabinet class-II (BSC-II). The diagnosis methods that not require growth of the virus, such as amplification of virus nucleic acid or sequencing and another routine lab. tests such as biochemical and serological tests should be performed in BSL-2, while diagnosis methods that require the growth of virus such as virus isolation, tissue culture or neutralization assays should be performed in BSL-3 [9,10].

In addition, procedure that require inoculation of virus in animals should be performed in BSL-3 animal facilities [11]. Any procedure generate aerosols should be performed at BSC-II [10]. WHO highly advises that national governments keep a list of licensed laboratories that keeping and working with suspected or confirmed specimens of SARS patients [14] and the laboratories are required to engage in external quality management programs [15] (Figure 1).

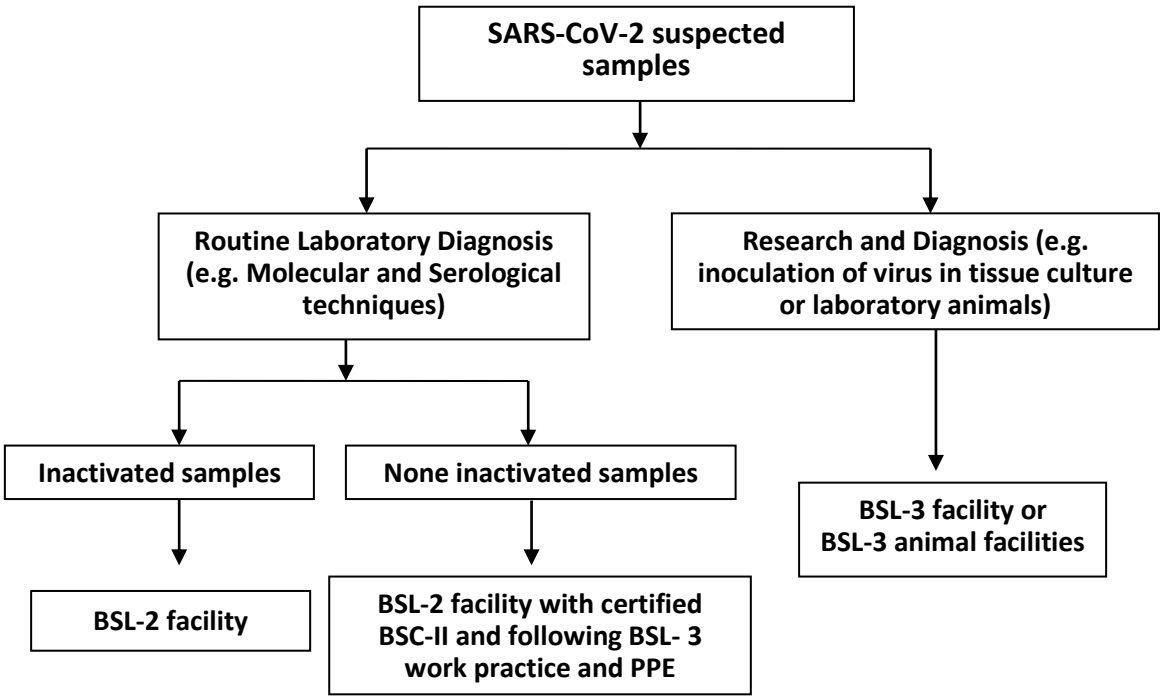

Figure 1. Building facilities required for SARS-CoV-2 suspected sample

The BSL-2 lab. should be physically segregated, with restricted access, from other operation areas in the same building. Staff should have access to a protective clothing dressing area. It is recommended to put the autoclave in a contained area [10,11], (Figure (2). The BSL-3 lab. should be physically isolated from other areas of operation or placed in a different building and have double doors that are self-closing. In the enclosed area, an electronically powered sink for hand washing and communication network with the outside should be present. There could be an emergency shower placed in the lab. Staff should have access to a protective clothing dressing area. To prevent the leakage of contaminants from inside to outside, the work area is kept at negative air pressure. After filtration via a high-efficiency particulate air (HEPA) filter, so air is exhausted from the lab. and can't be re-circulated inside the building unless a second HEPA filter is installed in the exhaust system [11,16].

5- Lab worker personnel

Laboratories should, as far as possible, encourage social distance within the workplace and monitor the likelihood of any daily exposure and health status of lab. staff [18]. Workers dealing with SARS-CoV or samples possibly carrying the virus should be educated on the signs of infection and recommended to report promptly to their boss any fever or respiratory symptoms [19]. After an observational time, personnel with prior BSL-3

expertise will be cleared to operate in the facility under supervision. The length of the training period depends on the skill and competence of the required techniques. In addition, those who working in the BSL-3 facility should be immunocompetent [16], therefore a high-risk group (e.g., over 70 years of age, pregnant, those with immune deficiencies or underlying medical conditions) should not work with SARS-CoV-2.

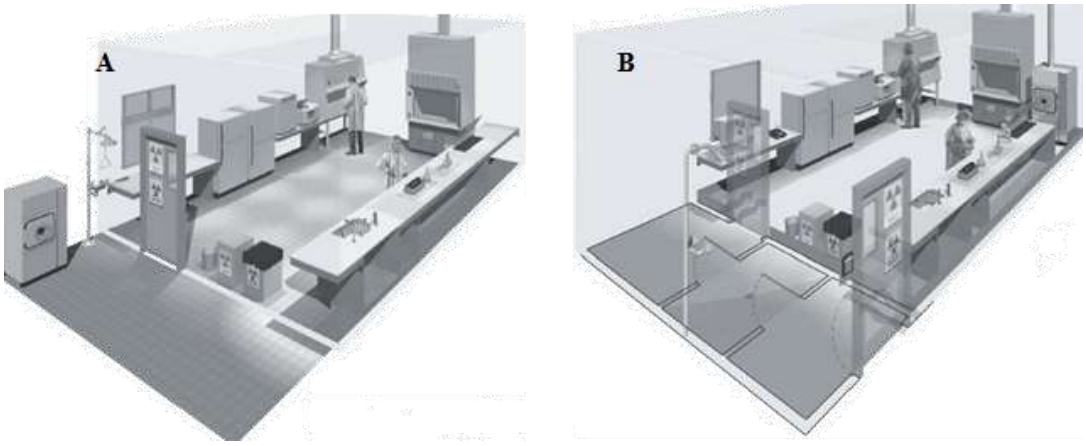

Figure 2. Biosafety level-2 (BSL-2) facility (A), Biosafety level-3 (BSL-3) facility (B) [17]

Institutions engaged in SARS coronavirus study should include the storage of a serum sample of individuals working with viruses or specimens containing viruses [19]. When real-time polymerase chain reaction (RT-PCR) findings are negative, the four-fold increasing in antibody titer between acute and convalescent phase sera could help COVID-19 diagnosis [20]. In addition, these organizations should establish and incorporate a clear occupational medical strategy. At a minimum, the strategy should include management procedures: identifiable breaks in lab. procedures; symptom-free employees; symptomatic employees within 10 days of exposure; and symptomatic lab. workers without known exposure [19].

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6- Personal Protective Equipment (PPE)

Due to less effectiveness compared to other approaches and high cost in the long term, the PPE is rated as the lowest in the infection control measure. PPE can only be used in combination with other regulatory and environmental control measures [8]. Full PPE including disposable gloves, lab. coat, class two filtering face-piece respirator and eye cover such as goggles or face shield should be worn by lab. staff while dealing with hazardous materials [7,10,21] and particular attention should be paid to hand hygiene after removal of gloves by regular hand washing with soap and water for at least 40 sec. and careful attention should be given to hand hygiene after removal of gloves [11,18]. Authorized filter respirator N-95 or higher standard (i.e., R95, P100, Powered air purifying respirator (PAPR) fitted with HEPA filters) should be used as recommended by the National Institute for Occupational Safety and Health (NIOSH); while the lower level of protection is not accepted [2,10,19] (Figure 3). However, it has been shown that the detection of influenza virus and coronavirus RNA in respiratory droplets and aerosols, respectively has been greatly reduced by surgical face masks [22].

Figure 3. Biosafety level-2 (BSL-2) facility (A), Biosafety level-3 (BSL-3) facility (B) [17]

There are various N95 mask brands from multiple manufacturers available. At 95 percent filtration efficiency, N95 is not resistant to oil. For oily solvents with 95 percent filtering efficiency, R95 is recommended. Higher designations like P100 mean that 99.99 percent of particles are flushed out. Fit checking for the N95 respirator is needed because it is a negative pressure respirator, so it should be perfectly shaved for N-95 users to match the mask fit test. However, fit checking for PAPR is not needed because the atmosphere of the breathing zone is under positive pressure and so a tight seal is not needed for proper operation, a PAPR can be used for users with beards [2]. After facing each patient, the instructions recommended the disposal of N95 respirators [23].

The lack of PPE forced hospitals to change infection control strategies after the COVID-19 pandemic. CDC endorsed N95 reuse techniques, even for the length of their shifts, workers reusing their own N95s. They tested decontaminate and reuse N95 at Duke University Health System using BSL3 facility Hydrogen Peroxide Vapor processing room to prolong their life and verified that the respirator already works for more than 50 times after 4 h of decontamination [24]. Also, Daeschler et al. (2020) observed that SARS-CoV-2 was inactivated by a single heat treatment for 60 min. at 70 ˚C at either 0 percent or 50 percent relative humidity in N95-respirators for more than 10 times. This is known to be a low-cost, rapid decontamination method for the secure reuse of disposable N95 respirators [25]. In addition, after disinfection with 1000 mg/L chlorine-containing disinfectant for 30 min, the PAPR could be reused after all sections repeatedly and uniformly cleaned with a soft cloth dipped in clean water [26].

7- Specimens collection, transport and storage

During sample collection, it iss crucial to adhere to the standard infection control practice and the use of complete PPE [20]. SARS-CoV-2 is an enveloped virus i.e., fragile, therefore care should be taken during sample collection and transportation. For molecular diagnosis, samples need to transported in viral transport medium (VTM). Specimens that would be directly sent to the lab. can be stored at 2-8 °C, but specimens will be frozen at -70 °C (dry ice) while there is a delay in specimens reaching the lab. [15,20]. Samples should be clearly labeled and stored frozen separately from other samples in restricted-entry locked freezers [11].

Specimens should be brought in special delivery tanks and boxes that satisfy the criteria for biosafety [26]. SARS specimens should be wrapped, secured and decontaminated as key containers for transportation within the facility [19]. But samples should be packaged in triple packs for shipment outside the facility in compliance with the WHO post-outbreak biosafety recommendations [14] and the International Air Transport Association (IATA) for hazardous

goods regulations [27]. The United Nation model regulations should be followed by international transportation [15].

8- Biosafety of laboratory diagnosis

While respiratory samples have the highest yield, in other specimens, including stool and blood, the virus can also be identified. Nucleic acid amplification test, such as RT-PCR, can used to screen the suspected cases for the virus [15], that targeting SARS-CoV-2 RNA-dependent RNA polymerase and E genes [7,28]. Serological testing could aid in the detection of a current pandemic of SARS-CoV-2, retrospective estimation of the occurrence rate of an outbreak, and could help the diagnosis of COVID-19 if the outcome of RT-PCR is negative [20,29].

Any operation with the ability to produce aerosols should be performed inside a certified BSC-II. Using enclosed centrifuge rotors for centrifugation, or a guard bowl with gasketed cover. Procedures carried out outside a BSC must be carried out in a way that minimizes the hazard of exposure to employees and release to the environment [19]. In the event of any accident during centrifugation, the lab. worker can pause the centrifuge, wear the BSL-3 PPE, wait half an hour for the centrifuge lid to release, and spray 75 percent ethanol. After that, the rotor with the specimen to be treated in the biosafety cabinet should be taken out [18].

After exposure to numerous widely used disinfectants and fixatives, a related coronavirus which causes SARS loses infectivity. However, acetone fixation for immunofluorescence assays at room temperature does not effectively kill the SARS virus until the acetone is cooled down to -20 ˚C [30]. Sera should be inactivated for 30 min. before analysis at 58 °C exposure. Tissue inactivated by formalin fixation for pathological analysis, fixation of smears for regular staining and microscopic tests and extraction of nucleic acid for PCR. Although it should be remembered that infectious RNA can be present in inactivated clinical samples [13]. Where appropriate, it is important to use automated instruments and analyzers. Aerosol-generating sample processing steps should be conducted in a BSC-II to manually treat non-respiratory specimens, wearing the recommended PPE [18].

9- Decontaminate equipment and surfaces

The virus sensitivity to inactivation is dependent on the environment and virus concentration [31]. Coronaviruses are enveloped viruses and generally thermo-labile [11]. Most disinfectants can easily affect the outer layer of the virus envelope [32]. The amount of time the virus is expected to survive depends on the sort of virus-containing substance or body fluid and different environmental factors, such as temperature or humidity [11]. It has shown that it is possible to kill a similar coronavirus that causes SARS at 56 °C at about 10000 units per 15 min [30]. Therefore, decontamination of work surfaces and disinfectant equipment is important using disinfectants at least every 3 h [10,32]. In addition, hazard specimens should be disinfected or autoclaved immediately [18].

It has been demonstrated that through disinfecting surfaces with 62-71 percent alcohol or 0.5 percent hydrogen peroxide bleach or household bleach containing 0.1 percent sodium hypochlorite, coronaviruses may be inactivated within minutes. Now a catalog of disinfectants that can be used against the SARS CoV-2 virus has been released by the US Environmental Protection Agency (EPA) [33]. The virus was destroyed in a report by Chin et al. (2020) by incubating a virus culture for 5 min with different disinfectants, such as 1:50 household bleach, 60-70 percent ethanol, 7.5 percent povidone iodine, 0.05 percent chloroxylenol or chlorhexidine and 0.2 percent to 0.4 percent benzalkonium chloride [34].

It is also recommended that an appropriate freshly prepared chlorine disinfectant (5500 mg/L) be used for > 30 min if the suspected specimen has leaked or generated BSC or

bench contamination. When lab. exposure is caused by positive specimens, the lab. room is closed to avoid contaminants from spreading, then the infected area should be cleaned with an appropriate chlorine containing towel (5500 mg/L) for > 30 min. Miscellaneous disinfectants (e.g., peracetic acid (2 g/m^3), H_2O_2 (3%), chlorine dioxide (100 mg/L)) may be used for overnight lab. fumigation, or aerosol disinfectants may be sprayed for 1-2 hours [18].

10- Waste management

Specimens and tissue culture should be disinfected or autoclaved and collected in leak-proof containers with their tops properly sealed prior to disposal [9]. Sharp items can be disposed of in a separate plastic box, sealed and sprayed with chlorine-containing 1000 mg/L disinfectant. Medical waste should be stored in a double-layer waste bag, wrapped in a gooseneck manner with cable ties, and sprayed with 1000 mg/L of chlorine containing disinfectant. Shift of waste to another facility with decontamination capability if decontamination is not feasible on site [9,26]. Bagged waste gathered into a collection box for hazardous waste, apply a special label for pathogen, completely enclose and transfer the box. Move the waste to a temporary medical waste collection point along the designated route at a set time point and store the waste separately at a fixed location until the licensed medical waste disposal contractor collects and disposes of it [26].

11- Biosecurity

There should be limits on entry to labs. A list of approved personnel engaged in the collection and archiving of COVID-19 samples should be preserved and circulated for bio-safety and biosafety purposes with the appropriate authorities. Sample storage zones, including those outside the main labs, should be guarded. Access to test databases, including storage locations and information, should be restricted to the relevant staff only [35]. It is desirable that two individuals work together while tissue culture is conducted in the BSL-3 lab. If it is not possible, however, it is mandatory that at least one other person be present directly outside the BL3 region of the lab. This person will be responsible for the continuous surveillance of the BSL-3 operation by means of internal video equipment [16].

12- Conclusion

It is essential to develop biosafety training and standard operative procedures for Iraqi laboratories, in addition establish emergency plans and practices for incidents that may occur in the lab. staff must be supplied with the necessary PPE that they need to comfortably do their work. A biosafety level must be determined based on the risk assessment in order to carry out research work on SARS-CoV-2. Moreover, due to special requirement needed to work with highly infectious viruses, the laboratories performing viral diagnosis in Iraq should be limited to only certified laboratories.

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Patterns of Laryngeal Nerves Injuries Following Thyroid Surgery in Al-Imamein Al-Kadhmein Medical City

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Abstract

Background Morbidity after thyroid surgery related to injuries to parathyroid, recurrent laryngeal nerve (RLN) and external branch of superior laryngeal nerve (EBSLN), mostly due to unawareness of the anatomical variations and lack of meticulous dissection added to that patient comorbidity and type of goiter.

Objective To assess the incidence of nerves injuries (RLN and EBSLN) and evaluation of the risk factors for nerves injuries.

Methods A prospective study included 250 patients admitted to the General Surgical Unit at Al-Imamein Al-kadhimein Medical City from the first of October 2014 to the end of October 2017. Evaluation of nerves injuries (RLN and EBSLN) with regard to thyroid pathology, types of resection, age and gender. Thyroidectomies were done by different surgical teams. Patients with recurrent goiter, prior cervical surgery and preexisting vocal cord paralysis were excluded. Vocal cords assessment was done preoperatively, intraoperatively at time of extubation and postoperatively. Patients with injury to the RLN were managed accordingly and followed up by serial examination for 6-12 month, while patients with symptoms of EBSLN injuries were followed depending just on clinical feature due to lack of diagnostic facilities.

Results There were 78 (31.2%), 172 (68.8%) male and females subsequently. The age range was (18-70) years and mean±SD was (44±11.9). Multinodular goiter was the main presentation in (168 patients), while thyroiditis was the least (6 patients). Injury to RLN was reported in 8 (3.2%) patients, 3 had unilateral and 5 had bilateral. Permanent damage documented in 2 patients. There was a significant RLN injury association with malignancy and total thyroidectomy. EBSLN injury have been reported in 55 patients (22%), it was transient in 43 patients and permanent in 12 patients.

Conclusion Nerve damage after thyroidectomy is not uncommon and affect significantly on quality of life. Careful dissection and awareness of anatomical variation is essential. Majority of injuries were transient and recovered spontaneously. Immediate intervention is not recommended unless airway compromisation ensure.

Keywords RLN, EBSLN, thyroidectomy

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List of abbreviations: EBSLN = External branch of superior laryngeal nerve, EMG = Electromyography, LEMG = Laryngeal electromyography, RLN = Recurrent laryngeal nerve, SLN = Superior laryngeal nerve, STA = Superior thyroid artery, V.C. = Vocal cord, ZT = Zuckerkandl's tubercle

Introduction

In the early nineteenth century, thyroid surgery was associated with high (40%) morbidity and (20%) mortality rates, due to

the lack of meticulous dissection techniques and sepsis [1]. With the advent of antiseptic techniques, refinement in surgical procedure, recognition of the presence of parathyroid, recurrent laryngeal nerve (RLN), and protection of the external branch of superior laryngeal nerve (EBSLN) resulted in lower morbidity and less than 0.1% mortality [1].

The complication which is most feared is trauma to the RLN estimated to occur in between 1 to 10% of operations. The nerve may be out, stretched or burnt, usually as a result of failure to recognize or dissect it properly. Unilateral paralysis of the RLN results in immobile vocal cord in the paramedian position which causes weak, cracked and breathy voice. Bilateral paralysis leads to severe airway obstruction necessitating an urgent tracheostomy in the majority of patients [2].

The external laryngeal nerve is traumatized more often than one supposes. Its close relationship to the superior vascular pedicle and an occasionally aberrant course predisposes it to damage [3].

Complications associated with thyroidectomy are related to the type of disease, extent of disease, removal approaches, surgeon's training, and experience. Several studies have shown that increased surgeon experience is significantly associated with decreases in complications after thyroid surgery [4-7].

This study aimed to assess the incidence of nerves injuries (RLN, EBSLN) in our center and to evaluate the risk factors of nerves injuries.

Methods

A prospective study included 250 patients admitted at the General Surgical Unit at Al-Imamein Al-kadhimein Medical City from the first of October 2014 to the end of October 2017; they have been assessed, investigated and prepared for surgery, all patients subjected to vocal cord examination pre, intra and post operatively.

Inclusion and exclusion criteria

All cases have been discussed with endocrinologist, referred, consented and agreement documented for enrolment in the study, they include: symptomatic euothyroid goiter not respond to medical treatment or patient wish for cosmetic purposes, toxic goiter (diffuse or multinodular) relapsed after medical treatment, suspected or malignant or goiter. While those with recurrent goiter, prior cervical surgery, associated parathyroid pathology and patient with preoperative vocal cord paralysis have been excluded.

The type of operations includes hemi-thyroidectomy, subtotal thyroidectomy, near total thyroidectomy and total thyroidectomy.

Patients with postoperative signs and symptoms of RLN injury were managed accordingly and had been followed by serial examination for 6-12 month. Patients with symptoms of EBSLN injuries were followed clinically due to lack of diagnostic facilities.

Nerve injuries (RLN, EBSLN) that resolved within 6 months considered as transient. While in cases that persist more than 6 months regard as permanent.

Results were analyzed statistically in relation to the risk factors using statistical package for social sciences (SSPS) version 26. A P-values of less than 0.05 accepted as significant.

Results

The study included 250 patients, 78 (31.2%) males and 172 (68.8%) females. The age range was between 18 to 70 years and the mean±SD was (44±11.9), as shown in figure 1.

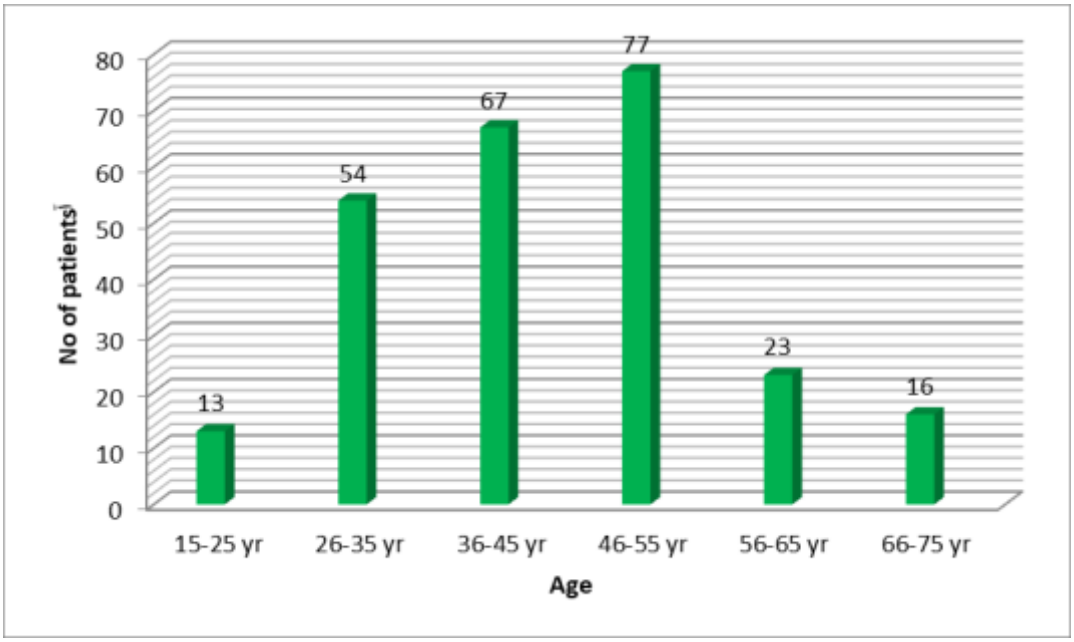

Figure 1. Age distribution

The majority of our patients were presented with symptomatic euothyroid multinodular goiter (MNG) goiter (168 [67.2%] patients), while the least were presented with thyroiditis (6 [2.4%] patients). Other pathologic distributions shown in figure 2.

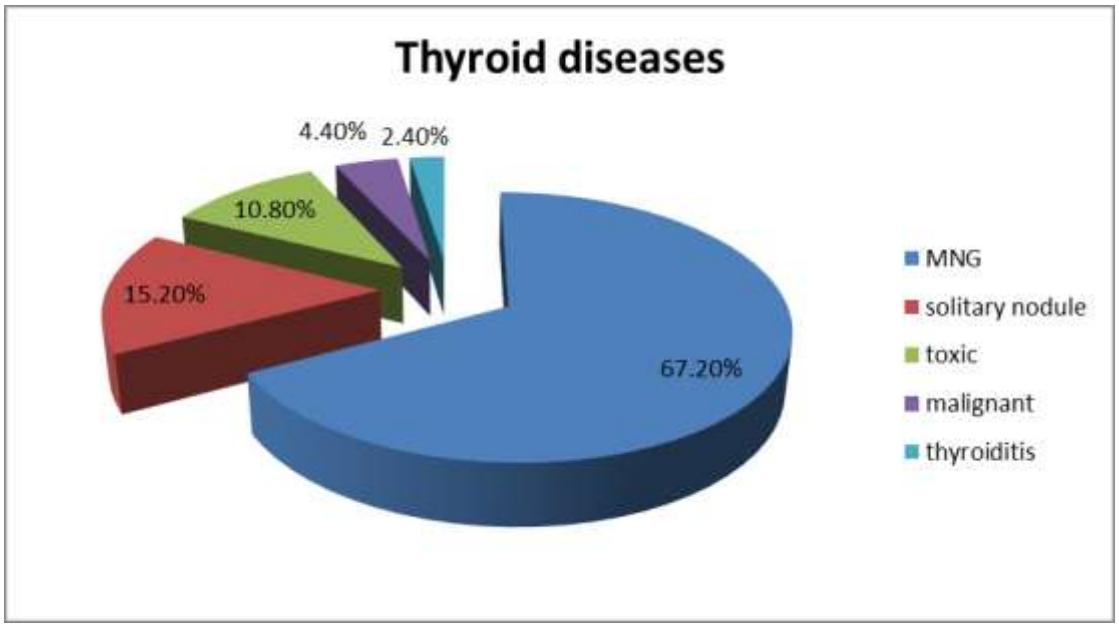

Figure 2. Types of thyroid diseases

Majority of patients underwent subtotal and near total thyroidectomies (36.8%), (34%) subsequently as in figure 3.

Injury to RLN was reported in 8 (3.2%) patients, 3 were unilateral, 5 bilateral, 6 transient and 2 permanents as shown in table 1.

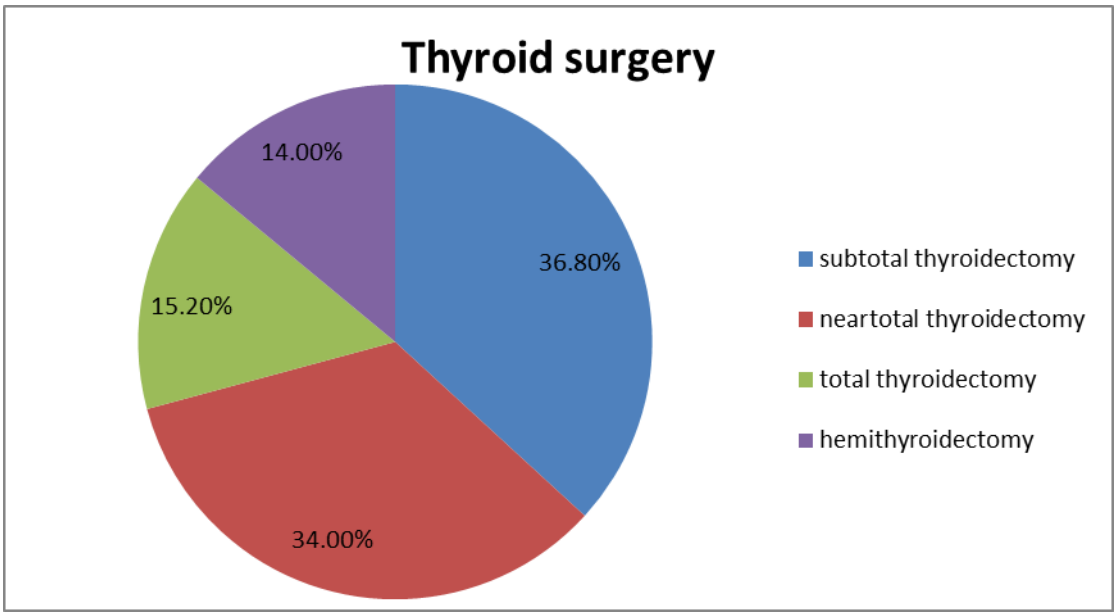

Figure 3. Types of thyroid surgery

Table 1. Types of recurrent laryngeal nerve injury

Type of injury	Unilateral	Bilateral	Transient	Permanent
No. of patients	3	5	6	2
Total No.	8		8	

The relation of RLN injury to the age and gender was insignificant, on the other hand there was a significant relation to malignant thyroid enlargement and to total thyroidectomy as table 2.

On the other side, EBSLN injury was reported in 55 patients (22%), transient injury occurred in 43 patients and permanent injury occurred in 12 patients (Table 3).

There was no significant association between EBSLN injury and malignancy (Table 4).

There was no significant association between EBSLN injury and the gender of the patients (Table 5).

There was no significant association between EBSLN injury and the age of the patients (Table 6).

Table 2. Relation of RLN injury to age, gender, pathology and type of thyroidectomy

Parameter		Total No.	No. RLN injury	P value
Gender	Male	78	2	0.7
	Female	172	6	
Age	≥ 44 yr	123	5	0.4
	< 44 yr	127	3	
Pathology	Benign	239	6	0.1
	Malignant	11	2	0.003
Type of operation	Total thyroidectomy	38	5	0.01
	Subtotal thyroidectomy	92	2	0.2
	Near total thyroidectomy	85	1	0.3

Table 3. Relation of external branch of superior laryngeal nerve injury with thyroid disease

Thyroid disease	No. of patients	No. of EBSLN injury	Transient	Permanent
Multinodular Goitre	168 (67.2%)	33 (19.6%)	27 (82%)	6 (18.0%)
Solitary nodule	38 (15.2%)	9 (23.6%)	7 (77.7%)	2 (22.3%)
Toxic Goitre	27 (10.8%)	7 (25.9%)	5 (71.4%)	2 (28.6%)
Malignant	11 (4.4%)	3 (27.1%)	2 (66.6%)	1 (33.3%
Thyroiditis	6 (2.4%)	3 (50.0%)	2 (66.6%)	1 (33.3%)
Total	250 (100%)	55 (22.0%)	43 (78.2%)	12 (21.8%)

Table 4. Relation of EBSLN injury with type of pathology

Pathology	No. of patients	No. of EBSLN injury	P value
Benign	239	52	0.6
Malignant	11	3	
Total No.	250	8	-

Table 5. Relation of EBSLN injury with gender

Gender	No. of patients	No. of EBSLN injury	P value
Male	78	18	0.7
Female	172	37	
Total No.	250	55	-

Table 6. Relation of EBSLN injury with age

Age	No. of patients	No. of EBSLN injury	P value
≥ 44 yrs	123	24	0.3
< 44 yrs	127	31	
Total No.	250	55	-

Discussion

Total thyroidectomy has replaced bilateral subtotal thyroidectomy in the last 25 years, and became preferred management for most of the patients with bilateral benign disease as MNG, Graves' disease, malignancy, the principal change in operative procedure has been the change from lateral dissection to capsular dissection [8].

Capsular dissection, sometimes referred to as Delbridge technique, involves hugging the gland and dividing the tertiary branches of the vessels, while dissecting the parathyroid glands with their vascular pedicles free from the thyroid surface, with minimal exposure of the recurrent laryngeal nerve or disturbance of its blood supply. When this technique is employed, the nerve is most often encountered within its sheath amongst the tracheo-esophageal groove [8].

The RLN may consist of multiple branches, which is closely associated with the inferior thyroid artery as it ascends to the middle third of the gland, so the inferior thyroidal artery regard as the most common used structure to identify the RLN, but it is not always a reliable guide, because it has a variable course even in the right and left side of the same patient [9].

Mechanisms of nerves injuries include traction, handling of the nerve, complete or partial transaction, contusion, burn, clamping, crush, and compromised blood supply and misplaced ligature [10]. The level of upper two tracheal rings regard as the most common site of accidental transaction where the Berry's ligament regarded as the area by which the nerve closely approximates thyroid gland [11]. The variable anatomical course of the nerve is another important risk factor for RLN injury, in addition to the absence of fixed relation of the nerve to close anatomic structures.

The surgical importance of Zuckerkandl's tubercle is that it must be dissected and excised in case of total thyroidectomy and its close relationship to the RLN, it is the posterolateral extension of thyroid lobes adjacent to RLN [12].

In this study, 172 (68.8%) were females, this is consistent with Zakaria et al. [13]. Thyroid diseases are more prevalent in women particularly between puberty and menopause, the epidemiological data suggest a role of estrogen in the pathogenesis of thyroid diseases and studies suggest that estrogen and progesterone may contribute to the pathogenesis of goiter [14,15].

In current study, the RLN injury reported in 8 patients (3.2%), transient in six 2.4% and permanent in two 0.8% patients. Aytac et al. reported an incidence of injury of 5.2% (1.4% permanent and 3.8 temporary) [16], while Zakaria et al. reported an injury of 4.1% (3.8 temporary and 0.29 permanent) [13]. The incidence of injury in this study is somewhat less than the others may be due to exclusion of recurrent goiter from the study.

Multinodular goiter was the most common indication for thyroidectomy in the current study; 67.2% (168 patient), only 4 patients had RLN injury (2.3%), all of them were bilateral and transient, two followed total thyroidectomy, one followed subtotal thyroidectomy and one followed near total thyroidectomy, which is comparable with injuries reported by Zakaria et al. [13] and Aytac et al. [16], 3.1% and 2.4% subsequently.

In toxic goiter, there were 27 (10.8%) patients and the injury was reported in one case (3.7%) following subtotal thyroidectomy, Aytac et al. study reported 8.7% injury [16].

RLN injury was higher in association with thyroid carcinoma (2 of 11 cases (18%), all

cases of thyroid malignancy managed by total thyroidectomy with central neck dissection, which explain the higher risk of nerve injuries. The incidence of RLN injuries in association with thyroid malignancy reported by Zakaria et al. [13] and Aytac et al. [16] were 12.8% and 20% respectively.

Total thyroidectomy was performed for symptomatic thyroiditis, none responding to medical treatment, RLN injury reported in (1 of 6 cases which means 16.6%) so this the second common pathology associated with nerve injury due to dense adhesion encountered [17].

Total thyroidectomy was more associated with RLN (15.2%), while subtotal and near total procedures associated with lower rate of injury (2.1% and 1.1%) subsequently. Zakaria et al. [13] report a 7.2%, 1.9% and Aytac et al. [16] 21%, 2.3% in cases of total and subtotal thyroidectomy subsequently. Near total thyroidectomy was performed in 85 patients, injury was reported in one patient (1.1%) while not in other studies [16,18].

Intraoperative nerve monitoring has been used by many surgeons to reduce incidence of RLN injury. The benefits of this method have been reported in the literature none of these shown any statistically significant decrease in the RLN injury [19,20].

Several minimal approaches thyroidectomy have been described, mini-incision procedure uses a 3-cm incision with no flap creation and video assistance can be used to improve the visualization via the small incision. Totally endoscopic approaches also have been described, via the supraclavicular, anterior chest, axillary, breast and transoral robotic-assisted approach to avoid neck skin incision. These methods are feasible, but clear benefits over the traditional approach have not been established [21].

On the other side, EBSLN injury was reported in 55 patients (22%), transient injury occurred in (78.2%). The prevalence of ESLN injury is unknown and often goes undiagnosed. This nerve has significant relevance surgically, as its rate of injury in thyroidectomy is reported to be as high as 58% [22].

There are several technical approaches to preserve the integrity of the EBSLN, of which isolating with individualize ligating the superior pole vessels closely adjacent to the thyroid capsule, identifying the EBSLN before securing the vasculature in the same manner and neuromonitoring of the EBSLN through thyroidectomy are the most popular methods, no one is superior [23].

The most effective method for preserving RLN is still controversial. Some surgeons claim that omitting nerve identification may cause little trauma. However, most literatures have proved no rule for identification and the knowledge of the anatomic course of the nerve during the operation and its variations is the secret to decrease the incidence of RLN injury [24].

Identification of RLN during operation make the surgeon more confident about the nerve integrity and eliminate tension while waiting for recovery of transient injury. Here the surgeon experience plays a major role in preventing iatrogenic injury, it was reported that a dissection started from avascular space of cricothyroid was the safest procedure for identification of RLN [25].

This study concluded that surgeon experiences and awareness of anatomical variation with careful dissection is the art of safe thyroidectomy. Most of the nerve injuries are transient and recover spontaneously, so immediate intervention is not recommended unless it interfere with breathing or swallowing. EBSLN injury is more common but functionally pass frequently unnoticed. Nerve injury is associated with thyroidectomies for malignancies or cases of thyroiditis.

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Author contribution

All authors participated in study design, performing surgeries, follow up of patients, data interpretation and manuscript organization and editing.

Conflict of interest

Authors declare no conflict of interest.

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Evaluation of HBx Antigen in Patients with Chronic Hepatitis B Virus Infection

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Abstract

Background Hepatitis B virus (HBV) is a serious public health problem worldwide and major cause of chronic hepatitis, cirrhosis, and hepatocellular carcinoma (HCC).

Objective To assess the association of the role hepatitis B x antigen (HBxAg) in chronic HBV; active and inactive patients, and complicated patients.

Methods Ninety blood samples from patients who had chronic HBV were enrolled in this study. Serum samples were screened by enzyme linked immunosorbent assay (ELISA) technique for the detection of HBxAg. Viral nucleic acid was extracted from these 90 samples, and plasma HBV viral load was investigated by real time-polymerase chain reaction (RT-PCR).

Results The overall prevalence of HBxAg was 26.7% with a significantly higher prevalence in active than inactive patients.

Conclusion The high HBxAg prevalence rates in active chronic patients raise the possibility of increasing risk of disease progression.

Keywords Hepatitis B virus (HBV), hepatitis B X antigen (HBxAg), hepatocellular carcinoma (HCC)

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List of abbreviations: AUC = Area under the curve, ELISA = Enzyme-linked immunosorbent assay, HBxAg = Hepatitis B X antigen, HBV = Hepatitis B virus, HCC = Hepatocellular carcinoma, ROC = Receiver operating characteristic, RT-PCR = Real time polymerase chain reaction

Introduction

Hepatitis B virus (HBV) is a small, enveloped virus with a partially double-stranded DNA genome of the family Hepadnaviridae [(1)]. Despite the availability of an effective prophylactic vaccine for nearly three decades, HBV remains the cause of a number of important public health problems [(2)].

Globally, there were an estimated 248 million persons with chronic HBV infection in 2010 and approximately 686,000 deaths were attributed to complications associated with chronic HBV infection in 2013 [(3)]. Chronic HBV infection remains the most important risk factor for hepatocellular carcinoma (HCC) worldwide, with the level of viremia being the number one risk factor [(4)].

Hepatitis B x antigen (HBxAg) is a protein of 154 amino acids, could cause enhanced colony formation or transformation of cells in vitro in various cell lines. This antigen has been shown to activate gene expression via oncogenic Ras

signaling by increasing TATA binding protein levels [5].

HBxAg has been reported to modulate the expression and activities of numerous genes, as well as epigenetic molecules (e.g., miRNAs and lncRNAs) and events (e.g., methylation and acetylation), leading to the deregulation of various pathways and function [6]. This antigen has been shown to associate with the tumor suppressor gene product p53 and inhibits its function.

The objective of this study is to assess the associated role of HBxAg in chronic HBV active and inactive patients and complicated patients.

Methods

In this case control study, blood samples were obtained from patients attending the Gastroenterology and Hepatology Center of Medical City from September 2018 to April 2019. This study was approved by the Institutional Review Board (IRB) of the College of Medicine, Al-Nahrain University, approval code number 64. The population consisted of ninety patients who had chronic HBV; divided into two groups: first uncomplicated 62 (68.9%) and second group with complicated HBV infection 28 (31.1%) (fibrosis, cirrhosis, and hepatocellular carcinoma (HCC)).

A total of 5 ml of venous blood for serum separation were withdrawn, all the 90 sample were tested for HBxAg by HBxAg enzyme linked immunosorbent assay (ELISA) Kit (My BioSource System San Diego, CA 92195-3308 USA).

Also, the viral load of all samples was quantified by first Viral Nucleic Acid Extraction Kit II (Geneaid-Tiwan), which was designed specifically for efficient purification of viral DNA and viral RNA from cell-free samples such as serum and plasma, and then Bosphore® HBV Quantification Kit (Anatolia-Turkey) have been used to detect and quantify hepatitis B virus DNA.

Detection of HBV DNA using quantitative real time polymerase chain reaction (RT-PCR)

Viral DNA extraction was conducted using Viral Nucleic Acid extraction kit (Geneaid-Tiwan) with two hundred µl viral DNA, which was extracted through three main steps lysis, nucleic acid binding, wash, and finally elute the purified nucleic acid. Hepatitis B virus was quantified by HBV Quantification kit (Bosphore®- Turkey). The kit contains positive and negative control, four external quantitation standards and PCR master mix, which contains a highly specific and accurate HotStarTaq DNA Polymerase, the PCR buffer, the dNTP Mix, the HBV-specific forward and reverse primers and a dual-labeled probe, and the internal control-specific forward and reverse primers and a dual-labeled probe.

Total volume of PCR preparation contains 15 µl of the master mix and 10 µl of DNA (sample/ standard/positive or negative control) was added into the PCR tubes or strips, then loaded into the RT-PCR machine (Magnetic Induction Cycler (MIC qPCR), BMS-Australia). The thermal cycler composed of an initial denaturation at 95 °C for 14:30 min, denaturation at 97 °C for 00:30 min, then annealing and synthesis at 54 °C for 01:30 min and hold finally at 22 °C for 05:00 min, and by the use of RT-PCR system software program calculated the baseline cycles and the threshold.

Detection of HBxAg by ELISA

Fifty µl from positive and negative control and 10 µl of testing sample were added to ELISA well then Sample Diluent 40 µl was added to testing sample well, one hundred µl of HRP-conjugate reagent was added to each well, incubated for 60 minutes at 37 °C., after that washed with washing solution, then Chromogen solution A 50 µl and chromogen solution B 50 µl were added to each well and incubated for 15 minutes at 37 °C. Finally, 50 µl Stop Solution was added and read at 450 nm using a microtiter plate reader.

Statistical analysis

The data were processed using statistical package for social sciences (SPSS) version 16.0.0, Microsoft Excel 2010, and Graphpad Prism version 7.04. Accordingly, the proper statistical tests were used. Student t-test and analysis of variance (ANOVA) test were used for parametric data to measure the significance of difference in means taking into account whether variables of analysis sharing different or equal variance. The diagnostic performance of a test or the accuracy of a test to discriminate true positive from false positive cases was evaluated using Receiver Operating Characteristic (ROC) curve analysis.

Results

The mean HBV viral load was $5.029x10^9$ copies/ml, with a range between 166.8 to $3.13*10^{11}$, according to Terrault *et al.* (2018) [7], chronic HBV patients were divided into active and inactive infections (median viral load was $4.9194*10^4$), and in this study 41 (45.6%) patient had active, and 49 (54.4%) patients had inactive chronic hepatitis B (CHB) infections, as shown in table (1).

Table 1. Frequency of patients according to the type chronicity

Type of chronicity	Frequency	%
Active	41	45.6
Inactive	49	54.4
Total	90	100.0

Results of ELISA study of HBxAg in patients with chronic HBV infection

This study found that HBxAg was positive in 24 (26.7%) of patients with chronic HBV infection. The current study revealed a highly significant correlation between positive HBxAg and active HBV infection p<0.001, as shown in table (2).

Analysis by ROC was carried out for estimating the sensitivity and specificity of the cutoff at which chronic hepatitis patients are considered positive for HBxAg when the viral load of HBV is equal or higher than the specified cut off value. The area under the curve (AUC) for HBV was 0.768, and P <0.001. The optimal cut-off value was 5115.9 with sensitivity and specificity 82.6%, 64.2% respectively, 95% confidence interval equal to 0.665-0.871 as illustrated in table (3) and figure (1).

Table 2. Correlation of HBxAg with sex, treatment, chronicity, and complication

Variables		HBxAg -ve		HBxAg +ve		Total	
		No.	%	No.	%	No.	%
Sex	male	45	80.4	11	19.6	56	100
	female	21	61.8	13	38.2	34	100
		Chi-sq: P=0.48					
		Correlation r=+0.2, P=0.054					
Treatment	No	56	70.9	23	29.1	79	100
	Yes	10	90.9	1	9.1	11	100
		Fisher exact: P=0.15					
		Correlation r=-0.14, P=0.16					
Chronicity	inactive	46	69.7	3	12.5	49	100
	active	20	30.3	21	87.5	41	100
		Chi-sq: P<0.000					
		Correlation r=+0.5, P<0.000					
Complications	No	44	71.0	18	29.0	62	100
	Yes	22	78.6	6	21.4	28	100
		Chi-sq: P=0.45					
		Correlation r=-0.08, P=0.46					

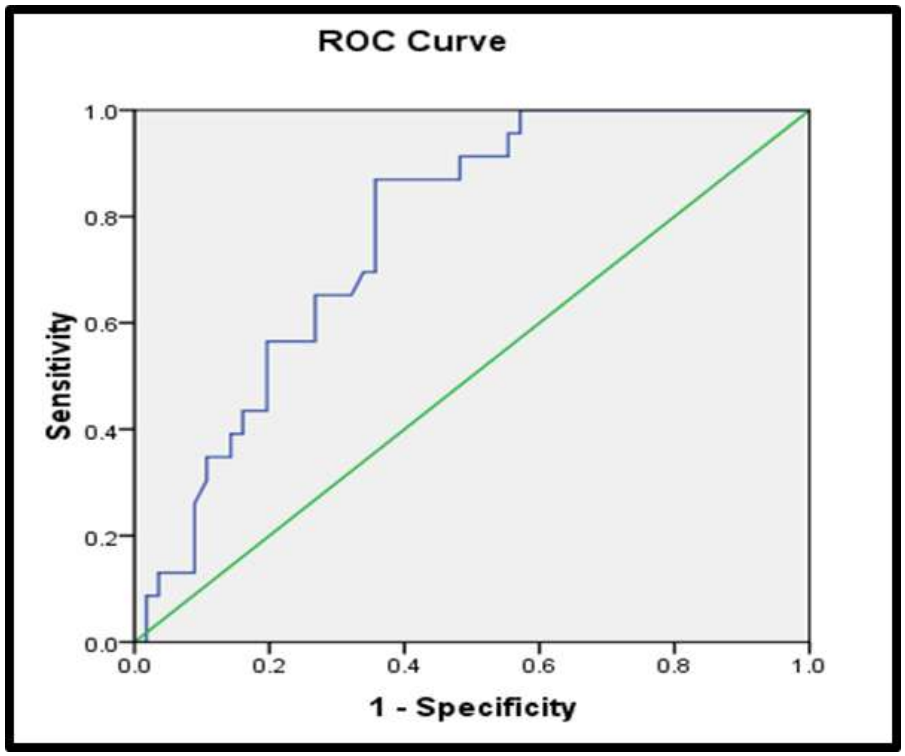

Figure 1. ROC curve for HBV viral load with HBxAg

Table 3. Association of HBV viral load with HBxAg (Area Under the Curve)

Area	Accuracy	Asymptotic Sig.	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
0.768	very good	0.000	0.665	0.871

Discussion

Globally, there were an estimated 248 million persons with chronic HBV infection in 2010 and approximately 686,000 deaths were attributed to complications associated with chronic HBV infection in 2013 [3]. Chronic HBV infection remains the most important risk factor for HCC worldwide, with the level of viremia being the number one risk factor [4].

In this study, 11 out of 25 patient had complications has chronic active hepatitis in whom HBV viral load more than $4.9194*10^4$, this agrees with a study suggested that HBV-DNA levels of 10^4 copies/ml or more are the strongest predictor of future cirrhosis or HCC risk, regardless of HBeAg status and serum alanine transaminase (ALT) levels at baseline [8]. A study of Bárcena Marugán and García Garzón (2009) [9], found that if patients have high DNA levels and normal ALT, without other unfavorable prognostic factors, it is advisable to follow the patients and not to treat them, because serum DNA levels are a prognostic factor, and contribute to define the phase of CHB infection, the treatment indication, and allow an assessment of the efficacy of antiviral therapy. High levels of HBV DNA are an independent risk factor for cirrhosis and HCC in Asia.

In this study, HBxAg has positive correlation R=+0.5, P<0.000 with active infection. Hepatitis Bx gene (HBx) showed progressive increase in expression with the increase in viral copy number and showed 50% expression in patients having high viral load from 41 patient with active HBV infection there was 21 positive HBxAg this suggested HBxAg as sensitive biomarker for HBV infection follow up, this high significant correlation between HBxAg and active chronic hepatitis can nominate it as a good prognostic marker for disease progression.

Previous study has shown that HBx can manipulate epigenetic mechanisms to regulate not only host gene expression in hepatocytes but also the replicative ability of HBV itself [10].

HBx can integrate into cellular DNA during chronic infection. Overexpression of HBxAg may alter signal transduction pathways of hepatocyte, and it can bind to and inactivate negative growth regulatory genes suggesting its role in hepatocarcinogenesis [11-13].

Al-Qahtani et al. study (2017) [14], supported the results of the current study in which expression of HBxAg correlates with the activity of the disease and the possibility of development of cirrhosis and fibrosis.

The HBx protein is expressed in chronic hepatitis, cirrhotic liver and HCC from individuals infected with HBV. It is localized in both the cytoplasm and nucleus, and can therefore interact with cell signal transduction pathways and transcription machinery. HBx has been reported to transactivate a variety of cellular genes. Also, HBx associates with the p53 tumor suppressor protein in vitro and in vivo, leading to p53 inhibition of its functions. Moreover, p53 inactivation by HBx has been implicated in liver carcinogenesis [15,16].

In conclusion, the presence of HBxAg could be an important indicator of the disease progression and possibility of developing complications in patients with chronic HBV.

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Author contribution

Dr. Mahdi: Collection of samples, DNA extraction and running the ELISA study of HBxAg. Al-Khezrachi: making the statistical analysis and writing the results. Dr. Shamran:

Final editing of the manuscript. Dr. Al-Obaidi: Detection of HBV DNA using quantitative real time polymerase chain reaction.

Conflict of interest

Authors declare that there is no conflict of interest.

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Incidence of Colorectal Carcinoma in Patients Undergoing Appendectomy After Age of 40 Years in Sulaimani Teaching Hospital

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Abstract

Background The appendiceal disease is one of the most common reasons for emergency hospital admission, and appendectomy is one of the most frequently performed emergency procedures. Obstruction of the appendiceal lumen is the usual cause of acute appendicitis. However, in elderly patients, it may also be due to a neoplasm of appendix, cecum, or even colorectal carcinoma and appendicitis can be its first manifestation. Of all the gastrointestinal tract malignancies, colorectal carcinoma is the most common one.

Objective To find the incidence of the carcinoma of colon in patients above 40 years of age who underwent appendectomy.

Methods Two groups of patients studied from Sulaimani Teaching Hospital and Kurdistan Center for Gastroenterology and Hepatology. Both groups underwent colonoscopy and one group only had appendectomy. Non-appendectomized group used as control. A 213 patients from a total of 545 patients studied from October 1st, 2018 to September 30th, 2019.

Results More than half of the patients (54%) had normal colonoscopy, others showed internal hemorrhoid (15.5%), polyp (15%), sigmoid mass (0.9%), rectosigmoid mass (0.5%) and gastrointestinal stromal tumor (0.5%). Histopathology results were tubular adenoma with low-grade dysplasia (36.4%), hyperplastic polyps (34.1%), adenocarcinoma (2.3%), and familial adenomatous polyposis (2.3%).

Conclusion The incidence of colorectal carcinoma is 4.76% in the studied group whom underwent appendectomy after 40 years of age.

Keywords Acute appendicitis, adenocarcinoma, colonoscopy, colorectal cancer, mucinous neoplasm of appendix, appendectomy.

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List of abbreviations: CRC = Colorectal carcinoma, CT = Computed Tomography, FAP = Familial adenomatous polyposis, GIST = Gastrointestinal stromal tumor, KCGEH = Kurdistan Center for Gastroenterology and Hepatology, SPSS = Statistical package for the social sciences, STH = Sulaimani Teaching Hospital, U/S = Ultrasound

Introduction

Colorectal cancer (CRC) is the most common gastrointestinal tract malignancy. CRC incidence reaches 10-fold variation throughout the world. Australia

and New Zealand have the highest incidence of 44.8 per 100,000 population in men and 32.2 in women; compared to Western Africa which has only 4.5 per 100,000 in men and 3.8 in women [1]. In the United States, there are over 140,000 new cases diagnosed annually and more than 50,000 fatalities each year, which ranks CRC as the third most lethal cancer in the United States [2].

In 2012, there were about 1.4 million new CRC cases and almost 700,000 deaths, however; it is predicted to grow by 60% to more than 2.2 million new cases and 1.1 million cancer deaths by 2030 [3].

CRC is more common with increasing age, and it is not so common before the fourth decade of life [4]. Nevertheless, lately, there has been a rise of CRC between the ages of 40 to 44 years [5].

Risk factors for developing CRC include aging, hereditary risk factors, environmental and dietary factors, and inflammatory bowel disease. Cigarette smoking, pelvic irradiation, and ureterosigmoidostomy are among other causes [6-8]. However, 70% of CRC are sporadic and minority is hereditary [8].

Most of the CRC develop from adenomatous polyps. Colorectal polyps can be classified as inflammatory (pseudopolyp, benign lymphoid polyp), hamartomatous (juvenile, Peutz Jeghers, Cronkite-Canada), hyperplastic and neoplastic (tubular adenoma, villous adenoma, tubulovillous adenomas, serrated adenomas/ polyps) [9].

It takes a decade for the adenoma-carcinoma sequence to develop. The majority of adenomas start as tiny polyps that grow and become dysplastic and at the end cancerous [10-11].

There are three categories of CRC screening including stool-based, imaging, and endoscopic tests. Stool-based tests such as the Guaiac-based fecal occult blood test and fecal immunochemical test can detect asymptomatic cancers at an early stage. Although they are not expensive and noninvasive but they are not capable of polyp detection and less sensitive for adenoma [12].

Imaging tests include double-contrast barium enema, computed tomographic colonography, and colon capsule endoscopy. The first two tests are capable of detecting polyps larger than 10 mm [13], while the latter is more expensive and cannot take biopsy.

Endoscopic tests include flexible sigmoidoscopy and colonoscopy. The distal gastrointestinal tract up to the splenic flexure can be visualized by the flexible sigmoidoscopy. But inability to visualize proximal colon makes it less sensitive. With colonoscopy, the entire length of the large bowel and distal small bowel can be visualized. It is considered the “gold standard” in screening CRC. It can visualize and take biopsy from cancerous and precancerous lesions. But it requires bowel preparation and sedation. Major complications include bleeding and perforation, which are more for therapeutic excisional biopsies.

The appendiceal disease is among the most common reasons for emergency hospital admission, and appendectomy is one of the most frequent emergency procedures performed. The lifetime risk of developing appendicitis is 8.6% for males and 6.7% for females, with the highest incidence in their twenties and thirties [14].

Classic physical findings such as pain and tenderness at McBurney's point, shifting pain from the central abdomen to right iliac fossa, anorexia, nausea, rebound tenderness, elevated temperature, leukocytosis, and shifting white blood cells to the left, have been used to make the diagnosis of acute appendicitis.

Pathologically, obstruction of the appendiceal lumen is the usual cause of acute appendicitis. However, in elderly patients, it may also be due to a neoplasm originating from appendix or cecum [15-17].

Ultrasonography (U/S) and computed tomography (CT) scan are the most commonly used imaging tests in patients with abdominal pain, particularly in the evaluation of possible

appendicitis. Multiple meta-analyses have been performed comparing the two imaging modalities. Overall, CT scan is more sensitive and specific than ultrasonography in diagnosing appendicitis. Imaging investigations like U/S and CT scan are increasingly used as a tool to exclude right-sided (non-appendiceal) colonic tumors on emergency admissions with clinical features of acute appendicitis especially in patients that are 40 years or older [18].

We suggest that if acute appendicitis is happening predominantly in young age population, and CRC is more common among middle and old age; then the patients present with a picture of acute appendicitis after the age of 40 may have CRC induced appendicitis.

Methods

This is a combined prospective and retrospective observational case series study conducted in Sulaimani Teaching Hospital (STH) between October 1st, 2018, and September 30th, 2019. Two groups of patients studied. We wanted to compare patients whom presented to emergency hospital and diagnosed as acute appendicitis and as a result of investigations or surgical operation CRC found; and to compare with a second group (control group) who didn't underwent appendectomy but underwent colonoscopy for other reasons.

Group A

Inclusion criteria

Includes 21 patients from a total of 86 patients who were above the age of 40 years and underwent appendectomy followed up for a duration of one to six months. They were contacted by phone three times (over a period of two months) to undergo colonoscopy. Only 15/21 patients were included.

Exclusion criteria

Patients who didn't respond, had no contact number, below the age of 40 years, or didn't want to do a colonoscopy were excluded.

Group B

Inclusion criteria

Data from Kurdistan Center for Gastroenterology and Hepatology (KCGEH) showed that the patients who were above the age 40 years and underwent colonoscopy were 198 patients from a total of 459 patients. Patients were contacted over the phone 3 times over a period of 3 months. Group B used as a control.

Exclusion criteria

Those who were below age of 40 years; didn't have contact number, didn't respond, have histopathology report of colonoscopy, or underwent colonoscopy after colorectal surgery, were excluded.

Figure 1 shows further details about the patients included in this study.

A questionnaire formulated and data regarding demography, age at the time of appendectomy, result of colonoscopy and histopathology, were collected.

Approval of ethics committee of University of Sulaimani, College of Medicine was granted on February 4th, 2020; number 108.

Statistical analysis was done using Statistical Package for the Social Sciences (SPSS) software version 25. Incidence, p-value, and odds ratio were calculated. A p-value of 0.05 or less considered statistically significant.

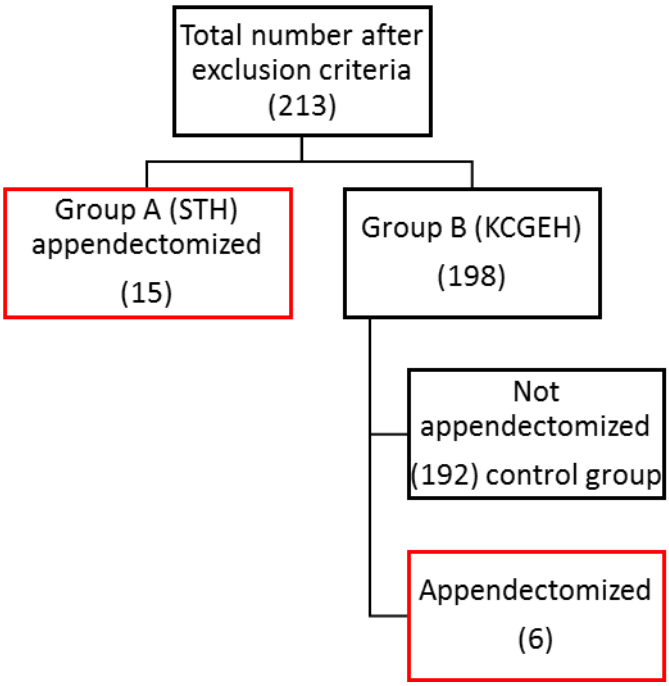

Figure 1. Flow chart of included patients. STH: Sulaimani Teaching Hospital, KCGEH: Kurdistan Center for Gastroenterology and Hepatology

Results

The mean age of patients in this study was 43±11.12 years with a range of 40-87. Females were 113 (53.1%) and males were 100 (46.9%). 21 patients (9.9%) underwent appendectomy and 192 patients (90.1%) were non-appendectomized as shown in table 1.

Table 1. General characteristics of patients

Variable		(mean±SD)	Range
Age (yr)		(43±11.12)	40-87
		Number	**Percentage**
Gender	Male	100	46.9%
	Female	113	53.1%
Appendectomy	Yes	21	9.9%
	No	192	90.1%

Table 2 shows the colonoscopic gross findings for the total 213 patients, and here are some of the findings: one patient (0.5%) had cecal mass, two patients had sigmoid mass (0.9%), one patient (0.5%) had rectosigmoid mass, one patient had gross finding, which confirmed later on biopsy to be gastrointestinal stromal tumor (GIST) (0.5%), 32 patients had polyp (15%), and 33 patients had internal hemorrhoids (15.5%). While 115 patients had normal colonoscopy (54%). GIST diagnosis was based on histopathological reporting from a tissue biopsy, which was taken during colonoscopy.

Table 2. Gross findings of colonoscopy

Colonoscopy (gross findings)	Number (Percentage)
Normal	115 (54%)
Internal hemorrhoid	33 (15.5%)
Polyp	32 (15%)
Diverticula	13 (6.1%)
Colitis	4 (1.9%)
Solitary Rectal Ulcer Syndrome	3 (1.4%)
Erosions	2 (0.9%)
Rectal nodularity	2 (0.9%)
Sigmoid mass	2 (0.9%)
Ileal ulcer	1 (0.5%)
Colonic ulcer	1 (0.5%)
Flat lesion	1 (0.5%)
Gastrointestinal stromal tumor	1 (0.5%)
Telangiectasia	1 (0.5%)
Rectosigmoid mass	1 (0.5%)
Cecal mass	1 (0.5%)
Total	213 (100%)

Histopathology assessment of the colonoscopy specimens (total 44 specimens) shows 16 patients (36.4%) had tubular adenoma with low-grade dysplasia, 15 patients (34.1%) had hyperplastic polyps, 1 patient (2.3%) had mucinous neoplasm of appendix with low-grade dysplasia, 1 patient (2.3%) had adenocarcinoma, and 1 patient (2.3%) had familial adenomatous polyposis (FAP). Normal colonoscopies, internal hemorrhoids, diverticula, and other conditions when no biopsy was taken, were not included in table 3.

Table 3. Results of histopathology of specimens taken during colonoscopy

Colonoscopy (Histopathology)	Appendectomized Number (Percentage)	Non-appendectomized Number (Percentage)	P value
Hyperplastic polyp	3 (42.9%)	12 (32.4%)	
Tubular Adenoma, Low grade dysplasia	2 (28.6%)	14 (37.6%)	
Colitis	0 (0%)	6 (16.2%)	
Familial adenomatous polyposis	0 (0%)	1 (2.7%)	0.288
Adenocarcinoma	0 (0%)	1 (2.7%)	
Data not available	1 (14.3%)	3 (8.1%)	
Mucinous neoplasm of appendix, low grad dysplasia	1 (14.3%)	0 (0%)	
Total	7 (100%)	37 (100%)	

Total number of patients with appendectomy was 21 (15 from group A, and 6 from group B). There was only one patient with mucinous neoplasm of appendix (Figure 1).

$$\frac{munious\ neoplasm}{Group\ A(15)+group\ B(6)} = \frac{1}{15+6} x100 = 4.76\%$$

Thus, the incidence of colorectal cancer among appendectomized patients is 4.76%.

Total number of patients without appendectomy was 192 (Group B). There were 2 patients with CRC (one patient with adenocarcinoma and one patient with FAP). (Figure 1).

$$\frac{FAP+adenocarcinoma}{Group\ B(192)} = \frac{2}{192} x100 = 1.04\%$$ Hence,

The incidence of colorectal cancer among non-appendectomized patients is 1.04%.

Mucinous neoplasm, FAP and adenocarcinoma from table 3 and patient numbers from figure 1 are used to calculate table 4. Details of the calculations are shown above.

Table 4. Incidence of carcinoma of colon among appendectomized and non-appendectomized patients

Data	Incidence of carcinoma of colon
Appendectomized	4.76%
Non-appendectomized	1.04%

Discussion

In this study, the mean age of appendectomy is 54 years, this is consistent with a study done by Khan et al. [19]. The majority of colonoscopies showed normal findings. The most common other colonoscopy findings were internal hemorrhoids and polyps (Table 2).

Female to male ratio is 1:1.13 while it was 1:1.06 in a similar study by Khan et al. [19].

One patient (14.3%) from appendectomized group A had low-grade mucinous neoplasm of appendix. Appendiceal mucinous neoplasm is rare dysplastic mucinous tumor, based on their cytologic features can be further classified into low-grade or high-grade. Diagnosed incidentally and clinical presentation is not specific. Low-grade appendiceal mucinous neoplasms don't invade the epithelium of appendix, but because they can grow into the muscularis propria, they can irritate and cause inflammation of the appendix, and can even cause the appendix to rupture. The best treatment for low-grade appendiceal mucinous neoplasms that are intact and confined to the appendix is an appendectomy. But if ruptured, spreading neoplastic cells through the peritoneum, leads to pseudomyxoma peritonei [20,21].

Two patients (28.6%) from appendectomized group A and 14 patients (37.6%) from nonappendectomized group B had tubular adenoma with low grade dysplasia. These tumors are classified as the low-risk group, which includes patients with 1-2 tubular adenomas of less than 10 mm with low grade dysplasia. Based on recommendations of the European Society of Gastrointestinal Endoscopy, it requires participation in national screening programs 10 years after the index colonoscopy. If no screening program is available, repetition of colonoscopy 10 years after the index colonoscopy is recommended [22].

It's well known that the adenoma-carcinoma sequence is a major role in the development of CRC [23,24]. In a study by Atkin and colleagues, they found out that colonoscopy surveillance was associated with a noticeable reduction in the incidence of CRC in the intermediate and high-risk adenomas compared with no surveillance [25].

Three patients (42.9%) from appendectomy group A and 12 patients (32.4%) from nonappendectomy group diagnosed with hyperplastic polyp. Hyperplastic polyps are the most common type of colorectal findings. They are considered to be benign tumors. In a study by Laiyemo and colleagues [26], they didn't find a strong relationship between hyperplastic polyps and recurrence of adenomatous polyps. Therefore, the current guidelines for surveillance colonoscopy is after 10 years, this resembles the same guidelines for the patients without any polyps.

Obstruction of the lumen of the appendix is the most common cause of appendicitis. Backpressure from CRC may cause inflammation of the cecum and occlusion of the appendix [8]. Acute appendicitis can also develop through inflammation and edema of appendiceal wall or as a consequence of obstruction of its lumen. Another pathology is immune mediated lymphoid hyperplasia of malignancy resulting in obstruction of the lumen of appendix [27].

Our results show that incidence of CRC among appendectozied patients is 4.76%, while among non-appendectomized (control group) patients is only 1.04. This means patients whom undergo appendectomy after age of 40 years are more likely to develop CRC.

Statistical analysis of group A and group B using SPSS software done and odds ratio calculated. An odds ratio is a measure of relationship between exposure and outcome. It is most commonly used in case-control studies; however, they can also be used in cross-sectional and cohort study designs [28].

The results of the present study were an odds ratio of 2.4. This means incidence of CRC among appendectomized patients is 2.4 time higher. In a study of 1873 patients by Lai et al it was 38.5 [29].

This study is limited by some factors that include a single center experience, and the small number of colonoscopies in the appendectomized group. That small number of colonoscopies was due to the fact that the majority of patients either refused colonoscopy, mostly due to their histopathology results that didn't mention malignancy, or they had job restriction in the morning, or they were afraid that the investigation might find a tumor.

The absence of electronic medical records was another obstacle. Every patient had to be contacted and asked for their history, investigation papers, and follow up. We contacted 71 appendectomized patients to undergo colonoscopy but only 21agreed.

In fact, one of the patients who underwent colonoscopy was a 45-year-old female housewife who became anxious after our phone call, she consulted two general surgeons because she was afraid that we might have found some malignant pathology. Later she became so anxious that her family consulted a psychiatrist who put heron anxiolytic medication.

The current study found that the incidence of colorectal carcinoma is 4.76% in the studied group whom underwent appendectomy after 40 years of age, while among non-appendectomized (control group) patients is only 1.04.

Postoperative colonoscopy within 3 months is of diagnostic significance for smaller polyps and colorectal tumors.

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Author contribution

Dr. Abdulla: created the study and carried out the design, data curation, analysis, interpretation of data, writing the draft and critical revision under supervision of Dr. Faraj. Histopathological reporting of tissue biopsies carried out by Dr. Abdulqader. Follow up of the patients done by Dr. Mardan, Dr. Mohammed, Dr. Mahmood, Dr. Hussein and Dr. Shareef. All authors read and approved the final manuscript.

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None to declare.

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The Relevance of *Helicobacter pylori* Infection to Iron Deficiency Anemia in Duhok City

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Abstract

Background *Helicobacter pylori* (*H. pylori*) infection had been criticized for many deleterious effects and had been amalgamated to iron deficiency by many authors, frequently based on correlative rather than direct relationship studies and often opposed by others.

Objective To evaluate the role of *H. pylori* infection in the etiology of iron deficiency anemia and to study the impact of the bacterial eradication on the response to iron therapy.

Methods The current study represents an interventional prospective study and involved 52 non-pregnant females with iron deficiency anemia. All patients were tested for the presence of active *H. pylori* infection by stool antigen test and they followed after one month of iron therapy. Patients with positive *H. pylori* infection followed for another month after eradication of *H. pylori* and iron therapy.

Results Fifteen patients (28.85%) were positive for *H. pylori*. Hematological and biochemical data were not different among both groups (*H. pylori* positive and negative) at presentation despite significant better response among *H. pylori* negative individuals. Continuation of iron therapy after eradication of *H. pylori* infection improve the response to therapy significantly.

Conclusion Eradication of *H. pylori* enhances the response to iron therapy significantly.

Keywords Iron deficiency anemia, *H. pylori*, iron therapy, eradication

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List of abbreviations: *H. pylori* = *Helicobacter pylori*, Hb = Hemoglobin, IDA = Iron deficiency anemia

Introduction

Anemia is one of the common disorders that disturbs a quarter of the population worldwide, and the higher prevalence is found among preschool-age children and the menstruating females [1-3]. As the hemoglobin (Hb) is the most abundant iron-containing protein in humans, and anemia is a characteristic trait of iron deficiency, thus, iron deficiency anemia (IDA) is considered to be the most common nutrient deficiency and the most common cause of anemia globally [4]. Iron deficiency anemia by many researchers has been related to a longer stay in hospital and reduced life expectancy [5]. IDA is a consequence of depletion of the iron stores because of either diminished iron uptake or increased iron loss/use. Body iron homeostasis is mainly through controlling iron entrance to the body rather than controlling its excretion as the body has limited excretion capacity and the gastrointestinal tract is a common site of blood loss and their diseases may cause malabsorption of iron [6].

Some studies showed that *Helicobacter pylori* (*H. pylori*) infection is linked to the increased probability of diminished iron storage, and *H.*

pylori eradication therapy may be beneficial in increasing ferritin levels. Several reported data supported the efficacy of *H. pylori* treatment in moderately to severely anemic patients compared to those with mild anemia. Nonetheless, it should be noted that some studies show negative correlations between *H. pylori* infection and IDA. It is now recommended that *H. pylori* infection must be tested and treated in patients with unexplained IDA [7].

A conflicting data exist about the relation of *H. pylori* infection to the etiology of IDA and the response of patients to therapy; thus, this study had been initiated to evaluate the role of *H. pylori* in IDA particularly in our locality in Iraq.

Methods

The current study represents a quasi-experimental interventional prospective study and performed at Azadi Teaching Hospital at Dohuk City, Iraq. Sample collection was done in a period of six months, from September 1st, 2019 to February 29th, 2020. The study was approved by the Ethical Committee at the Directorate of Health/ Duhok. A total of 52 patients (females), diagnosed as IDA by clinical and laboratory screening were enrolled in this study. All patients were from Duhok Governorate. Any female with age lower than 18 years, gastrointestinal bleeding, pregnancy, lactation, heavy vaginal bleeding, or chronic or significant diseases including chronic inflammatory diseases like rheumatoid arthritis or systemic lupus erythematosus were excluded from the current study.

At first, the process was explained to the patients and informed consent taken from all enrolled individuals. Then 3 ml of peripheral venous blood was taken by a clean and appropriate venipuncture technique from each patient. One milliliter in a sterilized tube containing EDTA, mixed well and subjected for complete blood count using Swelab Hematology Analyzer (Ds Biomed, Sweden) and the remaining 2 milliliters of blood in a gel tube with clot activator for the biochemical test including serum iron, unbound iron-binding capacity, transferrin saturation and serum ferritin using Cobas 6000 (Roche-Germany). All selected patients were tested also for the presence of active *H. pylori* infection by stool antigen using the One-Step *H. pylori* Antigen Test Kit (Plasmatec laboratory products, UK); infected patients were received oral iron therapy then re-assessed after one month and then received eradication therapy with oral iron therapy and re-assessed after another month, while patients with negative *H. pylori* infection received only oral iron therapy and assessed after one month. After data collection, data were analyzed using statistical package for social sciences (SPSS), version 24.0 (2016). Student t-test was used for comparison of continuous variables, and chi-square or Fisher exact tests were used for comparison of categorical variables. Two-sample paired t-test was used to assess the response and the P-value were considered to be significant if it's less than 0.05.

Results

Demographic data of all individuals are shown in (Table 1) and it reveals a total of 52 females with IDA had been enrolled in the current study with age ranging from 18-45 years (median 30±7.58 years). From these, 15 (28.85%) patients had *H. pylori* antigen in the stool and the remaining 37 (71.15%) were negative for *H. pylori* antigen.

Their hematological data reveals that the majority had mild 24/52 (46.15%), to moderate 21/52 (40.39%) anemia and only 7/52 (13.46%) had severe anemia. Regarding the severity of anemia among both groups; a majority of patients with *H. pylori*-positive had moderate 6/15 (40%) to severe anemia 4/15 (26.67%) in comparison to *H. pylori*-negative females who had mild 19/37 (51.35%) to moderate 15/37 (40.54%) anemia. No significant difference was seen among both groups (P= 0.09). There were no statistically significant differences in the Hb, packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), iron, total iron binding capacity (TIBC), transferrin saturation (Ts), and ferritin levels between patients with or without *H. pylori* infections with P-value consistently more than 0.05 for the above-examined parameters.

Table 1. Characteristic of patients with *H. Pylori* positive and negative

Parameters	*H. pylori* positive (N = 15) Mean ± SD	*H. pylori* Negative (N = 37) Mean ± SD	P value
Age (Years)	32.20 ± 7.514	28.81 ± 7.486	0.152
≤20 (Years) (No.)	1	7	
21-30 (Years) (No.)	6	15	
31-40 (Years) (No.)	6	12	
>40 (Years) (No.)	2	3	
Hb (g/dl)	9.15 ± 1.19	9.78 ± 1.158	0.095
10-11.9 (g/dl) (No.)	5	19	
8.0-9.9 (g/dl) (No.)	6	15	
<8.0 (g/dl) (No.)	4	3	
PCV (%)	29.00 ± 3.41	30.92 ± 2.881	0.070
MCV (fl)	67.03 ± 4.28	68.59 ± 4.597	0.247
MCH (pg)	21.15 ± 2.03	21.65 ± 2.226	0.562
IRON (µg/dl)	31.37 ± 1.79	28.30 ± 6.916	0.521
TIBC (µg/dl)	405.07 ± 62.05	447.51 ± 50.390	0.539
Transferrin saturation (%)	6.95 ± 2.38	6.30 ± 1.777	0.320
Ferritin (ng/ml)	6.00 ± 2.21	7.14 ± 3.002	0.118

After one-month oral iron therapy in *H. pylori*-negative patients and using a two-sample paired t-test, results showed a statistically significant difference in the all examined parameters including Hb, PCV, MCV, MCH, Iron, TIBC, Ts and ferritin (Table 2).

Table 2. Hematological and biochemical parameters difference after Oral iron therapy among H. pylori negative (N = 37)

Parameters	Before Treatment	After treatment	Mean difference	P value
Hb (g/dl)	9.78±1.16	11.97±1.02	2.20	< 0.001
PCV (%)	30.93±2.82	36.38±3.08	5.47	< 0.001
MCV (fl)	68.65 ± 4.60	78.53±4.79	9.88	< 0.001
MCH (pg)	21.69 ± 2.18	25.82 ± 1.99	4.13	< 0.001
IRON (µg/dl)	31.55 ± 1.59	32.85 ± 1.38	1.30	<0.001
TIBC (µg/dl)	419.22 ±51.38	298.78±59.34	120.43	< 0.001
Transferrin saturation (%)	6.31 ± 1.74	18.61 ± 9.70	12.30	< 0.001
Ferritin (ng/ml)	7.16 ± 2.91	17.24 ± 5.88	10.08	< 0.001

The result among *H. pylori*-positive showed lesser changes in all examined parameter, though the statistically significant difference in the all examined parameters including Hb, PCV, MCV, MCH, Iron, TIBC, Ts and ferritin (Table 3) and with the eradication of *H. pylori* further significant increment seen per the second months but still lower than *H. pylori*-negative patients (per month), however, after considering the response of the two months of therapy, the result showed highly significant changes with normalization of all parameter including the S. ferritin.

Table 3. Hematological and biochemical parameters difference among H. pylori positive (N = 15) after first month; second month (after *H. pylori* eradication) and both months together following oral iron therapy

Parameters	Before Treatment	After treatment First month			After second month treatment and *H. Pylori* eradication			Two months taken together	
			Mean difference	P value		Mean difference	P value	Mean difference	P value
Hb (g/dl)	9.15 ± 1.19	10.88 ± 1.05	1.73	< 0.001	12.53 ± 0.72	1.65	< 0.001	3.38	< 0.001
PCV (%)	29.00 ± 3.41	33.79 ±3.5.79	4.79	< 0.001	37.49 ± 2.24	3.69	< 0.001	8.49	< 0.001
MCV (fl)	67.03 ± 4.28	73.49 ± 2.87	6.45	< 0.001	81.14 ± 2.95	7.65	< 0.001	14.11	< 0.001
MCH (pg)	21.15 ± 2.03	23.66 ± 1.05	2.51	< 0.001	27.1 ± 1.26	3.44	< 0.001	5.95	< 0.001
IRON (µg/dl)	31.37 ± 1.79	32.19 ± 1.25	0.83	0.037	75.07 ± 22.63	28.13	<0.001	44.67	0.002
TIBC (µg/dl)	405.07 ± 62.05	367.67 ± 56.83	67.8	< 0.001	339.0 ± 51.37	28.67	0.107	96.47	< 0.001
Transferrin saturation (%)	6.95 ± 2.38	13.08 ± 6.05	6.14	< 0.001	21.91 ± 7.28	8.83	< 0.001	14.97	< 0.001
Ferritin (ng/ml)	6.00 ± 2.21	12.25 ± 3.61	6.26	< 0.001	21.76 ± 5.67	9.51	< 0.001	15.76	< 0.001

Tables 4 shows the average increment in the Hb, PCV, MCV, MCH, S. Iron and S. ferritin and decrement in the TIBC in both *H. pylori*-negative and *H. pylori*-positive (the first month before *H. pylori* eradication; the second month of iron therapy following eradication of *H. pylori* and two months together) patients and they reveal significant higher response among *H. pylori*-negative patients to iron therapy in the first month in MCV, MCH, S. Iron and S. ferritin; significant higher response in the second month in Hb, PCV, and TIBC; and significantly better response among H. pylori-positive patients in the Hb, PCV, MCV, MCH, S. ferritin if 2 months taken together.

Table 4. Mean changes in the hematological and biochemical parameters among *H. pylori* negative following iron therapy and *H. pylori* positive (first moth of iron therapy alone); (second month of iron therapy + *H. pylori* eradication) and (combined first- and second-months including *H. pylori* eradication)

Parameters	*H. pylori* Negative (N = 37) Mean ± SD	*H. pylori* positive (N = 15) Mean ± SD					
	First month only	First month only	P value (1)	Second month only	P value (2)	Both months together + *H. pylori* eradication	P value (1+2)
Hb (g/dl)	2.20 ± 0.78	1.73 ± 1.24	0.105	1.65 ± 0.69	0.022	3.38 ± 1.32	<0.001
PCV (%)	5.457 ± 2.78	4.793 ± 3.87	0.482	3.69 ± 2.19	0.032	8.49 ± 3.00	0.001
MCV (fl)	9.88 ± 4.14	6.45 ± 5.00	0.013	7.65 ± 3.11	0.066	14.11 ± 4.62	0.002
MCH (pg)	4.12 ± 1.42	2.51 ± 1.81	0.001	3.44 ± 1.35	0.115	5.95 ± 2.43	0.001
IRON (µg/dl)	37.41 ± 25.86	16.53 ± 16.63	0.005	28.13 ± 20.11	0.219	44.67 ± 17.98	0.326
TIBC (µg/dl)	-79.97 ± 46.20	-67.8 ± 43.02	0.385	-28.67 ± 64.43	0.002	-96.47 ± 77.34	0.346
Transferrin saturation (%)	12.30 ± 9.59	6.14 ± 5.05	0.02	8.83 ± 5.48	0.195	14.97 ± 5.88	0.322
Ferritin (ng/ml)	10.08 ± 4.84	6.26 ± 4.04	0.009	9.51 ± 4.68	0.697	15.76 ± 6.03	<0.001

Discussion

The prevalence of *H. pylori* shows large geographical variations reaching up to 50% of the population in some developing countries, while the prevalence of *H. pylori* in industrialized countries generally remains under 40% and is considerably lower in children and adolescents than in adults and elderly people [8].

The current study revealed no significant difference between *H. pylori*-positive and negative patients in Hb, PCV, MCV, MCH, S. iron, Ts, TIBC and ferritin; similar results were shown in studies from Turkey and from Iran in which they suggest no correlation between *H. pylori* infection and IDA without assessing treatment response [9,10]. Another study from Egypt has shown almost same results but they found significant results after treatment among *H. pylori*-infected patients [11] and their data augment the current results of a significant better response to iron replacement therapy among *H. pylori*-negative individuals, however a study from Alaska found a significant association between low serum ferritin levels and the prevalence of *H. pylori* infection, also a German study found significantly lower levels of hemoglobin in pregnant women suffering from *H. pylori* infection [12,13]. Moreover, an American study found that those who were seropositive for *H. pylori* infection had significantly lower serum ferritin levels compared with seronegative individuals [14]. This variability in studies could be due to differences in the geographical and ethnic distribution of patients, age, sample size, sampling procedures, methods of detecting anemia, and methods of detecting H. pylori infection.

Following one month of therapy with iron, the changes in MCV, MCH, S. iron, Ts, and ferritin were significant, while they were non-significant in Hb, PCV, and TIBC and these changes were concordances with data from Egypt, China and from Israel [11, 15-17].

After *H. pylori* eradication and continuous therapy with oral iron in *H. pylori*-positive group patients, results showed a statistically significant difference in the Hb, PCV, MCV, MCH, iron, TIBC, Ts and ferritin and with the consideration of the results of the last month after eradication of *H. pylori*, the significant difference still can be observed with the continuation of the iron therapy in all examined parameters (HB, PCV, MCV, MCH, iron and ferritin) except TIBC, this is considered the most reliable evidence for a cause-effect relationship between *H. pylori* infection and IDA. This result corroborated many other studies that showed that the eradication of *H. pylori* shows significant improvement in iron stores with or without hematological response such as from Israel [17,18], Egypt [11], China [15,16], and from India [19], which support our results. However, some other studies show a negative relation between *H. pylori* eradication and IDA from the USA [20] and Bangladesh [21].

Different mechanisms had been claimed to explain the impaired response to iron therapy among *H. pylori*-positive individuals including mucosal lining penetration by the bacteria to establish infection [8], chronic gastritis with impaired absorption, peptic ulcer with continuous iron loss [22].

In conclusion, hematological and biochemical parameters were not different among both *H. pylori*-positive and -negative patients. Patients without *H. pylori* infection show significantly better response to iron therapy than patients with *H. pylori* infection and eradication of *H. pylori* improve the response to iron therapy significantly.

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Author contribution

Dr. Eissa: Analysis of data and manuscript preparation. Dr. Mirza: data collection.

Conflict of interest

The authors declare that they have no competing interests in this work.

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Factors Related to Delayed Recovery After Anesthesia

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Abstract

Background Delayed recovery from anesthesia is one of the important challenges that face anesthesiologist. The time of delayed recovery from anesthesia is related to anesthetic agent, patient, duration and type of surgery.

Objective To determine the factors from the patients and other things related to delayed recovery after giving anesthesia.

Methods A total of 286 patients were observed in Shar Medical Hospital and Cardiac Center in Sulaimani city, Iraq between November 2019 and March 2020. Post-operative evaluation and observation were collected for delayed and normal recovery after anesthesia. P value less than 0.05 means significant relationship were obtained between variables.

Results From the 286 patients, 180 (62.94%) were male, in which 66 of them were have delayed recovery and significant relation was found p=0.005. Most of the patients were >50 years-old 126 (44.05%) and there was significant relation between age and delayed recovery of consciousness p=0.01. Delayed recovery was found among overweight more frequently than the underweight and normal weight (51 from 73 for overweight, 33 from 45 and 7 from 168, respectively). Also, significant relationship was found p=0.001.

Conclusion Old age, male, obese patients and cigarette smokers, diabetes mellitus, cerebrovascular accident, history of myocardial infarction, hyperlipidemia, and neurological diseases are high risk factors for delayed recovery.

Keywords Anesthesia, delayed recovery, post-operative care, risk factors

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List of abbreviations: GA = General anesthesia, $PaCO_2$ = Partial pressure of carbon dioxide, SPSS = Statistical Package for Social Sciences, $\chi 2$ = Chi-square

Introduction

Anesthesia is used to relieve pain during diagnostic procedures, and operative procedures. It is blocking the pathway that produces the pain from nerves in human body and leading to unconsciousness. It can be given to different parts of the body by various ways.

Types of anesthesia

There are three main types:

Regional anesthesia: Regional anesthesia is injection of the anesthetic agents to local area around the major nerves supplied to the area in the body this means that the person doesn't have to be sleep. It is divided in to three types according to injection; spinal, epidural and regional nerve block. The area of nerve blockage includes; thigh, ankle, forearm, hand, shoulder, and abdomen. Also, regional anesthesia uses to nerve locating device like nerve stimulator. It is producing numbness and tingling that leads to loss of movement and sensation in that part [1].

General anesthesia: general anesthesia means putting the patient unconscious by giving

anesthetic agents such as drugs or gases, which are deep enough, the patient will not feel the pain. Also leads to changes in breathing and circulation. This needs consistently monitoring the patient and always manages the breathing and circulating systems. After operation the consciousness and sensation return back gradually, this means recovery [(1)].

Local anesthesia: local anesthesia means putting anesthetic agents in to a small part of the body or near the surgical site. It causes numbness of this part which is injected, also used for short or simple operation such as dental and skin lesion or some injuries. In this type the patient is fully conscious. In spite of this, sedation also can be used to reduce the patient's consciousness and relieve the pain during the procedure that is unpleasant or uncomfortable such as in gastroscopy and colonoscopy [(1)].

Recovery could be divided into three phases after anesthesia

Immediate recovery; it means return of consciousness, recovery of airway protective reflex and return of motor action which continues for short time. Intermediate recovery; it means regain little movement with coordination and feel of dizziness. This phase goes on for one hour after anesthesia, but the patient may be discharged and considered fit. Long term recovery; it means full recovery of the patient and mental function. This phase lasts for hours to seven days after anesthesia [(2,3)].

Delayed recovery from anesthesia is one of the important challenges that face anesthesiologist. The time of delayed recovery from anesthesia is related to anesthetic agent, patient, duration and type of surgery. These factors and pre-medications are responsible for prolonged or delayed recovery. Non-pharmacological factors play important role in recovery, which may cause many serious sequels and metabolic disorders such as hyperglycemia, hypoglycemia and electrolyte disturbance, hypoxia, hypernatremia, hypertension, liver diseases, uremia and hypothyroidism. Early diagnosis of delayed recovery after operation should be done because delayed recovery may be due to hypoxia, hemorrhage, thrombosis and embolism in the brain. Emergency management should be used to maintain airway, breathing and circulation. On the other hand, the risk factors and causes, of delayed recovery depend on the previous experience and information from the patient from previous operation [(4)].

Recovery from anesthesia especially, intravenous opioids and hypnotics are more difficult and extend more than recovery from respiratory inhalation drugs. After operation the patients should stay in operating room till they have stable airway and circulating parameters. More frequent post-operative respiratory and circulation systems complications occur when the patients leave the recovery room early [(2)].

Delayed recovery after general anesthesia is characterized by continuous somnolence; it means that the patients cannot be triggered. But patients how don't have any medical related condition for delayed recovery could be due to undiagnosed conditions. There are several risk factors responsible for delay recovery (Table 1) [(4)].

Development of successful implementation and management of delayed recovery after surgery or post-operatively occur by a process that makes a climate for change to delegate and promote the successful implementation and management [(5)].

Management, team training and safety materials are useful considerations to change this magnitude. Creating a specialty may progress recovery pathway this process done throughout learning from other expert anesthetists or by correlating collaborative network of colleagues, also by continued review of the articles related to the anesthesia especially, recovery from anesthesia [(6,7)].

Table 1. Factors responsible for delayed recovery [4]

Patient factors	Drug factors	Surgical factors	Metabolic factors
Gender	Dosage	Duration of surgery	Hyper/hypoglycemia
Old age	Metabolism	Type of muscle relaxant uses	Hyper/hypernatremia
Variation of gene	Time of administration	Hypotension	Hypothyroidism
Central nervous system disorders	Excretion	Hypoxia	Hyperthermia
Co-morbidity	Drug interaction	Embolism	Acidosis
Body habits	Fluid disturbance	Neuro and cardiac surgery	Coagulates
Cognitive dysfunction	Anesthetic agent toxicity	Regional techniques with sedation	Central anticholinergic problems
Seizures	Gas solubility in the body	Stimulation of pain	Renal or hepatic failure

Any patient before anesthesia will need pre-operative evaluation to identify health conditions that affect management during operation or post-operatively especially recovery after general anesthesia [2]. It is known that some of the physical and psychological factors may affect delayed recovery from surgery and anesthesia [8]. Pre-operative anxiety for most patients was shown and associated with delayed recovery [9]. Premedication drugs to relieve anxiety should be administrated which is improving, helping and minimizing post-operative complication and delayed recovery [10].

On the other hand, surgical stress affects catabolic state that raises cardiac demand for oxygen, raises insulin resistance, change profile of coagulation and modify gastrointestinal and pulmonary functions [11]. This process leads to dysfunction of most of the organs in the body and increase morbidity which leads to delayed recovery [12]. Thromboembolism, nosocomial infection and long-term dysfunction of life may result from delayed recovery, also delayed recovery increases cost of health care which is another major problem [13].

Post-operative care has essential criteria for the patient's evaluation, assessment, documentation and monitoring especially during recovery periods. Recovery status started when the patients were assessed, and evaluated following return of consciousness and system functions comes from additional guidance review from American Association of Nurse Anesthesiology [14]. Also, the patient will return to the normal life activity few hours after operation [15,16]. The recovery staff should asses the patient's condition and must be satisfied that the patient recovered safely from anesthesia and all of the vital signs were normal such as pulse rate and blood pressure after that the patient can go to the ward [17]. There are many previous studies revealed that many risk factors lead to delayed recovery of the patients after anesthesia.

Patients factors

Age; geriatric patients or elderly patients are more sensitive to anesthetic drugs. They will return their consciousness slowly because of decline in their central nervous system function. Previous study showed that request for opioids decreased by more than 50% in elderly patients. The effect of midazolam in old patents can depress mental function and lead to delayed recovery and delayed hospital discharge [17,18].

But another study revealed that pediatric age also reported delayed recovery because of slow drug metabolism and heat loss resulting in hypothermia. Genetic factors related to the patient affect the response and disposition of the drugs especially, anesthetic drugs, and play important roles in drug adverse effect determination [4]. A study by Apfelbaum et al. revealed that male have more delayed recovery than female 1.4 times [19].

Previous study shown that obese patients more likely to get delayed recovery because of the increased fat, which require high drug concentration in plasma than a normal body weight patient [17].

Chronic or previous cardiac and respiratory diseases require more specific pre- and post-operative care and reducing anesthetic drug doses to avoid complications and delayed recovery. Previous study demonstrated that lung diseases, congestive heart failure and renal failure were the most risk conditions which lead to delayed recovery from anesthesia [20]. Also, hypothyroid patients have 1-10-times delayed recovery than the normal persons [21].

Psychological and central nervous system diseases may cause post-operative somnolence. Patient with Parkinson's disease is more likely to get delayed recovery because of low brain dopamine concentration [18]. A study by Sahoo et al. revealed that patients with long term seizure have delayed recovery more than 3.5 hours when medicated by Rocuronium and Phenytoin [22]. Pain after operation plays important role to speed up recovery. Also, the recovery becomes delayed when analgesics given to the patients [20].

Drug factors

Drug administration commonly can cause delayed recovery after over dose of anesthetic agents [23]. Drug dosage has different effects on the same patient, a high drug dose in general anesthesia can cause delayed recovery [24].

Previous study revealed that injection of benzodiazepines and opioids, also non-anesthetic drugs such as antihypertensive, antihistamines, some antibiotics, immunosuppressant's, and alcohol cause depression of central nervous system which led to delayed recovery [25].

Drug interaction is another factor can cause delayed recovery resulting in hypo/hypertension and coma after operation. Many drugs interact with the neuromuscular or anesthetic agents like diuretics, oral contraceptive pills, aminoglycosides, which cause prolonged neuromuscular junction block [26].

Duration of anesthetic used

Anesthetic drug administration techniques responsible for the duration of awareness after anesthesia [27]. A study by Garg et al. revealed that delayed recovery after long time laparoscopic procedure for hernia repair by general anesthesia occurs [28]. Also, repeated administration of anesthetic agents for long term surgery is another cause for delayed recovery [29]. Early respiratory depression caused by Neuraxial opioids leads to delayed recovery. Previous study shown that benzodiazepines combined with opioids leads to respiratory depression and causes coma that prolong recovery [30]. Various intravenous anesthetic agents determine the time of recovery, previous study demonstrated that duration of consciousness depends on the agents were given, Propofol has a short half-life about 10-70 minutes which is metabolized by liver has fast recovery [31].

Metabolic causes

Hypo and hyperglycemia play an important role in delayed recovery, diabetic ketoacidosis may cause delayed recovery [32]. On the other hand, hypoglycemia was another factor results due to prolonged fasting before operation which leads to delayed recovery [21].

Imbalance of electrolytes

Electrolytes change and acid-base disturbance in pre-operative period were the most

important factors, which lead to delayed recovery after anesthesia. A study by Razvi et al. shown that sodium loss by the kidneys resulting in hypernatremia can cause delayed recovery [33].

In old aged patients, hypothermia, if suspected temperature less than 33 °C with the anesthetic drugs may reduce minimum alveolar concentration value and lead to decreased drug metabolism and delayed recovery occurs [21]. But another study revealed that temperature more than 40 °C can cause loss of consciousness, which leads to delayed recovery [4]. Post-operative respiratory dysfunction leads to muscular dysfunction and hypoxia resulting in hypoxemia and affects cerebral functions and may cause damage of the cerebral cells followed by delayed recovery when $PaCO^2$ raised above 90-120 mm Hg [29]. Complications of the central nervous system or neurological disorders during operation such as hemorrhage, hematoma and cerebral infarction also, long period of hypoxemia may cause cerebral dysfunction and lead to delayed recovery [28]. During improperly sitting position of patient hypo perfusion can occur and leads to obstruction of blood flow in the carotid and vertebral circulation in the neck of the patient resulting in hypoxia and leads to delayed recovery [17]. Adequate analgesia was necessary for post-operative period to return the patient to normal activities; the aim of this procedure is to reduce pain. A study by Ramsay conducted that post-operative analgesia leads to low incidence of complications and decreases the delayed recovery status [34].

The aim of this study is to determine the factors from the patients and other things related to delayed recovery after giving anesthesia. Reducing those factors plays important role to enhance patient quality life.

Methods

During the study period, 286 patients were observed in Shar Medical Hospital & Cardiac Center in Sulaimani city between November 2019 and March 2020. The study population includes those patients undergoing admitted to the hospital for Surgery. A structured data sheet was used to collect the information's from the patients. Demographic characteristics of the patients, history of previous diseases, medications and type of surgery were taken. Anesthesia drugs include: Propofol, Benzodiazepines, Opioids (Fentanyl or Sufentanil), Atracurium, Thiopental and Scolin. Post-operative evaluation and observation were collected for delayed and normal recovery after anesthesia. Data analyzed by SPSS (Statistical Package for Social Sciences) software version 22. Frequencies, percentage, mean were used for statistical correlations. Chi-square ($\chi 2$) test was used for significant association between the variables. P-value less than 0.05 means significant relationship were obtained between variables.

Results

The present study includes 286 patients undergoing surgery, 195 (68.91%) of them were with normal recovery after anesthesia and 91 (31.81%) were with delayed recovery after anesthesia (Table 2).

Table 2. Patients distribution according delayed recovery

Recovery	No.	%
Delayed recovery	91	31.81
Normal recovery	195	68.19
Total	286	100

From the 286 patients, 180 (62.94%) were males, in which 66 of them were have delayed recovery and significant relation was found p=0.005. Patients from rural area are more

frequent 174 (60.84%). The mean age was 42.8 ± 21.6 years started from 6 months to 91 years-old. Most of the patients were >50 years-old 126 (44.05%) followed by <18 years old and there was significant relation between age and delayed recovery of consciousness p=0.01. delayed recovery was found among overweight more frequently than the underweight and normal weight (51 from 73 for overweight, 33 from 45 and 7 from 168, respectively). Also, significant relationship was found p=0.001. From 86 smoker patients 62 of them have delayed recovery from consciousness and the association between smoker and recovery is statistically significant p=0.003. Also, for alcoholic patients the significant relationship was found p=0.03 (Table 3).

Table 3. Patients characteristics in relation to recovery

Variable		No. (%)	Delayed recovery No.	p-value
Gender	Male	180 (62.94)	66	0.005
	Female	106 (37.06)	25	
Residency	Urban	112 (39.16)	36	0.6
	Rural	174 (60.84)	55	
Age group	< 18	104 (36.37)	27	0.01
	18 – 50	56 (19.58)	10	
	> 50	126 (44.05)	54	
Body weight	Underweight	45 (15.74)	33	0.001
	Normal	168 (58.74)	7	
	Overweight	73 (25.52)	51	
Smoker	Yes	86 (30.07)	62	0.003
	No	200 (69.93)	29	
Alcohol use	Yes	31 (10.84)	22	0.03
	No	255(89.16)	69	
Total		286 (100)	91	

Table 4 shows diseases in relation to delayed recovery from anesthesia. In this table there were significant association between recovery of consciousness after surgery / anesthesia and diabetes mellitus (p=0.001), cerebrovascular accident (p=0.03), history of myocardial infarction (p=0.002), hyperlipidemia (p=0.003), neurological diseases (p=0.001), hypothyroidism (p=0.001), history of previous surgery (p=0.05) and mental disorders (p=0.001). But for hypertension, renal diseases, liver diseases and hyperthyroidism the significant association was not found (p=0.2, p=82, p=0.16 and p=0.9 respectively).

From 286 patients, 162 (56.65%) of them have anxiety before operation, in which 45 of them suffered from delayed recovery. 171 (39.21%) of them with short time fasting; significant relationship was not found between anxiety and short time fasting with delayed recovery (p=0.86 and 0.64). But the majority of patients who used analgesia during surgery 195 (68.19%) have significant association with delayed recovery (p=0.03) (Table 5).

Table 4. Distribution diseases related to delayed recovery from anesthesia

Diseases		No. (%)	Delayed recovery No. (%)	p-value
Diabetes mellitus	Yes	62 (21.68)	62	0.001
	No	244 (78.32)	29	
Hypertension	Yes	71 (24.83)	44	0.2
	No	215 (75.17)	47	
Cerebrovascular accident	Yes	12 (4.2)	8	0.03
	No	274 (95.8)	83	
History of myocardial infarction	Yes	16 (5.6)	12	0.002
	No	270 (94.4)	79	
Hyperlipidemia	Yes	122 (42.66)	86	0.003
	No	164 (57.34)	5	
Neurological diseases	Yes	14 (4.9)	9	0.001
	No	272 (95.1)	82	
Renal diseases	Yes	48 (16.79)	12	0.82
	No	238 (83.21)	49	
Liver diseases	Yes	9 (3.15)	2	0.16
	No	277 (96.85)	89	
Hypothyroidism	Yes	49 (17.14)	33	0.001
	No	237 (82.86)	58	
Hyperthyroidism	Yes	14 (4.9)	2	0.9
	No	272 (95.1)	89	
History of previous surgery	Yes	65 (22.73)	24	0.05
	No	221 (77.27)	67	
Mental disorders	Yes	6 (2.1)	4	0.001
	No	280 (97.9)	87	
Total		286 (100)	91	

Table 5. Distribution of risk factors

Risk factors		No. (%)	Delayed recovery No. (%)	p-value
Anxiety	Yes	162 (56.65)	45	0.86
	No	124 (43.35)	45	
Short time fasting	Yes	115 (40.21)	12	0.64
	No	171 (39.21)	79	
Analgesia uses during surgery	Yes	195 (68.19)	72	0.03
	No	91 (31.81)	19	
Total		286 (100)	91	

Table 6 shows history of drug administration in relation to recovery from anesthesia. The association between drug administration and delayed recovery from anesthesia were found for Aspirin/Clopidogrel, Beta blockers, Diuretics, Insulin or other ant diabetic drugs, Calcium channel blockers, Angiotensin and Non-steroidal anti-inflammatory drugs (p=0.04, 0.001, 0.02, 0.001, 0.001, 0.02 and 0.05 respectively).

Table 6. History of drug administration in relation to recovery

Drugs	No.	Delayed recovery	Normal recovery	p-value
Aspirin/clopidogrel	112	69	22	0.04
Beta blockers	132	87	4	0.001
Diuretics	86	65	26	0.02
Insulin and anti-diabetic drugs	92	81	10	0.001
Calcium channel blockers	82	12	79	0.001
Angiotensin	46	5	86	0.02
Non-steroidal anti-inflammatory drugs	97	66	25	0.05

Discussion

In our study we found that male, old and pediatric age groups, overweight patients, cigarette smokers and alcoholics were high risk factors for delayed recovery. Similar result was found in the previous study which is conducted by Faritous et al. [35]. Previous study demonstrated that anesthetic drugs are removed or metabolized by 50% in old age patients because of decreasing plasma protein binding capacity resulting in increasing level of free plasma concentration of anesthetic drugs [17]. Regarding gender, female have lower sensitivity to the hypnotic effects of drugs especially, anesthetic drugs because of this, female quick recover from anesthesia [36]. Also, overweight patients have increased amount of fat in their bodies, who require high doses of drugs to get acting in plasma protein than the normal body weight persons [37].

In the current study, significant association between recovery of consciousness after surgery/anesthesia and diabetes mellitus, cerebrovascular accident, history of myocardial infarction, hyperlipidemia, neurological diseases, hypothyroidism, history of previous surgery and mental disorders were shown. The previous study is nearly consistent with the current study which is reported that cerebrovascular accidents and neurological disorders are related to the delayed recovery [38].

In the current study, significant association was found between delayed recovery and previous drug usage by the patients. When these drugs (antihypertensive, antidiuretics, etc.) are used for prolonged periods especially, in elderly people, will interact with anesthetic drugs and this leads to delayed recovery because the metabolism of the anesthetic drugs will be delayed. This interaction between these drugs will cause toxic effects on the circulatory, respiratory and nervous system in the body [22].

In the current study, patients with diabetes mellitus are more likely to have delayed recovery after anesthesia. Previous study agreed with this study and mentioned that high level of blood glucose causes osmotic diuresis and dehydration in diabetic patients. The dehydration can cause drowsiness because of acidosis resulting in delayed recovery from consciousness [27].

The study shown that use of analgesics during operation was the common reason for delayed recovery. A study by Rastogi et al. revealed that interaction between analgesics and opioids was the commonest reason which can cause delayed recovery [39].

Regarding, respiratory system, we found that patients with respiratory diseases have significant relations with delayed recovery. Similar result was found in the previous study which revealed that pulmonary diseases result in hypoxemia and will decrease cerebral function and causing cell damage [29].

Smoking may cause hypoxia in the cerebrum and destroy the function of the brain, which cause cerebral cell damage due to free radicals' accumulation and production of lactic acid. Also, smokers need more doses of muscle

relaxants during operation to maintain neuromuscular blockade, which will lead to delayed recovery [40].

In conclusion, old age, male, obese patients and cigarette smokers were high risk factors for delayed recovery. Diabetes mellitus, cerebrovascular accident, history of myocardial infarction, hyperlipidemia, neurological diseases, history of previous surgery and mental disorders also were common risk factors for delayed recovery from anesthesia. We recommend that pre and postoperative patient care can ensure the patient safety.

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Conflict of interest

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Zizyphus spina christi Effect on Pentylenetetrazole-Induced Kindling

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Abstract

Background Epilepsy is a common disorder of brain that is not well controlled via drugs available for it. Gephyrin, a well-known inhibitory synaptic regulator, has a role in epilepsy. Additionally, significant development of oxidative stresses tends to be involved in epileptic seizure pathophysiology that giving rise to neuronal cell death. *Zizyphus spina christi* is a vastly available Iraqi tree and its leaves have several beneficial effects on central nervous system level.

Objective To investigate the possible neuroprotective effect of crude *Zizyphus spina christi* extract in kindling model induced.

Methods The removed brains of forty albino male mice from four separate groups were exposed to immunohistochemical test for gephyrin, glutathione, and NeuN expressions.

Results The data found that pretreatment with *Zizyphus spina christi* crude extract shows highly significant difference (P value <0.001) in immunohistochemical scores for all studied parameters as compared with those being of pentylenetetrazole-kindled group.

Conclusion These findings suggest that crude *Zizyphus spina christi* extract of leaves is effective in protection against kindling that induced via pentylenetetrazole when applied orally for male mice.

Keywords Gephyrin; glutathione; kindling; NeuN; pentylenetetrazole; *Zizyphus spina christi*

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List of abbreviations: AEDs = Antiepileptic drugs, CNS = Central nervous system, DPX = Dibutylphthalate polystyrene xylene, GABA = Gamma aminobutyric acid, GSH = Glutathione, HPLC = High performance liquid chromatography, IHC = Immunohistochemistry, NeuN = Neuronal Nuclei, NMDA = N-methyl-D-aspartate, PTZ = Pentylenetetrazole, ZSC = *Zizyphus spina christi*

Introduction

Epilepsy is a complex etiology disorder of brain that makes individual more susceptible to having unprovoked and repeated seizures. Gephyrin is the base scaffolding protein present in miscellaneous brain regions and a master regulator of neuronal activity at inhibitory sites [(1)]. Notably, one of pathological mechanisms of epileptic seizure is production of massive reactive oxygen species, which is responsible for death of neuronal cells [(2)].

The antagonist of gamma aminobutyric acid (GABA) receptor, pentylenetetrazole (PTZ), is utilized experimentally as a chemoconvulsant where its frequent sub-convulsant doses [(3)] resulting in kindling, which is an animal model applied for investigation of the pathophysiology of epilepsy and inspection of anti-epileptic drugs [(4)].

Zizyphus spina christi "Sidr" is a significant plant species and one of well-known Iraqi

trees. Different portions of this plant have been widely utilized in folk medicine.

On the central nervous system (CNS) level, some of laboratory studies have shown that the extract of *Zizyphus spina christi* has antianxiety, neuroprotective as well as central inhibitory effect on rodents [5-8].

Thus, current study aimed to investigate the neuroprotective effect of crude *Zizyphus spina christi* extract against PTZ-induced kindling.

Methods

Animals and experimental design

Forty albino male mice weighing (27.6-34.8) grams were involved in the current study. Mice were housed in standard cages in a room with controlled environment and had free access to food and water. The forty male mice utilized in this study were randomly allocated into the following four groups of ten mice each: Group I served as normal control and got only distilled water. Group II, III, and IV were exposed to induction of kindling via intraperitoneal PTZ injection of 35 mg/kg dose every second day for 23 days. Thirty minutes post each PTZ injection group III and IV were received diazepam (2 mg/kg, orally) and crude extract of *Zizyphus spina christi* leaves (50 mg/kg, orally) respectively.

PTZ induced-kindling

PTZ were purchased from Sigma-Aldrich, USA; its solution was freshly prepared at the day of administration by dissolving 2 mg of it in 1 ml of 0.9% saline [4,9]. For kindling induction, a 35 mg/kg of PTZ was applied intraperitoneally every second day. After each PTZ injection, mice were observed for thirty minutes to record the seizure score [4]. In the present study, a total of 12 injections (23 days) were needed to acquire kindling.

Preparation of brain tissues

At 24th day of study, the intact brain of mice was rapidly removed under deep anesthesia and kept in (10%) formalin containing cup for immunohistochemical evaluation.

Immunohistochemical evaluation of gephyrin, glutathione, and NeuN expression in brain tissues

The 5 µm thicknesses of tissue blocks were affixed on adhesive positively charged slide, dewaxed; rehydrated; put in retrieval solution bath; heated; and treated with peroxidase block for 20 minutes. The slides were rinsed; with wash buffer; treated with protein block; incubated; exposed to diluted antibody; and incubated again for overnight. At the second day, the slides were washed; incubated with horse radish peroxidase polymer; washed with washing buffer. Then, the slides subjected to 3, 3'-diaminobenzidine (DAB) chromogen/substrate reagent; incubated; washed with buffer wash; immersed in Meyers' hematoxylin; washed again; dehydration using ascending grades of 50%, 70%, 90%, and absolute ethanol; immersed in two changes of xylene for ten minutes; mounted with DPX and then covered slipped and left to dry. Immunostaining for expression of each of gephyrin, glutathione, and NeuN were evaluated were based on the percentage of positively stained cells per section 10: Score 0: less than 5%; score 1: 5-25%; score 2: 26-50%; score 3: more than 50%.

Statistical analysis

Data were presented as median, 25% percentile, 75% percentile, mean as well as standard deviation and analyzed using SPSS 20.0. Mann-Whitney U test utilized and the statistical significance was considered as $P \leq 0.05$.

Results

Effect of crude *Zizyphus spina christi* extract on immunohistochemical expression of gephyrin

As showed in table 1 and figure 1, IHC scores and brain expression in PTZ-kindled group declined significantly in median and mean ± SD (1.00, 0.57 ± 0.53) as compared with normal group. In contrast, IHC scores of diazepam (2.00, 2.43 ± 0.53) and crude extract of *Zizyphus spina christi* (2.00, 2.29 ± 0.48)

previewed highly significant increment (P ≤ 0.001) in relation to those being PTZ-kindled group.

Table 1: Immunohistochemical scores of gephyrin

Statistics	Normal control (N=10)	Crude extract (N=10)	Diazepam (N=10)	PTZ (N=10)
Median	2.00	2.00 NS *	2.00	1.00
25% Percentile	1	2	2	0
75% Percentile	3	3	3	1
Mean	2.00	2.29	2.43	0.57
SD	0.57	0.48	0.53	0.53

Mann-Whitney test
NS, not significantly different from corresponding diazepam score, P > 0.05
*, Highly significant different from corresponding PTZ score, P ≤ 0.001

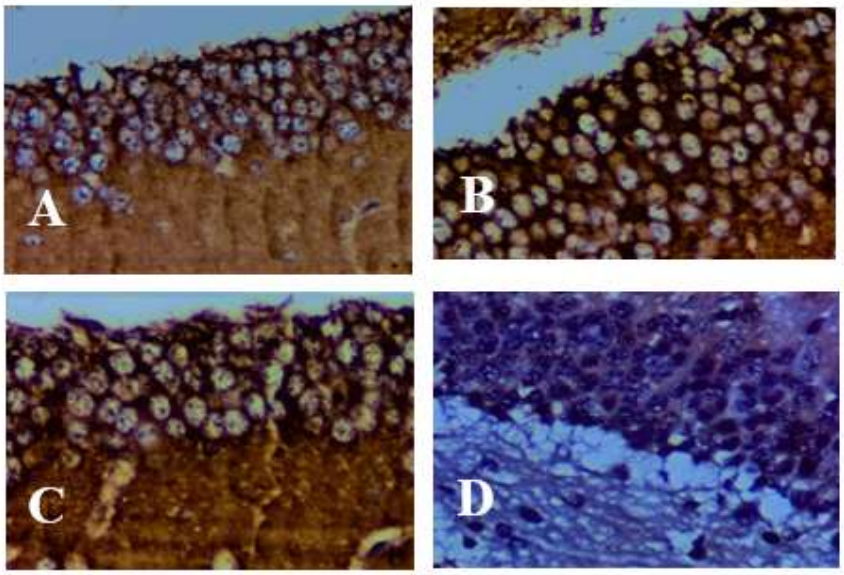

Figure 1. Immunohistochemical expression (40X) of gephyrin for (A) distilled water, (B) crude extract of *Zizyphus spina Christi*, (C) diazepam, (D) pentylentetrazole-kindled tissue

Effect of crude *Zizyphus spina christi* extract on immunohistochemical expression of glutathione

IHC scores and brain expression in PTZ-kindled group declined with great statistically significant (P ≤ 0.001) in median and mean ± SD (1.00, 0.57 ± 0.53) as compared with apparently healthy group. Contrariwise, IHC scores of diazepam (3.00, 2.71 ± 0.48) and crude *Zizyphus spina christi* extract (3.00, 2.86 ± 0.37) previewed highly marked increment (P ≤ 0.001) in median and mean ± SD of glutathione when compared with those of kindled group. All data are presented in table 2 and illustrated in figure 2.

Table 2: Immunohistochemical scores of glutathione

Statistics	Normal control (N=10)	Crude extract (N=10)	Diazepam (N=10)	PTZ (N=10)
Median	2.00	3.00 NS *	3.00	1.00
25% Percentile	2	2	2	0
75% Percentile	3	3	3	1
Mean	2.43	2.86	2.71	0.57
SD	0.53	0.37	0.48	0.53

Mann-Whitney test
NS, not significantly different from corresponding diazepam score, P > 0.05
*, Highly significant different from corresponding PTZ score, P ≤ 0.05

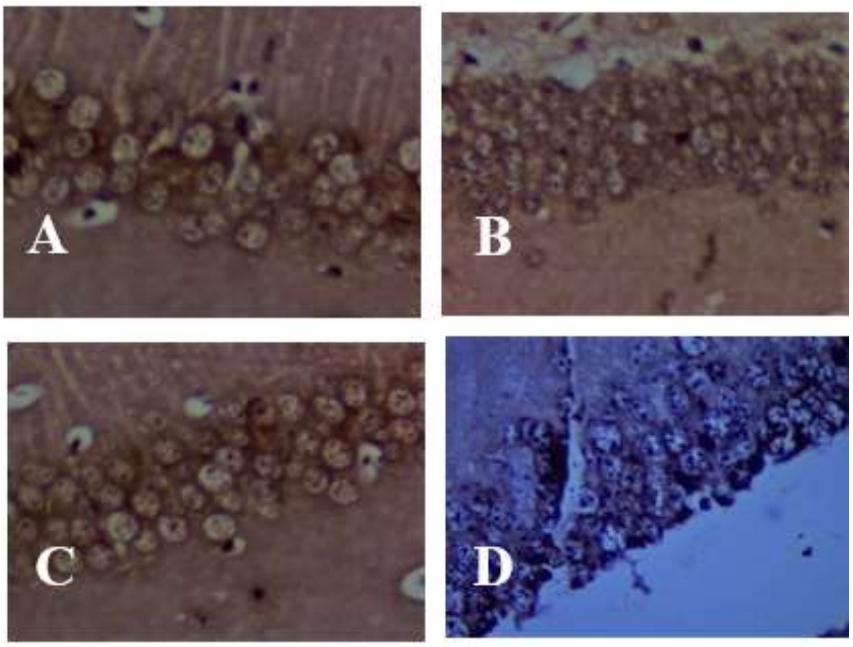

Figure 2. Immunohistochemical expression (40X) of glutathione for (A) distilled water, (B) crude extract of *Zizyphus spina Christi*, (C) diazepam, (D) pentylentetrazole-kindled tissue

Effect of crude *Zizyphus spina christi* extract on immunohistochemical expression of NeuN

IHC scores and staining are shown in table 3 and figure 3 respectively. There was highly significant suppression (P ≤ 0.001) of NeuN expression in brain tissues of PTZ-kindled group with median and mean ± SD of (1.00, 0.67 ± 0.50) in relation to normal group. In the opposite manner, the IHC scores of diazepam group (3.00, 2.57 ± 0.53) and crude *Zizyphus spina christi* extract group (2.00, 1.71 ± 0.48) were increased remarkably (P ≤ 0.001) as compared with corresponding in PTZ-kindled group.

Table 3. Immunohistochemical scores of NeuN

Statistics	Normal control (N=10)	Crude extract (N=10)	Diazepam (N=10)	PTZ (N=10)
Median	3.00	3.00 NS *	3.00	1.00
25% Percentile	2	2	2	0
75% Percentile	3	3	3	1
Mean	2.89	2.71	2.57	0.67
SD	0.33	0.48	0.53	0.5

Mann-Whitney test

NS, not significantly different from corresponding diazepam score, P > 0.05

*, Highly significant different from corresponding PTZ score, P ≤ 0.001

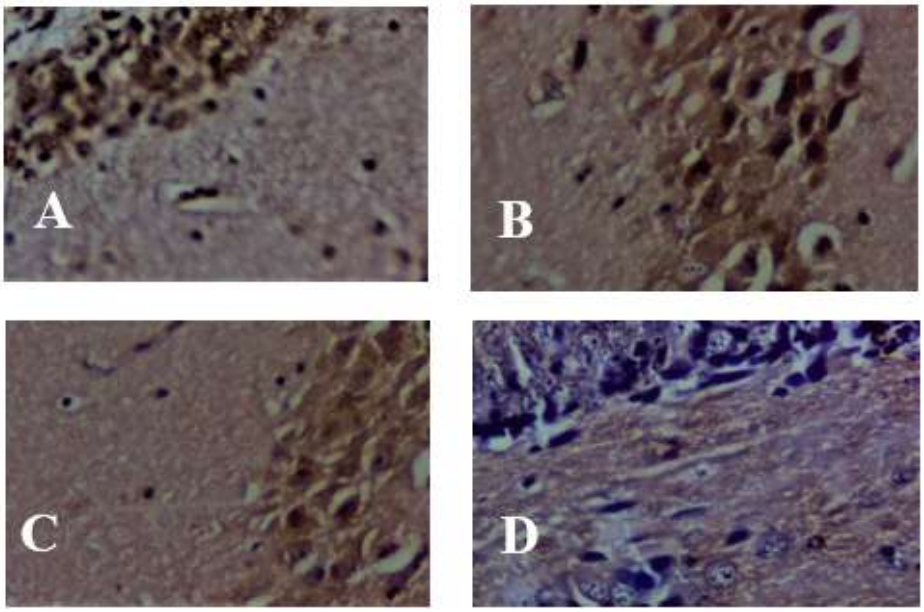

Figure 3. Immunohistochemical expression (40X) of NeuN for (A) distilled water, (B) crude extract of *Zizyphus spina Christi*, (C) diazepam, (D) pentylentetrazole-kindled tissue

Discussion

The kindling models are more look alike human epilepsy syndromes and have been widely utilized to reveal the disease modifying agents [11]. In PTZ-induced kindling, repeated administration of sub-convulsive doses of PTZ, a pro-convulsant, resulting in development of seizure activity and ultimately leads to occurrence of primary generalized tonic-clonic seizures [12,13]. PTZ originally acts as an antagonist on GABA receptor. Owing to presence of miscellaneous bioactive compounds such as alkaloids, flavonoids, steroids, phenols and saponins in *Zizyphus spina christi* [14,15], it was disclosed that its extract possess diverse pharmacological effects against the brain disorders such as anti-anxiety effect [5], attenuation of memory impairment induced via scopolamine [16], and neuroprotection against cerebral ischemia [6].

The present work found that the effects of pretreatment with crude extract exhibited a remarkable gephyrin expression in brain tissues similar to those of diazepam group as compared to those of PTZ-kindled mice. The finding reinforced the sedative effect of *Zizyphus spina christi* leaf extract and it is in harmony with that of previous study [5]. This

sedative nature of *Zizyphus crude extract* may be related to its peptide, cyclopeptide alkaloids, and saponin contents. The possible mechanism of alkaloids action may be through modulation of systems of neurotransmitter involving GABA receptors [17]. Meanwhile, the underlying mechanism of saponins might be related to its targeting GABAA receptors [18]. In the present work, the glutathione and NeuN expression witnessed a remarkable increment in mice pretreated with crude extract of *Zizyphus spina christi* leaf in comparison with that being in PTZ-induced kindled mice. This might be attributed to the presence of the alkaloids, saponins and flavonoids compounds in crude extract. In fact, epilepsy associated with N-methyl-D-aspartate (NMDA) receptors activation that giving rise to oxidative stress production and consequently damage of neurons and even death. The possible mechanism of action of phyto-constituents of plant extract could be due to modulation of neurotransmitter system as well as their anti-oxidant effects [16,17,19,20].

In conclusion, hydro-alcoholic extract of *Zizyphus spina christi* exhibited protective effects against PTZ-induced kindling via upregulation of gephyrin, glutathione as well as NeuN expression. However, further studies are required to investigate its therapeutic effects and to detect its underlying mechanisms.

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Author contribution

Dr. Al-Humaidhi: designed and conducted this research; Dr. Abd and Dr. Ghazi supervised the study and participated in its interpretation.

Conflict of interest

Authors declare no conflict of interest.

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Acute Severe Icteric Hepatitis Caused by the Novel Corona virus: A Case Report

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Abstract

Background Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) cases are being frequently reported nowadays and the objective of this clinical case report is to highlight its unique presentation as acute icteric sever hepatitis.

Case report A 28-year-old female patient presented with 3 days history of fever, abdominal pain, nausea, vomiting and jaundice. Lab investigations revealed positive COVID-19 reverse transcription-polymerase chain reaction test along with picture suggestive of acute severe hepatitis (Aspartate aminotransferase; 2772 U/L (N: <32), Alanine transaminase; 2522 U/L (N: <33), Alkaline phosphatase; 172 U/L (N: 35-104), direct bilirubin 4.2 mg/dl (N: 0-0.3)). The patient was admitted, monitored and started on supportive therapy for 5 days and was discharged well for outpatient follow-up.

Conclusion Requesting liver function test for COVID-19 patients who presents with gastrointestinal symptoms is a crucial decision that can guide us with the management of the case since many drugs used in the treatment of the new SARS-CoV-2 infection are hepatotoxic and should therefore be used with caution.

Keywords Hepatitis, COVID-19, SARS-CoV-2, COVID-19 hepatitis, icteric hepatitis

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List of abbreviations: aPTT = Activated partial thromboplastin time, ALT = Alanine transaminase, ALP = Alkaline phosphatase, AST = Aspartate aminotransferase, CRP = C-reactive protein, CMV = Cytomegalovirus, ESR = Erythrocyte sedimentation rate, INR = International normalized ratio, PCR = Polymerase chain reaction, PT = Prothrombin time, RT-PCR = Reverse transcription-polymerase chain reaction, SARS-CoV-2 = Severe acute respiratory syndrome coronavirus 2

Introduction

Coronaviruses are large, positive single-stranded RNA viruses with envelope which infect both humans and animals. They were named coronaviruses (Latin: corona = crown) because of their shape as they consist of spherical virions with a core shell and outer projections resembling a solar corona. Four sub-families of coronaviruses exist: alpha-, beta-, gamma- and delta-coronaviruses. Alpha- and beta-coronaviruses was found to be originated from mammals, particularly from bats while gamma- and delta-viruses originate from birds and pigs. The genome size ranges from 26 kb to 32 kb. Among the different subtypes of coronaviruses that can infect humans, the beta-coronaviruses may cause fatal disease complications, whereas alpha-coronaviruses maybe asymptomatic or cause mild infection. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) belongs to beta-coronaviruses specifically, the B lineage, and is closely related to the SARS-CoV virus [(1)]. SARS-CoV-2 is 96%

similar at the whole-genome level to the coronavirus found in a bat [2].

SARS-CoV-2 was found to be successfully transmitted from animals to humans in Wuhan, China, in a seafood market. The median incubation period was found to be 3 days (Range was from 0 to 24) [1]. Patients' clinical manifestations included fever, non-productive cough, dyspnea, myalgia, fatigue, normal or decreased leukocyte counts, and radiographic evidence of pneumonia. Organ dysfunction (eg, shock, acute respiratory distress syndrome (ARDS), acute cardiac injury, and acute kidney injury) and death can occur in severe cases [3].

Around 50% of COVID-19 patients presenting to the hospital are reporting digestive symptoms. Rarely, digestive symptoms may occur without any respiratory symptoms. Laboratory tests have shown that patients with digestive symptoms have higher liver tests and prolonged coagulation profile as compared to those without digestive symptoms [4]. On the initial presentation of COVID-19, the prevalence of abnormal liver function tests is still undetermined. Current approaches to COVID-19 therapies generally fall into two categories: antivirals — which prevent the virus from multiplying — and immune modulators — which help the immune system to fight the virus or stop it from overreacting dangerously. Some potential therapies act in a different way or via multiple mechanisms. In the EU, remdesivir is now licensed for the treatment of COVID-19 in adults and adolescents with pneumonia requiring supplemental oxygen [5]. This study reports a case of COVID-19 infection presenting solely as acute, icteric hepatitis.

Case presentation

A 28-year-old healthcare worker female with negative past medical history presented to our emergency department complaining of nausea, vomiting, epigastric pain, dark urine, pale stool, and jaundice for 3 days. Further questioning revealed that these symptoms were preceded by fever, which lasted for 3 days and was relieved by Paracetamol with no respiratory symptoms.

At the time of presentation, her temperature was 36.8, there was epigastric tenderness and jaundice. There was no hepatomegaly or splenomegaly. Her chest examination and x-ray were normal.

The laboratory results showed the following: COVID-19 polymerase chain reaction (PCR) test was positive, white blood cell count 3.22 * 10^3/µL (N: 4.0-10.0), lymphocyte count 1.22 * 10^3/µL (N: 1.0-3.0), aspartate aminotransferase (AST) 2772 U/L (N: <32), alanine transaminase ALT 2522 U/L (N: <33), alkaline phosphatase (ALP) 172 U/L (N: 35-104), albumin 4.2 g/dL (N: 3.5-5.2), total bilirubin 4.3 mg/dL (N: up to 1.2), direct bilirubin 4.2 mg/dL (N: 0-0.3), indirect bilirubin 0.1 mg/dL (N: 0-0.8), D-dimer 1.4 mg/L (N: <0.5), international normalized ratio (INR) 1.16 (N: 1-1.2), prothrombin time (PT) 14.5 s (N: 11-15), activated partial thromboplastin time (aPTT) 33.2 s (N: 25-37), erythrocyte sedimentation rate (ESR) 22 mm/1st hr (N: 0-20), C-reactive protein (CRP) 9.8 mg/L (N: <5.00), urea 23 mg/dL (N: 17-49), creatinine 0.5 mg/dL (N: 0.5-0.9). Screening for virology including: hepatitis viruses (A, B, C and E), Herpes virus, Cytomegalovirus (CMV), Human immunodeficiency virus (HIV) and Epstein-barr virus (EBV) were negative. Tests for autoimmune diseases and Wilson's disease came back negative as well. Abdominal ultrasound with Doppler study showed non-specific findings suggestive of acute hepatitis with normal vasculature.

Patient was admitted for 5 days and was given intravenous fluid 500 ml *4, Odanstron amp. 8 mg *3, Metoclopramide amp. 10 mg *3, Esomeprazole vial 40 mg *1. On the fourth day of admission, oral feeding started as the symptoms and lab results showed improvement (figure 1). On the following day the patient was discharged to out-patient clinic for follow up.

ALT = Alanine transaminase, AST = Aspartate aminotransferase, ALP = Alkaline phosphatase

Figure 1. Liver function test during the disease course

Discussion

In a study done in Hubei, China, 204 patients with COVID-19 were taken where most of them presented with fever or respiratory symptoms. About 18.6% presented with a gastrointestinal symptom like abdominal pain, vomiting and diarrhea. In six cases, there were gastrointestinal symptoms without any respiratory symptoms [(4)]. In our report, the patient presented with gastrointestinal symptoms without the involvement of other systems.

Investigations showed that the cause of gastrointestinal symptoms in our case were due to acute severe hepatitis caused by the SARS-CoV-2. This was supported by the documented positive COVID-19 RT-PCR test and the exclusion of other causes of acute sever hepatitis.

In a case report, a 59-year-old woman with a medical history of well-controlled human immunodeficiency virus (CD4 499 and viral load undetectable), hypertension, hyperlipidemia, Graves' disease, and a left facial paralysis secondary to previous actinomycosis infection, presented with acute, non-icteric hepatitis. Her laboratory results were as follow: serum bilirubin 0.6 mg/dL (N: up to 1.2), AST 1230 IU/L (N: <50), ALT 697 IU/L (N: <50), alkaline phosphatase 141 IU/L (N: <125) [(6)], whereas our patient had no significant past medical history and laboratory results revealed higher bilirubin, ALT, AST and ALP.

In the previous study, the patient received hydroxychloroquine 200 mg twice a day for 5 days. She got well and was discharged home after 8 days from hospital admission [(6)], while our patient received supportive treatment only and was discharged well after 5 days of admission.

The proposed mechanisms of liver injury caused by SARS-CoV-2 is thought to be either due to the angiotensin-converting enzyme 2 (ACE2) mediated hepatic injury, the destruction caused by cytokine storm or drug induced liver injury. Since our patient did not receive any medication except for low dose of Paracetamol tablet (total of 1.5 g/day for 3 days) and there was no evidence of cytokine storm, the main culprit of liver injury in our patient is the cytopathic effect of COVID-19 virus mediated by ACE2 receptors found in the liver and bile ducts.

In conclusion, it is important to recognize acute severe icteric hepatitis as a presentation of SARS-CoV-2 infection even in the absence of

respiratory symptoms and liver function test should be done especially in patients presenting with gastrointestinal symptoms. Since many of the drugs that are used in the treatment course of COVID-19 can exacerbate liver injury, it is of great importance to exclude hepatitis due to SARS-CoV-2 before commencing them.

Author contribution

Both authors contributed to the data collection, patient's follow-up and to the writing of the manuscript.

Conflict of interest

There are no conflicts of interest.

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Cervical Intraspinal Conduction Time by Magnetic Root Stimulation versus Conventional Electromyography in the Diagnosis of Cervical Radiculopathy

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Abstract

Background Cervical radiculopathy (CR) is a pathological procedure that encompasses root of the cervical nerve. Cervical disc herniation, accompanied by cervical spondylosis, is among the most common causes of radiculopathy. Clinical neurophysiology is a medical field focused mainly on assessing activity in the nervous system and muscles being an expansion of neurological assessment, using particular same anatomical localization criteria as clinical testing.

Objective To evaluate the sensitivity and specificity of conduction time of the intraspinal segment of peripheral motor neurons in the diagnosis of CR using cervical root magnetic stimulation and conventional electromyography (EMG) study.

Methods Fifty patients (15 males and 35 females) aged 46.02±9.76 years with reported CR were subjected to an electrophysiological examination and control group consisted of 50 (36 females and 14 males) healthy volunteers aged 36.24±12.09 years. Analysis of sensory and motor nerve conduction, conventional needle EMG test, and motor evoked potentials (MEPs) for all (recording abductor pollicis brevis (APB), abductor digiti minimi (ADM), biceps brachii (BB), deltoid muscles) was conducted to determine the peripheral nerves, muscles, sensory and motor pathways. The motor evoked potential (MEP) parameters studied for the median, ulnar musculocutaneous and axillary nerves include latency of the spinal root {peripheral motor conduction time} (PMCT).

Results During direct cervical root stimulation, the PMCT of the median nerve shows the highest specificity (59%) than that of the ulnar nerve (57%), while the intraspinal latency shows the same specificity for both nerves (57%). In comparison, PMCT by kimura formula recording ADM during direct stimulation of the cervical root have the highest sensitivity (56%) than that of PMCT by kimura formula recording APB. Additionally, the recording of direct cervical root stimulation amplitude after deltoid muscle showed the highest specificity and sensitivity (57.0%, 58.0% respectively). On the other hand, PMCT shows the highest specificity and sensitivity while the BB muscle is recoded (62.0%, 49.0%).

Conclusion Overall, the current motor evoked potential study shows abnormalities in less than 60% of patients with cervical radiculopathy relative to conventional EMG needle study abnormalities that reached 90%. The intraspinal latency of the median and ulnar show low sensitivity and specificity.

Keywords Cervical radiculopathy, electromyography, motor evoked potentials

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List of abbreviations: CV = Cervical radiculopathy, CNS = Central nervous system, CV = Conduction velocity, CMAP = Compound muscle action potential, EMG = Electromyography, Eps = Evoked potentials, SNAP = Sensory nerve action potential.

Introduction

Cervical radiculopathy (CR) is pathological process that involves cervical nerve root. This results from squeezing and

inflammation of the nerve root and or roots, which are at or adjacent to the cervical neural foramen. This happens periodically in 85 out of 100,000 people [1]. The cervical disc herniation followed by cervical spondylosis is most common cause for radiculopathy. It is less common than CR induced by intra spinal or extra spinal tumors, trauma with nerve root avulsion, synovial cysts, meningeal cysts, dural arteriovenous fistulae, or tortuous vertebral arteries [2,3]. CR is probable to take place without an identifiable cause. Upper limb nerve entrapment, shoulder disease, brachial plexus illnesses, and peripheral neuropathy are other situations that can simulate cervical radiculopathy; all ought to be incorporated in the distinctive diagnosis. The focus is about radiated pain that is subsequent to squeezing of (cervical nerve rootlets) by herniated disc element or pain that is linked with cervical spondylosis [4,5].

Clinical neurophysiology is a field of medical practice that fundamentally focused on evaluating function in the central nervous system, peripheral nervous system, and muscles as a complementary to neurological evaluation. It applies an identical anatomic origin of localization as clinical examination. It depends completely on the measurement of underway function that is either spontaneously, or in response to a defined stimulus by recording alterations in physiology as manifested by changes in electrical waveforms, electromagnetic fields, and force activities [6]. Nerve conduction readings are usually normal in radiculopathy; the electrodiagnosis is done by needle electromyography (EMG). Notwithstanding that nearly motor abnormalities are infrequently appreciated in radiculopathy, the further serious purpose to execute nerve conduction studies is to take away further conditions that may simulate radiculopathy particularly entrapment neuropathy and plexopathy [7].

Among the least accessible structures of the peripheral nervous system are roots. Late responses can be employed for estimating conduction along with the roots. Evaluation of F wave latency is proved to be very valuable in the field of clinical neurophysiology. It supplements routine nerve conduction study (NCS) especially for the proximal segment of the nerve which cannot be assessed via conventional NCS. F wave is essential to disorders that involve the nerve roots, plexuses and, evaluating patients with demyelinating polyradiculoneuropathies, and entrapment neuropathies [8]. However, the abnormalities, which are of late responses, are not specific to the diagnosis of radiculopathies because of their conduction over the entire length of peripheral motor pathways and lesions, which at any level, determine similar changes of late responses. Moreover, the measurement of the F wave latency is not very sensitive in revealing the slowing of the conduction that is along with the motor roots; this is because the slowing of conduction in the short segment of the compressed root is diluted in a much longer segment of normal conduction along all the remaining peripheral motor pathways. In root lesions, fibrillation potentials in the corresponding paraspinal muscles or myotomes may be documented by needle electromyographic studies; however, these electromyographic changes appear only after 2 to 3 weeks [9]. Because the proximal part of peripheral motor pathways is activated by noninvasive and painless magnetic paravertebral stimulation, it may be useful beside the aforementioned traditional techniques in the diagnosis of radiculopathies and in the assessment of those peripheral nerves, such as the thoracic nerves which cannot be directly explored by means of standard electrophysiological techniques [10]. The motor axons of peripheral nerves are activated by magnetic paravertebral stimulation at a point that is near their exit from the spine. This site is distal to that of root compression that is produced by disc disorders or spondylotic changes. Moreover, the latency of responses, which is evoked by magnetic

paravertebral stimulation, is normal in the case of root compression. The conduction time, along the proximal part of motor roots, can be estimated by subtracting the latency of motor responses, which is evoked via magnetic paravertebral stimulation, from the overall peripheral conduction time calculated by conventional EMG throughout a specific formula of Kimura [(F+M-1)/2] [(6)].

The goal of the present study is to evaluate the sensitivity and specificity of conduction time of the intraspinal segment of peripheral motor neurons in the diagnosis of CR using cervical root magnetic stimulation and conventional EMG study.

Methods

A case-control analysis was carried out for the period from August 2019 to February 2020 at the Neurophysiology Unit in Al-Imamein Al-Kadhimein Medical city. This research has been verified by the Institute Review Board (IRB) of the College of Medicine, Al Nahrain University. Informed consent for enrollment in the study was provided by each participant.

Subjects

Subjects enlisted in the study were split into two groups; control group and patients' group.

Control group

This group implicated of 50 (14 males and 36 female) apparently healthy volunteers, their mean age is 36.24±12.09 years; they were clinically examined by the neurosurgeon, orthopedician, rheumatologist to be included in the study.

Patients group

Fifty patients (15 males and 35 females), their mean age is 46.02±9.76 years, with certified CR diagnosis by a neurosurgeon, orthopedician, rheumatologist, or neurologist were substituted to enrolled in the study. Full history and the complete neurophysiologic study were achieved. Patients with a history of pacemaker or metal foreign body, epilepsy, pregnancy, peripheral neuropathy, brain surgery, stroke and cancer, chemo or radiotherapy were excluded from the study.

Methods

Neurophysiologic studies

The following neurophysiologic tests were done for all studied subjects:

1. Sensory nerve conduction study (SNCS) of the median, ulnar, and radial nerves (bilaterally).
2. Motor nerve conduction study (MNCS) and F wave studies of the median and ulnar nerves (bilaterally).
3. MNCS of musculocutaneous and axillary nerves (bilaterally).
4. Needle EMG studies of the deltoid, biceps brachii (BB), triceps, first dorsal interosseous (bilaterally).
5. Cervical root magnetic stimulation: this is accomplished by placing the center of the round coil over the C5, C7 spinous process for the commonly studied hand muscles (Abductor pollices brevis, Adductor digiti minimi) and often recommended for more proximal arm muscles (BB, deltoid) that may be recorded simultaneously by measurement of intraforaminal cervical spinal latency through the following methods:
   - Magnetic cervical root stimulation, peripheral motor conduction time PMCT = MEP (motor evoked potential) latency at the neuroforamina of upper limb muscles.
   - F wave technique, PMCT = (F + M - 1)/2
   - Intraforaminal cervical spinal latency = (F + M - 1)/2- MEP latency

(F: F wave latency; M: M-wave latency; 1, the time attributable to central delay at the level of spinal motor neurons).

Throughout the test procedures, the examination room temperature was set between 25-28 ^{o}C and skin temperature measured by a thermometer at the axilla and kept between 36-37 ^{o}C.

Instrumentation

For all electrodiagnostic tests, the following instruments were used: The EMG /EP machine,

Computerized EMG equipment (Micromed, 8-channel elecromyograph, B, model 1715, Italy) was used.

Electrophysiological Studies

Sensory nerve conduction study

In contrast to motor conduction studies, in which the compound muscle action potential (CMAP) reflects conduction along the motor nerve, neuromuscular junction (NMJ), and muscle fibers, in sensory conduction studies, only nerve fibers are assessed. Because most sensory responses are very small (usually in the range of 1-50 µV), technical factors and electrical noise assume more importance. For sensory conduction studies, the gain usually is set at 10-20 µV per division. A pair of recording electrodes (G1 and G2) are placed in line over the nerve being studied, at an interelectrode distance of 2.5-4 cm, with the active electrode (G1) placed closest to the stimulator. Recording ring electrodes are conventionally used to test the sensory nerves in the fingers. For sensory studies, an electrical pulse of either 100 or 200 ms in duration is used, and most normal sensory nerves require a current in the range of 5-30 mA to achieve supramaximal stimulation. This is less current than what is usually required for motor conduction studies. Thus, sensory fibers usually have a lower threshold to stimulation than do motor fibers. This can easily be demonstrated on yourself; when slowly increasing the stimulus intensity, you will feel the paresthesia (sensory) before you feel or see the muscle starts to twitch (motor). As in motor studies, the current is slowly increased from a baseline of 0 mA, usually in 3-5 mA increments, until the recorded sensory potential is maximized. This potential, the sensory nerve action potential (SNAP), is a compound potential that represents the summation of all the individual sensory fiber action potentials. SNAPs usually are biphasic or triphasic potentials. For each stimulation site, the onset latency, peak latency, duration, and amplitude are measured. Unlike motor studies, a sensory conduction velocity can be calculated with one stimulation site alone, by taking the measured distance between the stimulator and active recording electrode and dividing by the onset latency. No NMJ or muscle time needs to be subtracted out by using two stimulation sites [8].

Motor nerve conduction study and F wave

For motor conduction studies, the gain usually is set at 2-5 mV per division. Recording electrodes are placed over the muscle of interest. In general, the belly-tendon montage is used. The active recording electrode (also known as G1) is placed on the center of the muscle belly (over the motor endplate), and the reference electrode (also known as G2) is placed distally, over the tendon to the muscle. The designations G1and G2 remain in the EMG vernacular, referring to a time when electrodes were attached to grids (hence the G) of an oscilloscope. The stimulator then is placed over the nerve that supplies the muscle, with the cathode placed closest to the recording electrode. It is helpful to remember black to black," indicating that the black electrode of the stimulator (the cathode) should be facing the black recording electrode (the active recording electrode). For motor studies, the duration of the electrical pulse usually is set to 200 ms. Most normal nerves require a current in the range of 20-50 mA to achieve supramaximal stimulation. As current is slowly increased from a baseline, usually by 510 mA increments, more of the underlying nerve fibers are brought to action potential and, subsequently more muscle fiber action potentials are generated. The recorded potential, known as the CMAP, represents the summation of all underlying individual muscle fiber action potentials. When the current is increased to the point that the CMAP no longer increases in size, one presumes that all nerve fibers have been excited and that supramaximal stimulation has been achieved. The current is then increased by another 20% to ensure supramaximal stimulation [6]. The electromyographic setting was: 100-500 Hz frequency, 5 msec/division sweep speed, and sensitivity: 1 mV/division. The F wave response examination technique is practically the same

as that used for motor nerve conduction study (MNCV). The only difference is that the stimulating cathode was positioned proximally to prevent antidromic impulse anodal block. Manually adjusted the intensity of the stimulating current to activate a maximum muscle (M) response. After that, to ensure supermaximum stimulation, the intensity was increased by 20-30%. F wave latency measured from stimulus artifact to the beginning of the potential evoked. The electromyographic setting was: 16 Hz-16 kHz frequency, sweeping speed: 5-10 msec/division and sensitivity: 200 µV/division.

Electromyographic examination

They are studying the muscles at rest to detect spontaneous activity. The gain settings were 50 µv/cm, and 5-10 msec/cm sweep speed. Motor unit action potentials (MUAPs) were tested in order to activate 3-6 motor units with minimal muscle contraction. The gain was set at 200 µV/cm and was 3-5 msec/cm at sweep speed. Twenty or more single MUAP were separated, and the duration of the MUP, the amplitude of the MUAP, the percentage of polyphasia, and the places were investigated with a single needle puncture by progressing or removing the needle in small steps and adjusting the direction of the needle two or three times [(6)].

Evoked potentials

Done by placing the center of the round coil above the spinous C7 process for the commonly studied hand muscles [(11)], and is often recommended for more proximal arm muscles, which can be recorded simultaneously. The coil may also be placed lower at ~2 cm laterally at the T3 level, thus placing the C8 / T1 nerve roots under the coil's upper quadrant for optimal muscle recording of the APB. The optimal coil position for recordings from proximal arm muscles (BB and deltoid, C5, and C6 innervated) is 2-3 cm above C7, midline, or 2 cm lateral to this position [(12)].

Statistical analysis

The statistical analysis was accomplished applying the Statistical Package for Social Sciences (SPSS) version 23, and the 2010 Microsoft Office Excel. Whole data were represented as mean ± SD. For the measurement of discrepancies between groups, data from and patient and control groups were matched using an independent sample t-test. Within the same group, a paired t-test was used to compare the right and left sides. A P-value of 0.05 or lower was deemed significant.

Results

The duration, amplitude, and phases of MUAPs observed from both upper limbs' deltoid, BB, triceps and first dorsal interossi (FDI) muscles were significantly different between the patient and control using unpaired t test (Table 1).

Needle EMG study of the examined muscles shows no spontaneous activity in its variable types including positive sharp wave, fibrillation, and even fasciculation whereas interference pattern was significant for all muscles ($P<0.001$ for all muscles) (Table 2).

Table 1. Motor unit potential duration, amplitude and phases in patients with cervical radiculopathy

Muscle	Parameter	Patients N=100 Mean±SD	Control N=100 Mean±SD	P value
Biceps Brachii	Duration (ms)	15.36±2.23	10.75±1.11	<0.001
	Amplitude (µV)	1.45±0.65	0.6±0.14	<0.001
	Polyphasia (%)	15.67±4.17	9.28±2.17	<0.001
Deltoid	Duration (ms)	15.4±2.19	11.83±1.68	<0.001
	Amplitude (µV)	1.46±0.66	0.46±0.17	<0.001
	Polyphasia (%)	26.62±6.24	12.49±4.25	<0.001
Triceps brachii	Duration (ms)	16.24±1.3	11.75±1.68	<0.001
	Amplitude (µV)	1.7±0.7	0.6±0.13	<0.001
	Polyphasia (%)	17.51±3.42	8.48±3.45	<0.001
FDI	Duration (ms)	8.98±3.6	7.56±0.89	<0.001
	Amplitude (µV)	0.75±0.18	0.68±0.1	0.001
	Polyphasia (%)	9.11±3.63	8.13±2.53	0.028

The data presented as mean ±SD, FDI = First dorsal interosseous

Table 2. Percentage of abnormal EMG findings in patients with cervical radiculopathy and controls

Parameters	Muscle	Patients N=100	Control N =100	P value
SA (PSW, FIBS)	Biceps	Normal	Normal	1.000
	Deltoid	Normal	Normal	1.000
	Triceps	Normal	Normal	1.000
	FDI	Normal	Normal	1.000
IP	Biceps	Reduced	Normal	<0.001
	Deltoid	Reduced	Normal	<0.001
	Triceps	Reduced	Normal	<0.001
	FDI	Reduced	Normal	<0.001

SA = Spontaneous activity, PSW = Positive sharp waves, FIBS = Fibrillation potentials, IP = Interference pattern, FDI = First dorsal interosseous

Concerning sensory and motor parameters of the median, ulnar and radial nerves; unpaired t- test showed major differences between the patient and the control limbs nerve data except for radial sensory latency (SL), radial CMAP and median nerve conduction velocities (Table 3).

For motor parameters of the musculocutaneous and axillary nerves; unpaired t test showing a significant difference between the patient and control limbs nerve data of the latency parameters (P=0.001 and <0.001), otherwise amplitude parameters were not significant (Table 4).

Table 3. Sensory and motor nerve conduction parameters in patients with cervical radiculopathy and controls

Parameters	Nerve	Patients N=100	Control N=100	P value
SL (ms)	Median	2.92±0.95	2.2 ±0.24	<0.001
	Ulnar	2.01±0.27	2.23±0.33	<0.001
	Radial	1.72±0.25	1.71±0.15	0.784
SNAP (µV)	Median	41.26±24.0	28.44±4.2	<0.001
	Ulnar	55.07±32.29	30.9±6.41	<0.001
	Radial	43.26±18.82	34.15±7.12	<0.001
SNCV (m/s)	Median	46.92±11.56	55.63±6.63	<0.001
	Ulnar	55.07±5.84	57.34±6.58	<0.011
	Radial	63.13±9.96	58.49±5.55	<0.001
DML (ms)	Median	3.36±0.82	2.88±0.41	<0.001
	Ulnar	2.31±0.44	2.51±0.44	0.001
	Radial	2.25±0.56	2.47±0.2	0.001
Distal CMAP (mV)	Median	13.18±4.57	7.16±2.37	<0.001
	Ulnar	12.84±2.68	10.21±3.23	<0.001
	Radial	6.95±2.82	7.16±2.37	0.582
Proximal CMAP (mV)	Median	11.33±4.35	8.42±2.42	<0.001
	Ulnar	11.75±3.02	9.52±2.39	<0.001
	Radial	6.96±2.73	9.26±1.99	<0.001
MNCV (m/s)	Median	58.72±8.7	56.48±7.43	0.051
	Ulnar	61.84±8.37	57.52±7.57	<0.001
	Radial	61.68±9.28	56.72±7.26	<0.001

The data presented as mean±SD, SL = Sensory latency, SNAP = Sensory nerve action potentials, SNCV = Sensory nerve conduction velocity, DML= Distal motor latency, CMAP = Compound muscle action potential, MNCV = Motor nerve conduction velocity

Table 4. Motor nerve conduction parameters of axillary and musculocutaneous nerves in patients with cervical radiculopathy and controls

Parameters	Nerve	Patient N=100	Control N=100	P value
DML (ms)	Musculocutaneous	4.37±0.51	2.63±0.49	<0.001
	Axillary	3.96±0.84	2.83±0.37	<0.001
CMAP (mV)	Musculocutaneous	7.34±3.13	7.15±1.72	0.603
	Axillary	8.04±4.04	7.73±2.17	0.490

The data presented as mean±SD, DML= Distal motor latency, CMAP = Compound muscle action potential

Table 5 illustrates the data of MEPs of the patients and controls. No significant difference was observed between the two groups except (F+M-1\2) of ulnar nerve, recording ADM (0.013) were significant.

Table 5. Cervical root magnetic stimulation - motor evoked potentials data recorded from the median and ulnar nerves of cervical radiculopathy patients

Parameters	Muscles	Patient N = 100	Control N = 100	P value
F+M-1\2 (ms)	APB	14.34±1.53	14.03±1.17	0.108
	ADM	13.55±1.13	13.18±0.97	0.013
Spinal latency (ms)	APB	13.38±1.52	13.05±1.06	0.077
	ADM	12.7±1.26	12.41±1.12	0.090
Intraspinal latency (ms)	APB	0.96±0.75	1.05±0.8	0.402
	ADM	0.84±0.61	0.74±0.57	0.209

The data presented as mean±SD, F = F response, M = CMAP latency, 1 = Time in the anterior horn cell, APB = Abductor policies brevis, ADM=abductor digiti minimi

The cervical root evoked potential of BB and deltoid latency and amplitude were differed significantly between the patient and control groups in deltoid amplitude and BB latency (p = 0.019; 0.027; respectively) (Table 6).

Table 6. Motor evoked potentials data recorded from the musculocutaneous and axillary nerve of patient with cervical radiculopathy

Parameters	Muscles	Patient N=100	Control N=100	P Value
Amplitude (mV)	Deltoid	6.6±2.49	7.42±2.41	0.019
	Biceps	5.92±2.47	6.23±2.64	0.395
Latency (ms)	Deltoid	8.02±1.13	7.74±0.98	0.061
	Biceps	11.37±1.76	10.85±1.57	0.027

Table 7 illustrates specificity and sensitivity of EMG parameters according to the cut-off values of the prolonged MUAP durations, higher amplitudes, and polyphasia recorded from previously selected muscles. The MUAP duration of it, show the highest specificity and sensitivity than that of other muscles, while the amplitudes of it show the highest specificity and sensitivity than that of the other muscles, moreover, the polyphasia of deltoid muscle show highest specificity and sensitivity than that of other muscles.

Table 7. Area under curve, sensitivity, specificity and cut-off value of conventional needle EMG study parameters

Muscle	Parameter	AUC	Sensitivity	Specificity	Cut-off value
Biceps brachii	Duration (ms)	0.946	81.0%	90.0%	12.2
	Amplitude (mV)	0.966	90.0%	92.0%	0.85
	Polyphasia (%)	0.887	79.0%	94.0%	11.0
Deltoid	Duration (ms)	0.896	78.0%	95.0%	14.95
	Amplitude (mV)	0.982	93.0%	94.0%	0.75
	Polyphasia (%)	0.940	87.0%	91.0%	19.5
Triceps Brachii	Duration (ms)	0.984	96.0%	100%	14.95
	Amplitude (mV)	0.999	99.0%	100%	0.95
	Polyphasia (%)	0.961	54.0%	100%	15.5
First dorsal interossi	Duration (ms)	0.582	54.0%	59.0%	7.6
	Amplitude (mV)	0.597	44.0%	71.0%	0.725
	Polyphasia (%)	0.558	52.0%	52.0%	9.75

AUC = Area under curve

Table 8 showed specificity and sensitivity of median and ulnar nerves cervical root (F+M-1)/2-APB and ADM, spinal and intraspinal latency according to the cut-off values of the prolonged latencies, recorded from APB and ADM muscle. The spinal latency of the median shows the highest specificity than that of the ulnar nerve, whereas, the intraspinal latency show the same specificity for both nerves. Moreover, the cervical root (F+M-1)/2-ADM shows the highest specificity than that of cervical root (F+M-1)/2-APB. As regards the intraspinal sensitivity of the median nerve which was the highest than that the other sensitivities, whereas, cervical root (F+M-1)/2-ADM has the highest sensitivity than the other sensitivities.

Table 8. Area under curve, sensitivity, specificity and cut-off value of the median and ulnar motor evoked potentials

Muscle	Parameter	AUC	Sensitivity	Specificity	Cut-off value
APB	F+M-1\2 (ms)	0.540	47.0	49.0	14.15
	Spinal latency (ms)	0.538	50.0	59.0	13.15
	Intraspinal latency (ms)	0.532	53.0	57.0	0.85
ADM	F+M-1\2 (ms)	0.594	56.0	56.0	13.35
	Spinal latency (ms)	0.568	54.0	57.0	12.7
	Intraspinal latency (ms)	0.549	51.0	57.0	0.75

AUC = Area under curve, APB = Abductor pollicis brevis, ADM = Abductor digiti minimi, F = F response, 1 = Time in the anterior horn cell

Table 9 illustrates the specificity and sensitivity of musculocutaneous and axillary nerves direct cervical root stimulation according to the cut-off values of the prolonged latencies, lower amplitudes recorded from BB and deltoid muscles.

Table 9. Area under curve, sensitivity, specificity and cut-off value of the musculocutaneous and axillary motor evoked potentials

Muscle	Parameter	AUC	Sensitivity	Specificity	Cut-off value
Biceps brachii	Amplitude (mV)	0.522	50.0	50.0	5.9
	Latency (ms)	0.585	49.0	62.0	11.15
Deltoid	Amplitude (mV)	0.600	58.0	57.0	7.05
	Latency (ms)	0.562	47.0	61.0	8.05

AUC = Area under curve

Discussion

Conventional motor and sensory nerve conduction study

Though normal motor and sensory conduction studies are typically common in CR, they remain an integral part of their diagnostic evaluation. Mononeuropathy, polyneuropathy, and plexopathy may all need to be excluded before electrodiagnosis of CR can be performed; all of these require relevant motor and sensory conduction studies [6,13]. For CR patients, motor and sensory conduction tests were within the control group's normal limits and had no side-to-side difference, finding that was similar to other researchers' results [14,15]. In this study, the sensory and motor parameters of the median, ulnar and radial nerves, showed significant differences in nerve data between the patient and the control limbs except for radial SL, radial CMAP, and median velocities of nerve conduction. Besides, the motor parameters of the musculocutaneous and axillary nerves; shows a substantial difference in latency nerve data between the patient and the control limbs, otherwise amplitude parameters were not significant. These changes in CMAP were due to the chronic effects of CR contributing to secondary axonal degeneration, and changes of DML, SL, and SNAPs due to peripheral entrapment neuropathies.

The conventional needle EMG study

In the current research, for CR patients, some upper limb muscle EMG needle (FDI, BB, TB, and deltoid), shows no spontaneous activity in its variable types including Positive sharp waves (PSW), fibrillations, and even fasciculation although IP was significant for all muscles. While the duration, amplitude, and phases of MUAPs examined from the deltoid, BB, TB, and FDI muscles of both upper limbs were significantly different among the patients and controls. Such results suggest a variable degree of secondary axonal degeneration and have been identified by Pezzin as signs of denervation due to compression of the nerve root [16].

Cervical magnetic root stimulation study

In this study, MEP parameters reported after stimulation of median, ulnar, musculocutaneous and axillary nerves and recording from (APB, ADM, Biceps, and Deltoid respectively), other than the cervical spinal root were not significantly different in CR patients except for ADM (F+M-1\2) [17]. Similarly, musculocutaneous and axillary nerve data indicate substantial variations between CR patients and control subjects for cervical spinal root-BB latency and deltoid amplitude. No substantial difference between patient CR and control group was observed in the current study.

The sensitivity and specificity of conventional needle EMG study

Specificity and sensitivity of traditional needle EMG analysis of selected muscles (biceps brachii, triceps, deltoid, and first dorsal interossei) based on the cut-off values of prolonged MUAP durations, higher amplitudes, and polyphasia reported by needle EMG from previously selected muscles. The MUAP duration of triceps shows the highest specificity and sensitivity (96.0%) than that of other muscles, while the amplitudes of triceps show the highest specificity and sensitivity (100%,

99%) than that of the other muscles, Moreover, the polyphasia of deltoid muscle show highest specificity and sensitivity (91%, 87%) than that of other muscles table [18].

Sensitivity and specificity of MEPs

Median and ulnar nerves

During direct cervical root stimulation, the spinal latency of the median and ulnar nerves shows low specificity not exceedingly more than (59%), while for both nerves, the intraspinal latency shows the same specificities (57%). In addition, the cervical root PMCT by Kimura formula recording ADM and APB during direct stimulation of the cervical root displays low specificity of less than (56%). As for the median nerve's intraspinal latency sensitivity which was low of less than (53%) as the other sensitivities; this is in agreement with other researches [19-21].

Musculocutaneous and axillary nerves

Direct cervical root stimulation amplitude and latency following recording of the deltoid muscle exhibits of low specificity and sensitivity of both nerves (of less than 62%). Overall, the current motor evoked potential study shows abnormalities in fewer than 60% of patients with cervical radiculopathy relative to conventional needle EMG study abnormalities that reached 90%. This is in agreement with findings of other researchers [19-21], who found that low sensitivity and specificity of MEPs in comparing with conventional EMG in the diagnosis of cervical radiculopathy.

In conclusions, the study of motor and sensory conduction in CR could be normal, and no significant side to side difference was found. Conventional EMG findings revealed various abnormalities that denote an inconsistent degree of secondary axonal degeneration and were considered as signs of denervation due to nerve root compression. During magnetic cervical root stimulation, the spinal latency of the median and ulnar nerves during direct cervical root stimulation shows the low specificity and sensitivity. The intraspinal latency of the median and ulnar show low sensitivity and specificity. Direct cervical root stimulation amplitude following deltoid and BB muscle was recording shows the low specificity and sensitivity. In more than 90 % of CR cases, EMG reported abnormalities compared to less than 60 % for MEPs.

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Author contribution

Dr. Abdulhameed: Collection of patients, performing the electrophysiological tests, writing the manuscript. Dr. Kaddori: supervised the study, and final revision of the article.

Conflict of interest

Authors declare no conflict of interest.

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Post-Traumatic Stress Disorder and School Performance Among Adolescents Students in Baghdad

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Abstract

Background Post-traumatic stress disorder (PTSD) before adulthood has long-lasting effects on school performance among students, thus, early recognition and treatment are vital.

Objective To identify the effect of war trauma among secondary school students, test the rate of PTSD among students, and investigate the relationship between exposure to war trauma and effect on school performance.

Methods Total 108 (third intermediate class) students of both sex at two secondary schools. They screened by modified war trauma Questionnaire scale (CRIES-13) to diagnose PTSD and used impact on school performance scale.

Results The percentage of students who had >11 and above traumatic events during the preceding seven years was 38.9%. PTSD rate is 27.8% (above the cutoff point of 17 on children revised impact of events scale-13). PTSD rate is higher in females (63.3%) than 36.7% of males. The rate of impact on school performance is 43.5% (above the cutoff point of 21). The rate of impact on school performance is 83.3% among students with PTSD.

Conclusion The exposure to war trauma increases risk of PTSD among school children, number of traumatic events are high among adolescents, rate of PTSD is high in relation to events and higher in females than male and effect on school performance is high among students with PTSD.

Keywords Children, PTSD, school performance

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List of abbreviations: CRIES = Children revised impact of events scale, DSM IV = Diagnostic and statistical manual, fourth edition, PTSD = Post traumatic stress disorder, PTSS = Post traumatic stress symptoms, WWI = First world war, WWII = Second world war

Introduction

Post-traumatic stress disorder (PTSD) is categorized as a response to terrifying or disturbing events outside the range of usual human experience. It also has been considered a normal response to an abnormal situation, though when core symptoms of intrusion, avoidance and hyperarousal persist, the response risks becoming maladaptive. Some believe repeated exposure to excessive stress is among the criterion link to PTSD response in trauma survivors [(1)].

Iraqis witnessed and still witness the painful bloody and horrible sequence of explosive cars, which caused the death of hundreds of Iraqi people and many injuries in the country. In addition to explosion, many events happened in last seven years, for example; kidnapping, murder, armed robberies, torture, rape, kidnapping for ransom and sectarian problem.

A child may personally be the victim of physical violence and injury parent, sibling and other important people may be killed, injured, or

may disappear from his life without explanation, chronic fear, insecurity and chaos may result in trauma, loss of faith in the future, loss of trust in those who previously were his caretakers, even after the shooting stops. The child will be dealing with grief, trauma and demoralization and grow up around him [2]. So, PTSD is a syndrome that develops after a person sees, is involved in, or hears of an extreme traumatic stressor [3].

In Diagnostic and statistical manual, fourth edition (DSM IV) [4], PTSD is defined by four variables:

1. Exposure by personal experience or by witnessing an event which threatened or caused death and severe injury to self or other.
2. The person reacts to this experience with fear and helplessness.
3. Persistently relives the event through flashbacks, nightmares.
4. Try to avoid being reminded of it.

To make the diagnosis, the symptom must last more than one month after the event and most significantly affect important areas of life such as family, study and work.

The stressors arise from experience in war, torture natural catastrophes, assaults, rape, kidnapping and serious accidents [5].

PTSD in children and adolescents

Most studies have focused on adult high rates of PTSD have been documented in adolescents exposed to combat and other war related, community violence and natural disaster [6].

PTSD in adolescents may closely resemble PTSD in adults, however, there are few features that have been to differ, children may engage in traumatic play following a trauma. Adolescents are more likely to engage in trauma reenactment, in which they incorporate aspects of trauma into daily lives [7,8] .

In addition, adolescents are more likely than younger children or adults to exhibit impulsive and aggressive behaviors [9].

One important measure of functioning in children is school performance although some have minimized the impact of trauma on academic functioning, several studies have found diminished scholastic performance in children positive for PTSD or PTSD symptomatology [10].

Re-experiencing symptoms are common in traumatized children, although avoidance numbing and arousal may be more frequent in some situations, the avoidance/numbing cluster and arousal may be more pathognomonic [11].

Epidemiology

The lifetime prevalence of PTSD is estimated to be about 8% of the general population, epidemiological rates in adults for current and lifetime PTSD are 0.4 and 1.3%. [16], an additional 15% of adults are said to have many of the symptoms of PTSD. Through few studies have been conducted on the PTSD rate in children and adolescents, the National Center for PTSD [17] estimate that 15 to 43% of girls and 14 to 43% of boys have experienced at least one traumatic event. Of those children and adolescents, it is estimated that between 3 and 15% of girls and 1 to 6% of boys meet full criteria for PTSD [18].

In the case of Iraq, the population has been estimated to have elevated PTSD symptoms level by expert and non-expert alike but a figure has yet to be realized an important consideration for researchers and clinician is the likelihood that multiple trauma exposure will be common experience for many. Research on displaced Iraqi adolescents reported between four and five high magnitude stressors per individual e.g., Experience for bombardment, physical assault, attempted kidnapping and witness dead bodies [1].

High rates of PTSD have been documented in children exposed to such life-threatening events as combat and other war-related trauma, kidnapping, loss of parent(s). Studies on young victims or witnesses to criminal assault, and community violence have revealed high psychiatric morbidity «include post-

traumatic stress» following exposure to violence [11].

Specific factors that vulnerability to PTSD increase when prior traumatization compounded with successive traumatic events, these risks are also influenced by gender and individual difference such as preexisting psychiatric disorder, inadequate social support and genetic predisposition, low self-esteem, separation from parents before the age 10, being female [20].

The current study aimed to identify the effect of war trauma among secondary school students who were exposed to traumatic events of war and continued violence during preceding seven years, to find out the rate of PTSD among students and to investigate the relationship between exposure to war trauma and impact on school performance.

Methods

study place

The study was conducted in two intermediate schools for both sexes in Al-Mustansiriyah district, east of Baghdad during the period between 21-24 December 2009 at two schools.

a) Palatine intermediate school for boys; total number of students there was 850 (the school was exposed to bombardment since 2006 leading to death of two students and about forty injured and a teacher severely injured, so students remember the accident and everybody in school had experience with shell, bombing, shooting at close distance and witnessed dead bodies).
b) Al-Bashair intermediate school for girls in the same district; this school had (350) students as a total number.

Study sample

We decided to recruit the intermediate class students for both schools for the study, as they are mature enough to understand and answer the questionnaire which were basically designed for this age group and those have willingness to participate in study.

Two classes from each school were taken as blocks to avoid sampling bias. The total number of students who were recruited at the end for this study was 108, including 61 males and 47 females. Their age ranged from 14-16 years.

1. Two classes from Palestine school for boys, in which these were six classes at same level except for those who declined to do so or those who did not complete questionnaires.
2. Two classes from Al-Bashair school for girls. This situation was easier as there were only two classes at this level of 3rd intermediate, so we include all girls who were there except those who were not willing to be involved in the study or those who did not complete the questionnaires.

Ethical issues

1) Permission to conduct the study was taken from the authority in Baghdad (Ministry of education).
2) Consent form to perform the study was taken from the school director to facilitate the implementation of study.
3) Students were talked about the study and told they are in a research study exploring the effect of war trauma on school performance in their age, so given them enough time to talk to family and get their approval.
4) Verbal consent was taken from the students to participate in the study.
5) All questionnaires were coded a day before they were distributed to the students. The researcher was the only person who had the codes.

Tools

1. Modified war trauma questionnaire [32], this is a (44 item) questionnaire, that asks about traumatic events experienced by the student's subjects, and used to measure event that can happen in the life of adolescents or family. Respondents were required to indicate (yes or no). If the event happens to them or family in past years. (appendix 1)
2. The children's impact of event scale (CRIES-13): Self-report questionnaire was originally developed by Horowitz et al. (1979) to

monitor the main phenomenon of re-experiencing and of avoidance of that event and the feeling to which it gave rise [33]. It was not originally designed to be used with children, but it has been successfully used in a number of studies with children aged 8 years and older. However, studies found that number of items are misinterpreted by children, these studies identified identical factor structures of the impact of event scale and these were used to select eight items that best reflected the underlying factor structure and so produce shortened versions, the impact of event scale (CRIES - 13) for children. It is clear that post-traumatic stress symptoms in children are more similar across cultures than they are different, intrusion and arousal are robust factors of the impact of event scale in children from different cultures. Not at all =0, rarely =1, sometime=2, often=3. If the sum of the score on these two scales is 17 or more, then the probability is very high that the child will obtain a diagnosis of PTSD [34].

3. Impact on school performance scale: Self-report questionnaire, this is a (17 item) used in children as young as to measure school performance. There are 17 items that are scored on a four-point scale; not at all = 0, rarely =1, sometime =2, often =3. There are four reverse items in the scale [6,13,14,17]. If the sum of the scores on these scales is 21 or more, then the probability that child will have an impact on school performance. The scales were used previously in many studies on adolescents and translated into Arabic.

Results

The study sample consists of 108 students third intermediate class rang age 14-16 years, 61 males and 47 females.

Types of traumatic events were multiple; most of them experience shooting at very close distance 73.6%, witnessed explosion 63.6%, witnessed someone injured 47.2%, witnessed someone who was killed 44.5%, family received threat to life 36.3%, witnessed someone tortured 29.0%, kidnapped 13.6% (Table 1).

Table 1. Types of events among students

Type of event	No.	%
Experienced shooting at very close distance	81	73.6
Experienced shelling on bombing or a car explosion at every close distance	70	63.6
Eye witnessed someone who was injured	52	47.2
Eye witnessed someone was killed	49	44.5
Family received threats to life	40	36.3
Have been in a situation during the war when you thought you would be killed	40	36.3
Eye witnessed that someone was tortured	32	29.0
Eye witnessed massacres (the killing of many people at the same time)	17	16.3
Forced by violence to leave your home	16	15.4
Been kidnapped	15	13.6
Helped, carried or been contact with injured or killed person	13	11.8
Home been attacked or shelled	7	6.3

Number of students = 108

The number of frequencies of traumatic events are single in 5.6% and multiple in 94.4% of study sample, eleven and above traumatic events was the most frequent of multiple 38.9% (Table 2, Figure 1).

Table 2. Frequency of traumatic events during past 7 years (from 2003-3009)

No. of incident	No. student	%
1-2	6	5.6
3-4	10	9.3
5-6	13	12.0
7-8	17	15.7
9-10	20	18.5
11 and above	42	38.9
Total	108	100

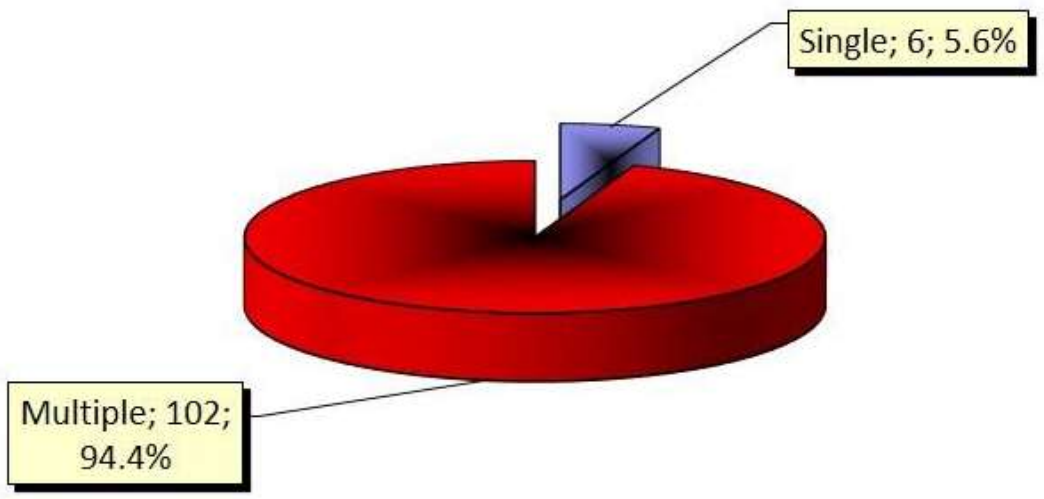

Figure 1. Rate of single and multiple traumatic events

The rate of positive cases of PTSD among students was 27.8% according to (CRIES-13) see (Figure 2).

The number of students who are exposed to multiple trauma events more than eleven are 42 students of study sample and 20 (47.6%) of them diagnosed as PTSD but 22 (52.1%) of them not having disorder (Table 3).

The rate of PTSD in student sample according to sex was higher in 63.3% in females and 36.7% in males (Table 4).

The rate of impact on school performance among student samples is 43.5% (Figure 3).

The rate of impact on school performance among students with PTSD is 83.3%. The number of students whose impact on school performance is 47 (43.5%) of the total sample and 25 (83.3%) of those diagnosed as PTSD but 22 (28.2%) of them not diagnosed as PTSD (Table 5).

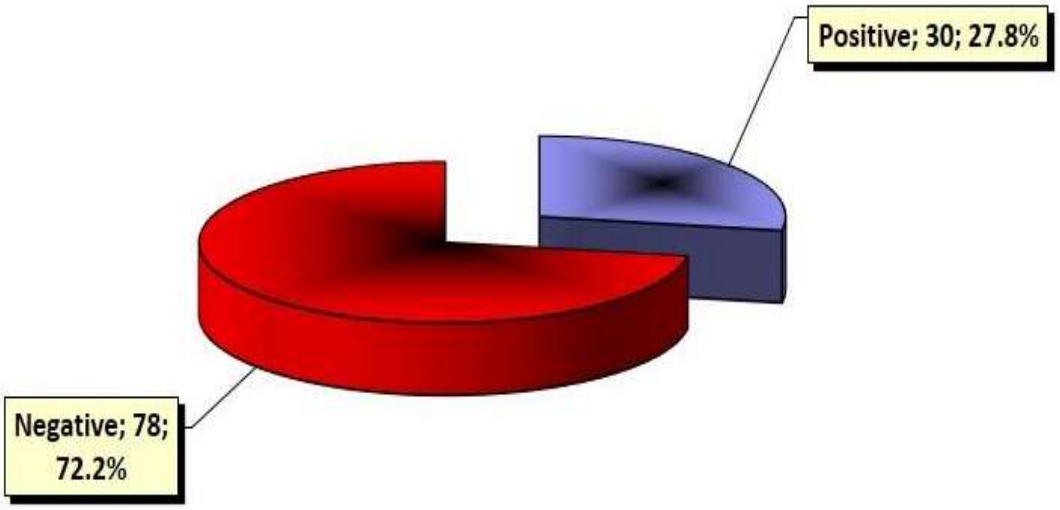

Figure 2. Rate of post-traumatic stress disorder among students' sample

Table 3. Frequency of traumatic event in relation to post-traumatic stress disorder

Trauma event	PTSD		Non-PTSD		Total	
	No.	%	No.	%	No.	%
1-10	10	15.2	56	84.8	66	61.1
11 and above	20	47.6	22	52.4	42	38.9
Total	30	27.8	78	72.2	108	100

P=0.0002 (Highly significant) using Pearson chi-squared test at 0.05 level of significance

Table 4. Rate of post-traumatic stress disorder among students' sample in relation to sex

Sex	PTSD		Non-PTSD		Total	
	No.	%	No.	%	No.	%
Male	11	36.7	48	61.5	59	54.6
Female	19	63.3	30	38.5	49	45.4
Total	30	27.8	78	72.2	108	100

P=0.020 (Significant) using Pearson chi-squared test at 0.05 level of significance

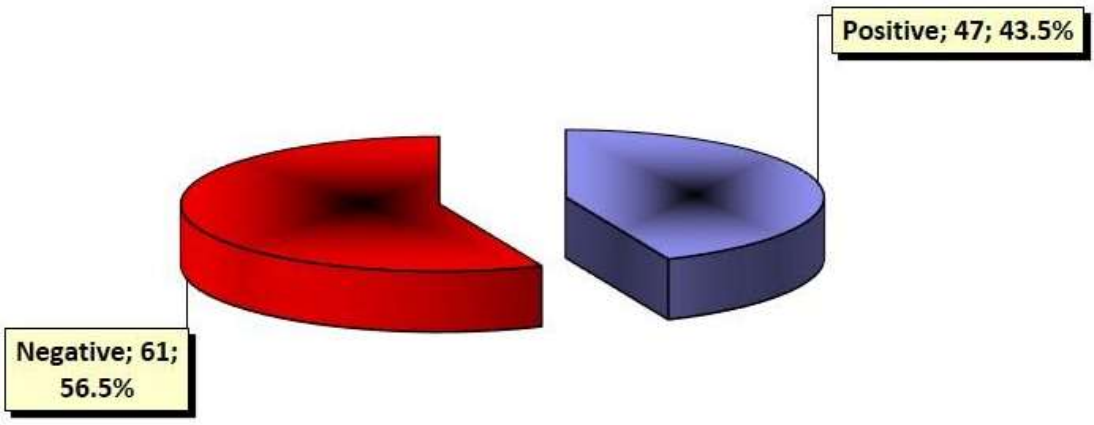

Figure 3. Rate of impact on school performance among student sample

Table 5. Rate of impact on school performance among student with post-traumatic stress disorder

Student	Impact on school performance				Total	
	Positive		Negative			
	No.	%	No.	%	No.	%
PTSD	25	83.3	5	16.7	30	27.8
Non-PTSD	22	28.2	56	71.8	78	72.2
Total	47	43.5	61	56.5	108	100

P=0.0001 (Significant) using Pearson chi-squared test at 0.05 level of significance

Discussion

The present study revealed that the rate of PTSD in students sampled according (CRIES-13) is 27.8%, and this result is concomitant with the studies of the National Center for PTSD, which estimate that 15-43% have experienced at least one traumatic event [(4)].

PTSD was higher in rate among students who had multiple traumatic experiences, 42 (38.9%) of the sample had more than eleven traumatic events during the preceding seven years and 20 (47.6%) of them diagnosed as PTSD but 22 (52.4%) of them not diagnosed as having PTSD.

Not everybody who is exposed to severe trauma will develop symptoms of stress reaction, and the majority of those who develop the acute stress reaction will get better in a few months while a minority will continue to suffer.

In this study, the percentage of PTSD response is higher in females than males (63.3% and 36.7% respectively), which is concomitant will all studies that have investigated gender as risk factor have found that females are more likely than males to develop PTSD.

The rate was found to be relevant in comparison with other studies from Iraq and other countries, which measured the rate of PTSD among adolescents. A study carried out in Iraq by Salim (2005) [(35)] studied the prevalence of PTSD among secondary schools' students in Baghdad, sample was 389 secondary schools' students for both sexes, mean age 16 years using semi structured interview base on (DSM-IV) criteria, the study showed that the rate of PTSD is 25.4% and higher in females.

The relative difference between the present study and Yousif, was that he studied the rate of acute PTSD after two months of severe trauma (explosion of three cars in Hay-Alamil city in Baghdad) and using in his study a semi

structured interview based on (DSM-IV) criteria for diagnose acute PTSD.

In other study carried out by Snell & Ali (2008) [32] on displaced Iraqi adolescent living in Amman and who have arrived to Jordan after the war in 2003 showed that rate of PTSD was 15% using CRIES-13.

The present study showed that rate of impact on school performance among students of PTSD is higher in comparison to (Snell & Ali) [32], this finding may be due to change in place to avoid (avoidance and hyperarousal)) situation and less rate of trauma exposure than present study.

In other study of adolescents carried by Peltzer (1998) [36] studied for traumatic experiencing and PTSD in South African using child PTSD checklist and life event questionnaire adolescent's version, high-rate PTSD of violence exposure, ranging from 67% to 95%, with 8.4% to 40% of children less than 17 years of age fulfilling PTSD diagnostic criteria. This study shows a positive relationship between extent of exposure to trauma and development of PTSD and higher rate of PTSD than present study. In present study, not everybody who is exposed to severe trauma developed PTSD because most people relied on helping themselves, helping each other and getting support from their religion and the society, at the same time avoiding being stigmatized or labeled as mental ill.

Also study the percentage of impact on school performance according to impact on school performance scale is 47 (43.5%) and reveal that impact on school performance among students is higher in students with PTSD. Students whose impact on school performance is 47 (43.5%) of total sample and 25 (83.3%) of those diagnosed as PTSD but 22 (28.2%) of them not diagnosed as PTSD, so other causes of impact on school performance rather than PTSD like depression and anxiety because student are living under continuous stress and fear about their own safety, fear of being shot, fear of being killed in cross fire and fear of car bombs that spare no place or time.

School refusal, which in most cases is fear of the real danger of explosion, multiple fear (fear of arms and fear of darkness), enhanced startle reaction, conduct disorder, hyperactivity, aggression; these problems of change behavior and concentration, attention and social interaction lead to decrease school performance.

Limitation of this particular study: the sample size was relatively small because each paper was filled by the interviewer when he was reading and clarifying each question in the questionnaire. Also, there is the likelihood that some of the students did not answer all the questions correctly as those questions, which may be reminders of the traumatic situation, which they may experience, may increase their stresses and they may avoid answering them correctly.

In conclusion, the study revealed the following:

1. The rate of PTSD among adolescent students is 27.8%, and 38.9% of the student sample had more than eleven traumatic events during the preceding seven years.
2. The rate of PTSD is higher among females than males (63.3% and 36.7%).
3. The rate of PTSD was affected by exposure to traumatic events, parent death, closed family members killed and high in students with personal loss of family or injury in the traumatic events.
4. The rate of impact on school performance among students is 43.5% and higher in those students with PTSD which is about 83.3%.
5. The difference between percentage of impact on school performance and percentage of PTSD among students reveal that other causes effect on school performance rather than PTSD like anxiety, depression and widespread symptomatic traumatic behavior ranging from nightmares and increased aggression and hyperactivity as well as decrease at tension span and concentration capacity.

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Author contribution

All authors participated in study design, acquisition of data, analysis, interpretation of data and drafting the manuscript.

Conflict of interest

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Atrial Flutter, The Commonly Misdiagnosed Arrhythmia as Supraventricular Tachycardia or Sinus Tachycardia

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Abstract

Background Atrial flutter is a common arrhythmia in structurally normal or abnormal heart. The electrocardiographic features of it can be mistaken for sinus tachycardia or supraventricular tachycardia. By careful electrocardiogram (ECG) inspection or by electrophysiological study differentiating atrial flutter can be reliably done.

Objective To differentiate atrial flutter from supraventricular tachycardia and sinus tachycardia.

Methods Twenty-one patients, ten females and eleven males, collected over 6 years at the author's practice in Sulaymaniyah, diagnosed as sinus tachycardia or supraventricular tachycardia and then found to be in atrial flutter were included in this study. The atrial flutter differentiated by careful inspection of the 12 leads ECG or with electrophysiological study.

Results Ten patients were misdiagnosed as sinus tachycardia and eleven patients as supraventricular tachycardia. Eleven diagnosed by careful ECG inspection and ten by electrophysiological study where ablation therapy was done in 8 patients. In 13 patients, drug therapy was applied where 4 reverted to sinus rhythm and in 6 patients reverted by synchronized DC shock. Two failed to revert to sinus rhythm.

Conclusion Differentiating atrial flutter from sinus tachycardia and supraventricular tachycardia is essential for management strategy decision.

Keywords Atrial flutter, misdiagnosis, sinus tachycardia, supraventricular tachycardia

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List of abbreviations: AFl = Atrial flutter, AT = Atrial tachycardia, AVC = Atrio-ventricular conduction, CCW = Counter clockwise, CTI = Cavo-tricuspid isthmus, ECG = Electrocardiogram, EPS = Electrophysiological study, ST = Sinus tachycardia, SVT = Supraventricular tachycardia

Introduction

Atrial flutter (AFl) is clinically commonly encountered arrhythmia in normal heart, structural heart diseases and after cardiac surgery [(1)]. It is commonly misdiagnosed arrhythmias because of non-identification of the characteristic flutter waves in the 12 leads electrocardiogram (ECG) classically described as saw teeth appearance [(2)]. The flutter waves may be mistaken for a P wave of sinus rhythm and accordingly sinus tachycardia (ST) is over diagnosed [(3)]. When a single P seen after the QRS, a supraventricular tachycardia (SVT) or atrial tachycardia (AT) is over diagnosed and if two P seen in between the QRS an atrial tachycardia with 2:1 AV conduction (AVC) is diagnosed which led to miss-management [(2,3,4)]. The mechanism of AFl is a macro re-entry circuit passing through the right atrial wall, around the tricuspid annulus and the narrow slow part of the circuit is at the cavo-tricuspid isthmus (CTI) [(3,4)]. The atrial rate in AFl is about 240-350 bpm, while the ventricular rate varies widely from 50-200

depending on the AV node conduction [1,3,4]. Surface ECG can diagnose AFl if carefully inspected but it might be not easy to differentiate it from ST or other SVT [3,4,5]. Quantitative ECG analysis may help to differentiate AFl from other arrhythmias [°]. When AFl is conducted in 2:1 pattern, the resulting ventricular rate is around 125-175 per minute (usually around 150); at this rate, it can appear that there is a P wave in front of each QRS and a T wave after each QRS. This causes the misdiagnosis of ST or SVT even with computer-based interpretation [6-8]. Differentiating AFl from ST and SVT is essential for management strategy and at certain clinical situations [9-14].

The objectives of this study are to differentiate AFl from ST and SVT and avoiding misdiagnosis of AFl by the surface ECG.

Methods

Patients misdiagnosed as ST or SVT and then diagnosed as AFl are included in this study. A total of 21 patients were collected from the author's practice at Sulaymaniyah, KRG, Iraq over 6 years' period from 2013-2019. The diagnosis of AFl is made by careful re inspection of the surface 12 leads ECG or by electrophysiological study (EPS) with back revision of the misdiagnosis through the ECG to evaluate the characteristic features and pattern of the flutter waves indicating AFl. The characteristic ECG features of AFl used in this study were the typical flutter waves and the undulating iso-electric line in between the flutter waves. The unclear flutter waves were clarified by slowing the ventricular rate by either carotid message or iv adenosine injection. Electrophysiological study applied when the tachycardia could not be surely identified by the surface 12 leads ECG and when catheter ablation is considered as a therapeutic option.

Results

Over a period of 6 years, a total of 21 patients were collected; ten females and 11 males. Age ranged from 26-75 yr. The main clinical presentation was palpitation in 15 patients and dyspnea with vague chest pain in 4 and in 2 patient's syncope was the presenting symptom. The AFl was misdiagnosed for ST in 10 patients and SVT in 11. In 8 patients, an EPS and CTI ablation done successfully after the diagnosis of counter clockwise (CCW), typical isthmus dependent AFl was confirmed. In the rest 13 patients, drug therapy with either amiodarone or flecanide was used; with reversion and stabilization in to sinus rhythm in 4 patients and reversion by DC shock in 6 and then maintained on antiarrhythmic drug, the other three advised for ablation therapy but they refused (Table 1).

The followings figures (1-5) are examples of the misdiagnosed cases of AFl

Table 1. The demographic features of cases.

Total number	Male	Female	Clin. pres.	ST	SVT	EPS +Abl.	Drugs
21	11	10	15 P 4 CP & SOB 2 S	10	11	8	4 SR 6 DCS 3 no Abl

Clin. pres.: Clinical presentation and type of misdiagnosis and treatment afforded. P: Palpitation, CP: chest pain, SOB: Shortness of breath, S: Syncope, ST: Sinus tachycardia, SVT: Supraventricular tachycardia, EPS: Electrophysiological study, Abl: Ablation, SR: Sinus rhythm, DCS: Direct current shock.

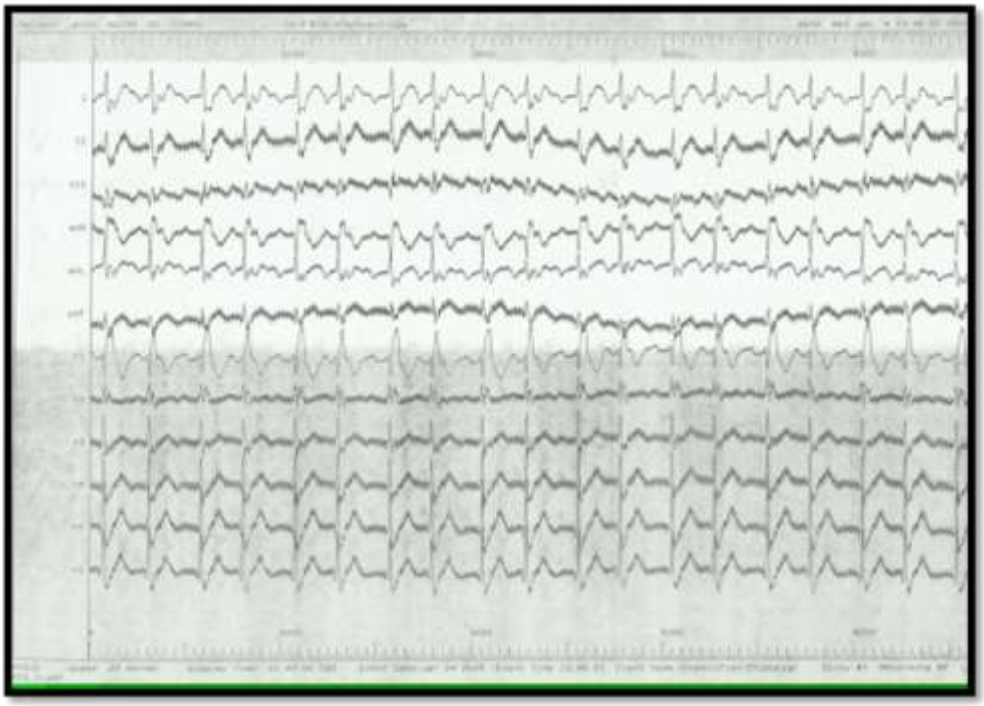

Figure 1A. A 55 yr male diagnosed as SVT for two years and treated with BB and amiodarone with no improvement. EPS confirmed CCW typical AFl. Careful inspection flutter waves and undulating isoelectric line seen in lead III. 2:1 AFl seen. QRS is of RBBB pattern. SVT: Supraventricular tachycardia, BB: Beta blockers, EPS: Electrophysiological study, CCW: Counter clockwise, AFl: Atrial flutter, RBBB: Right bundle branch block

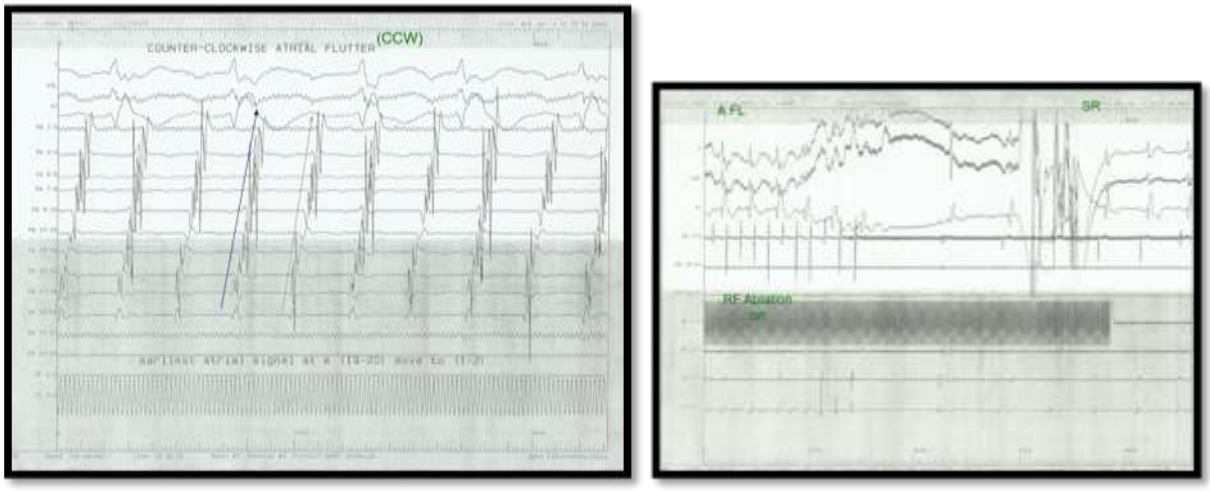

Figure 1B. Same patient in figure 1A, EPS confirmed 2:1 CCW typical AFl and CTI ablation reverted him in to SR. CTI: Cavo-tricuspid isthmus

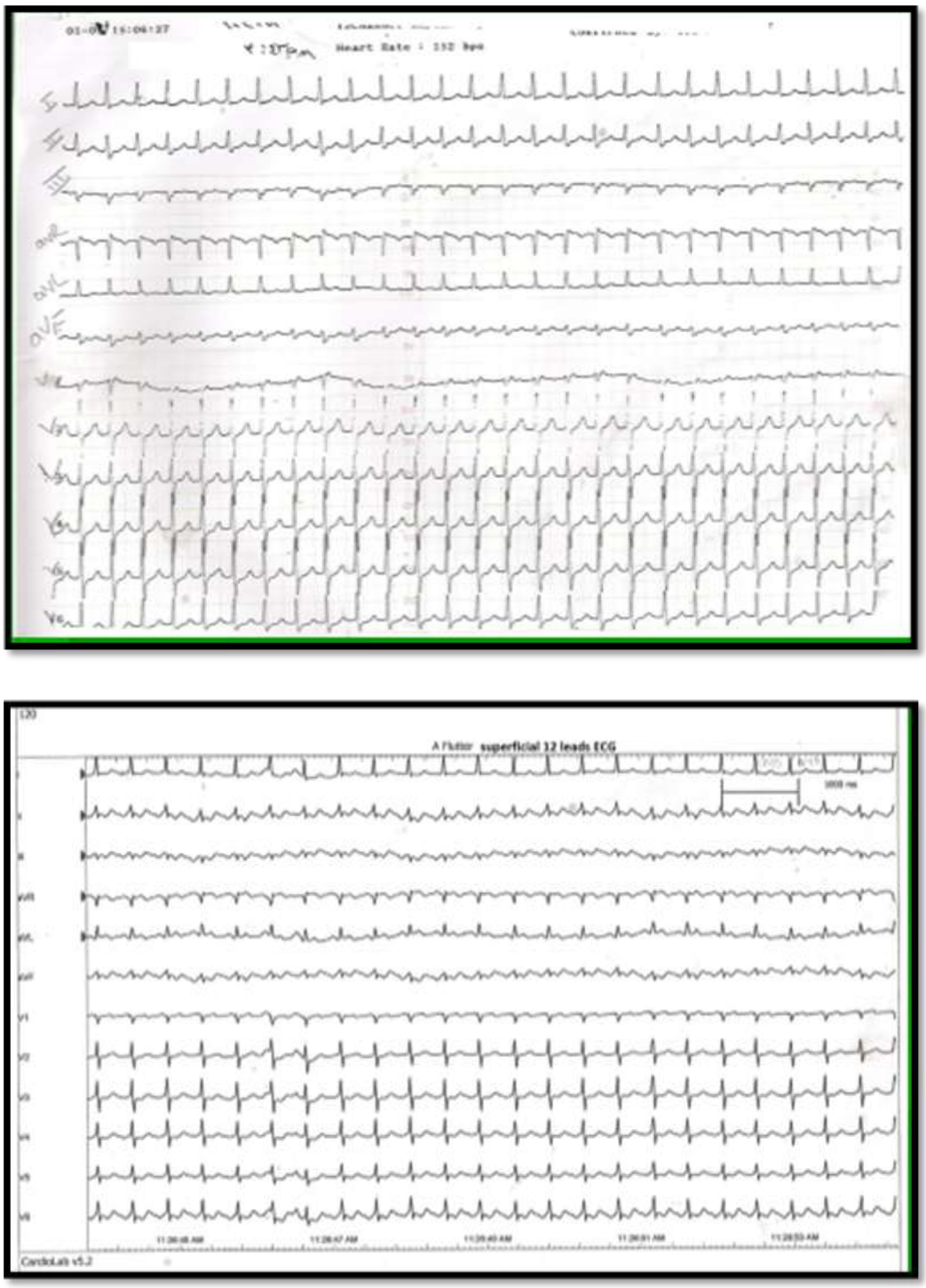

Figure 2. A 36 yr male, structurally normal heart with palpitation, diagnosed as SVT of short RP long PR, s/f AVNRT diagnosed for 4 years (top ECG). The bottom ECG showed undulant isoelectric line and 2:1 atrial flutter seen clearly in lead III and aVF

Figure 3. A 56 ye female with structurally normal heart with sustained tachycardia referred as extreme ST or SVT (top). Holter (lower left) clearly showed atrial flutter with variable AVC. EPS and CTI ablation (lower right) reverted her in to sinus rhythm

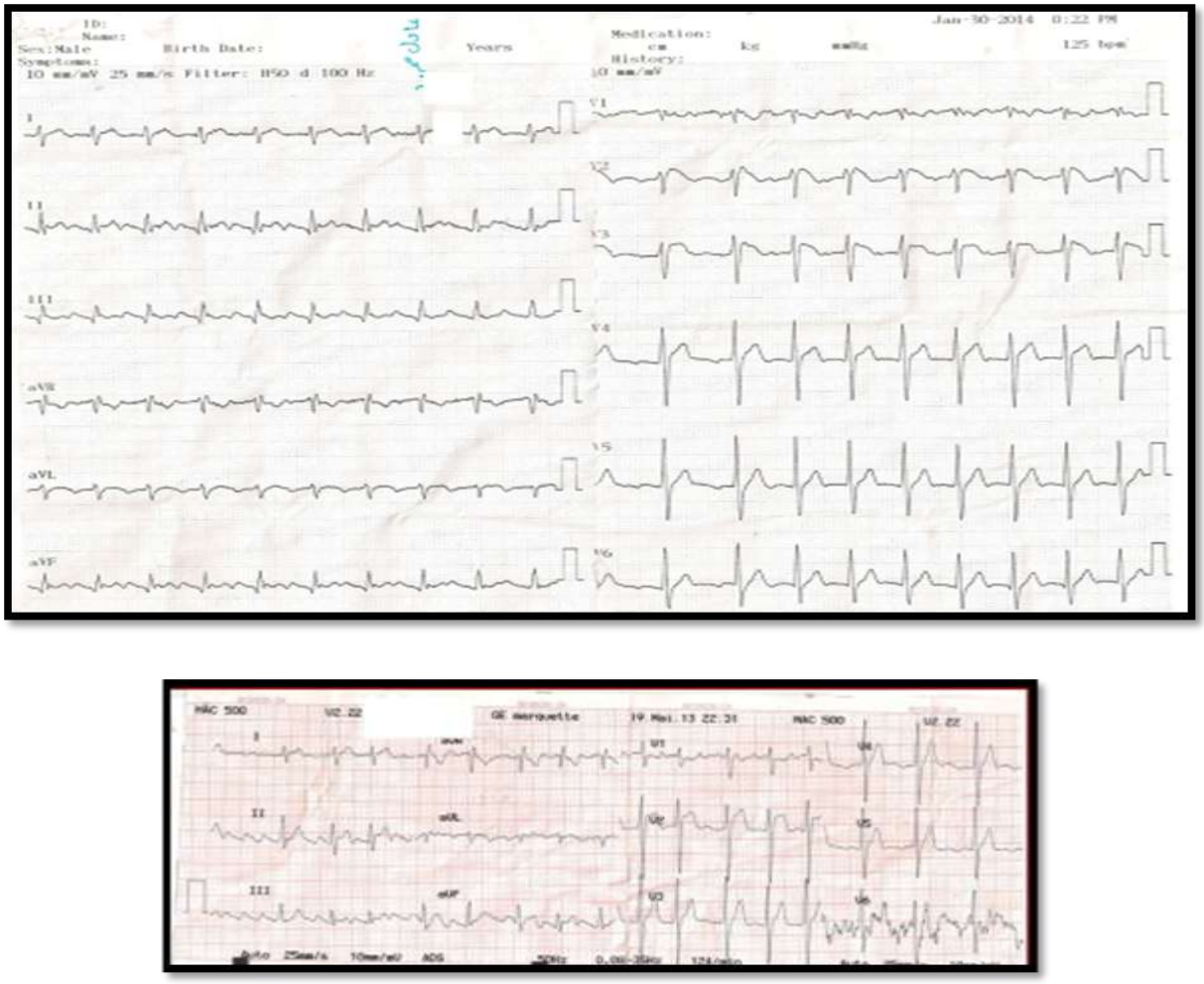

Figure 4. A 34 yr male presented with pre syncope and palpitation. ECG reported as sinus tachycardia where a P wave seen in II, III, aVF and V1 (Top). Bottom ECG showed clearly atrial flutter with 3:1 and 2:1 AVC in leads I, II and III. In V2, V3 a Brugada syndrome features

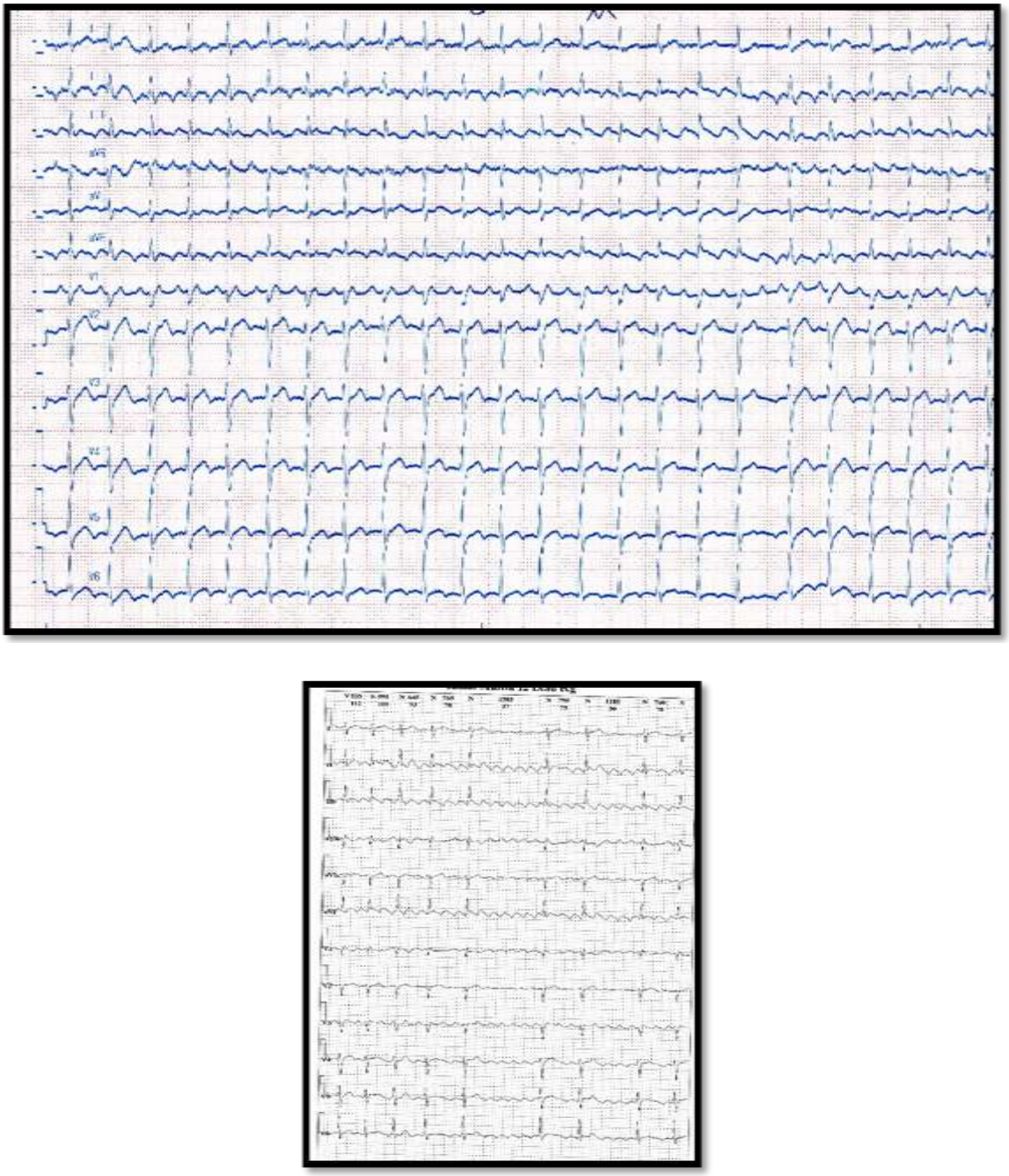

Figure 5. ECG of a 45 yr male diagnosed as extreme sinus tachycardia based on P wave at V1 before the QRS. Holter recording clearly showed atrial flutter with variable AVC

Discussion

The recognition of AFl from ST and other SVT is clinically important for management's decisions. Differentiating AFl from ST and SVT can be done by considering the clinical status, careful inspection of all the 12 leads ECG with long strips or if in doubt by EPS where ablation therapy can be offered at the same session.

A cardiac rhythm of about 150 bpm should raise the possibility of AFl mostly with 2:1 AVC, ST is a common suspect in this situation but it should be remembered that ST usually have an underlying clinical reason, is not sudden in onset and can fluctuate with activities like exercise or changing position, while AFl patients has no obvious reasons for ST and

shows a high rate suddenly, maintain it and may ends suddenly to go in to SR or continue in to persistent AFl.

The 12 leads ECG with long strips of selected leads is much superior than few leads ECG to interpret for differentiation because the flutter waves can be seen only in some leads than others where either the classical saw teeth appearance flutter waves and undulating isoelectric line are seen. Occasionally the 2:1 AVC may go in to 3:1 or more by itself, with carotid message or iv adenosine, which immensely help to diagnose AFl rather than ST or SVT; remembering that SVT and AT may conduct in 2:1 pattern but the isoelectric line is very stable and not undulant as in AFl. In our series of 21 patients, the differentiation of AFl could be done with careful inspection of the 12 leads ECG in 50% and by EPS in the other 50%.

In conclusion, AFl can be distinguished by careful ECG inspection of all the 12 leads and long strips of some leads or by EPS to avoid misdiagnosis as ST or SVT where management is different.

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Conflict of interest
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Outcome of Orbital Complications of Acute Rhinosinusitis in Children

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Abstract

Background Acute bacterial rhinosinusitis occurs commonly as a sequel to a viral upper respiratory tract infection. Spread of infection outside the sinuses to the surrounding brain and orbit results in complicated sinusitis. Complications are more common in children than adults due to anatomical and immunological factors.

Objective To identify the correlation of orbital manifestations with computed tomography (CT) scan findings in diagnosing and classifying the disease, the efficacy of various treatment modalities and the outcome of these complications in children.

Methods A prospective study of 16 patients who presented with orbital swelling and features of acute rhinosinusitis was conducted in the Otorhinolaryngology Department at Al-Imamein Al-Kadhimein Medical City from April 2014 to October 2015. A full history, ear, nose and throat (ENT) examination including naso-endoscopic examination (if possible), ophthalmological assessment and CT scan of nose and paranasal sinuses were done. Patients were classified to have either pre-or postseptal infection depending on CT findings. All patients were admitted to the ENT ward, received intravenous broad-spectrum antibiotics and supportive measures and kept under close observation with monitoring of ophthalmological manifestations and vital signs. Some patients responded to medical treatment and others required surgical intervention. All patients after recovery were discharged home on oral antibiotics for 2 weeks and followed up monthly for a total duration of 3 months.

Results The majority of patients were males (62.5%). Mean ages of patients was 8.9±2.9 years with a range (4 to 15) year. Preseptal cellulitis was more common (62.5% vs. 37.5%). Impaired visual acuity, impaired extraocular movement and proptosis were significantly associated with postseptal infections as the P values were 0.01, 0.003 and 0.003 respectively. Preseptal cellulitis responded well to medical treatment. Postseptal abscesses required surgical drainage using an external, endoscopic or combined approach. 94% of patients had complete recovery without any sequel.

Conclusion Orbital manifestations are strongly correlated with CT findings. Medical treatment is very effective for preseptal infection. The choice of surgical approach for managing postseptal infection will depend upon, the site and extension of the abscess and the state of the nasal cavity. Early and accurate management can reduce morbidity and mortality.

Keywords Orbital complications of sinusitis, pediatric rhinosinusitis, ethmoiditis

Citation Al-Hassani JMK. outcome of orbital complications of acute rhinosinusitis in children. Iraqi JMS. 2021; 19(1): 90-98. doi: 10.22578/IJMS.19.1.12

List of abbreviations: None

Introduction

Acute bacterial rhinosinusitis occurs commonly as a sequel of a viral upper respiratory tract infection. The spread of infection outside the sinuses results in

complicated sinusitis [1]. Complications of paranasal sinus disease are more common in children than adults. The spread of infection from the nose and paranasal cavities to the surrounding brain and orbit is facilitated in children by dehiscence in the common bony walls at the suture lines, the thinness of the cranial bones and the relative immunosuppression of the child under the age of five [2]. The proximity of the orbit to the paranasal sinuses especially the ethmoid sinuses and paper-thin medial wall of the orbit, namely lamina papyracea, make it the most commonly involved structure in the complications of sinusitis. The orbital septum, a reflection of the orbital periosteum, is an important structure because it is the only soft tissue barrier between the sinuses and the orbital contents. Orbital infections are divided into two groups based on this element of orbital anatomy; preseptal and postseptal infections [1]. Chandler et al. classified the orbital complications of sinusitis into five groups: preseptal cellulitis, orbital cellulitis, subperiosteal abscess, orbital abscess and cavernous sinus thrombosis. The first complication, preseptal cellulitis is a preseptal infection and the latter four are postseptal [3]. The difficulty for the clinician lies in distinguishing preseptal cellulitis from subperiosteal or orbital abscess at the initial presentation, with marked orbital edema and pain limiting the ophthalmic examination especially in children [2]. Furthermore, progression of infection could happen during the course of treatment with development of new complications making the clinical assessment more difficult [4]. Accordingly, imaging is often necessary to appropriately classify orbital complications. Computed tomography (CT) is considered the gold standard for sinus imaging. It has been shown to demonstrate good accuracy in diagnosing pediatric rhinosinusitis [5,6]. In preseptal cellulitis, CT reveals a diffuse increase in density and thickening of the lid and conjunctiva. Postseptal inflammation is characterized by a soft tissue density or low-attenuation area adjacent to the lamina papyracea [5,7]. Abscesses appear as hypodense areas with rim enhancement and mass effect [8].

The aim of this study is to identify the correlation of orbital manifestations with CT scan findings in diagnosing and classifying the disease, the efficacy of various treatment modalities and the outcome of these complications in children.

Methods

Ethical considerations

Verbal consent was taken from each patient parent after explaining (by the researcher) the purpose of the study.

Study population

Sixteen selected patients with orbital swelling and features of acute rhinosinusitis were included in this study with the following criteria.

Inclusion criteria

1. Patient within pediatric age group.
2. Orbital swelling, eyelid edema and eyelid erythema.
3. Features of acute rhinosinusitis:
   - Facial congestion.
   - Nasal obstruction.
   - Nasal discharge/purulence/discolored.
   - Purulence on nasal examination.
   - Duration of symptoms of less than 4 weeks.
4. CT scan findings suggesting rhinosinusitis (presence of sinus opacification or air-fluid levels), with features of orbital involvement.

Exclusion criteria

1. Patient with documented ophthalmological problems (as a result of infection of the eyelids and orbital adnexa, trauma including insect bites or a foreign body, dacryocystitis, history of surgery, odontogenic infection, and orbital tumors).
2. Immunocompromised patients.

Study design

A prospective study was conducted in Otolaryngology Department at Al-Imamein Al-Kadhimein Medical City over a period of 18 months (Apr. 2014 - Oct. 2015). Direct questioning of patients and/or his/her parents about the biographic data, chief complaint and duration, associated symptoms, other medical or surgical history, drug history and any relevant socio-economic history was done. General clinical examination, full ear, nose and throat (ENT) and head and neck examinations were done. Apart from nasal examination, nasoendoscope (where possible) was done for majority of patients using 2.7 mm and 4.0 mm rod Hopkins rigid nasoendoscopes or flexible fiberoptic nasopharyngoscope according to the patient age and nasal cavity status. A formal assessment of the full range of eye movements, degree of proptosis, pupillary light reflex, visual acuity (using a Snellen chart), color vision (using Ishihara plates) and inspection of the optic disc (where possible) were carried out by an ophthalmologist at time of presentation. Radiological examination included CT scan (axial and coronal views) of nose and paranasal sinuses were done for all patients (as a part of our study to confirm the diagnosis, to classify the disease and then to correlate the clinical presentation with CT findings). All patients were admitted to the ENT ward for medical treatment and close observation including twice daily monitoring of the full range of eye movements, visual acuity and proptosis in conjunction with regular temperature monitoring and pulse measurement. All patients received intravenous (IV) broad spectrum antibiotics (third generation cephalosporin 50 mg/kg/day and metronidazole 30 mg/kg/day), topical nasal decongestants, analgesia and nasal irrigation. Some patients were subjected to surgical intervention in the form of endoscopic sinus surgery, external ethmoidectomy, or combined approach to drain the pus and release pressure on the orbit. All patients were kept on IV treatment until complete improvement, discharged home on oral antibiotics for a total duration of 14 days, and followed up monthly for up to 3 months.

Statistical analysis

By using the statistical package for social sciences (SPSS) program version 20, data were entered and analyzed with appropriate statistical tests. Descriptive statistics were presented as frequencies, proportions, mean and standard deviation (SD). Paired t test was used to compare pre and post -operative mean VAS scores and Fisher's exact test was used to compare frequencies (proportions). Level of significance (P value) ≤ 0.05 considered as significant. Finally, all findings and results are presented in tables and/or figures with explanatory paragraphs accordingly.

Results

Ten patients were males and 6 were females (Fig. 1).

Patients ages ranged from 4-15 years, 8 (50%) of them were between 8-11 years and the mean age±SD was (8.9±2.9) years (Fig. 2).

Depending on CT scan findings, 10 patients were presented with preseptal and 6 with postseptal infection (Fig. 3).

Preseptal infection occurred more commonly in males and postseptal infection occurred in equal numbers in both genders (table 1).

Both types of complications occurred more commonly in patients over the age of 7 years (table 2).

Common orbital manifestations (Orbital swelling, eyelid edema and eyelid erythema) were observed in all patients, while specific features (impaired visual acuity, ophthalmoplagia and proptosis) were observed specifically as follows: for those with preseptal infection, 2 had visual acuity of 6/18, 1 had impaired extraocular movement, and no one had proptosis, while those with postseptal infection, 5 of them had visual acuity of 6/18 or worse, 5 had impaired extraocular movement, and 4 had proptosis and as shown in (table 3), these manifestations were significantly associated with postseptal infection as the P values were < 0.05.

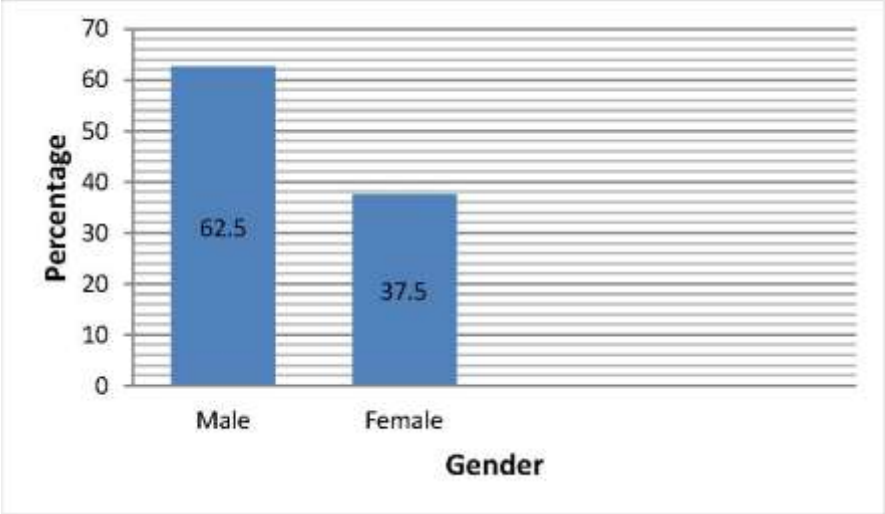

Figure 1. Gender distribution

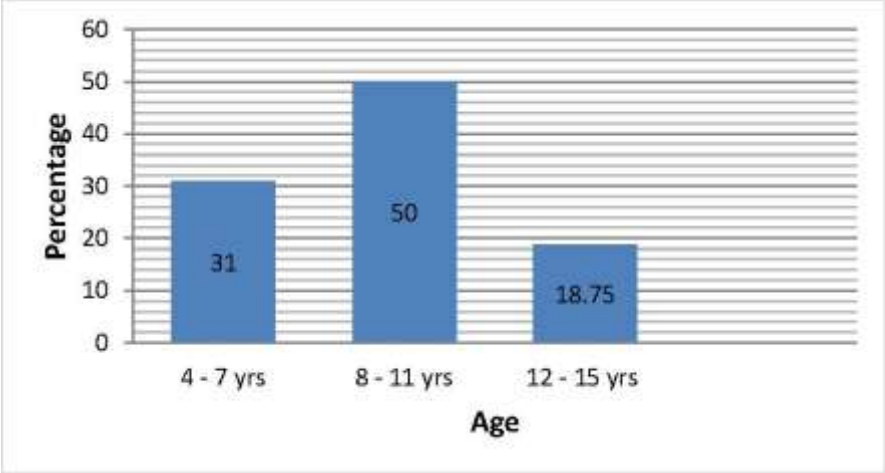

Figure 2. Age distribution

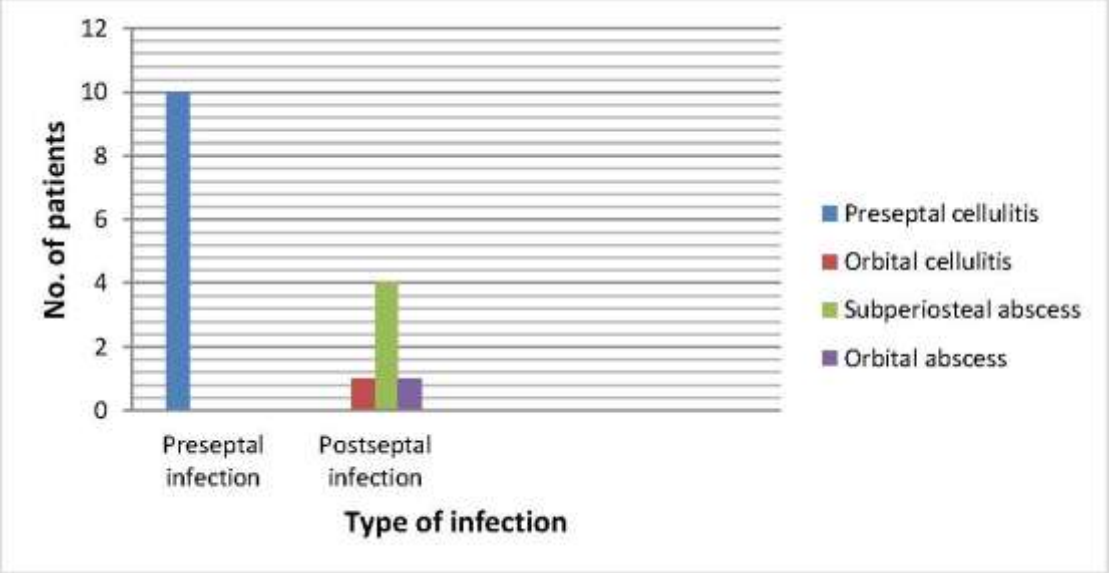

Figure 3. Types of orbital complications according to CT findings

Table 1. Gender of the patients and its relation to the type of orbital complication

Gender	No	%	Preseptal infecion	Postseptal infection	P value
Males	10	62.5	7	3	0.4
Females	6	37.5	3	3	
Total	16	100	10	6	

Table 2. Age of the patients and its relation to the type of orbital complication

Age (yr) (mean±SD)	No	%	Preseptal infecion	Postseptal infection	P value
≤7 (5.8±1.3)	5	31	4	1	0.3
7> (10.2±2.2)	11	69	6	5	
Total	6	10	100	16	

Table 3. Orbital manifestations and their relation to the type of orbital complication

Orbital Features	Preseptal infection			Postseptal infection			P value
	No. of affected patients	No. of total patients	%	No. of affected patients	No. of total patients	%	
VA* 6/18 or Worse	2	10	20	5	6	83.3	0.01
Impaired EOM**	1	10	10	5	6	83.3	0.003
Proptosis	0	10	0	4	6	66.7	0.003

* Visual acuity, ** Extraocular movement

Eleven patients were subjected initially to medical treatment alone (those diagnosed with preseptal cellulitis and orbital cellulitis without abscess), 9 (82%) of them responded well to medical treatment, and the other 2 (18%) showed progression of signs and symptoms within 24 hours of close observation. Seven patients (those who had abscess formation and those with outcome of orbital complications of acute RS in children 8 unimproved cellulitis and disease progression) required surgical intervention (Table 4). Six patients from the surgical group showed clinical improvement on surgical management and 1 patient (who had orbital abscess) developed blindness.

Table 4. Different surgical approaches required for management of orbital complications

Surgical Approach	Diagnosis				Total
	Preseptal cellulitis	Orbital Cellulitis	Subperiosteal abscess	Orbital abscess	
Endoscopic	1	1	1	---	3
External	---	---	1	---	1
Combined	---	---	2	1	3

Discussion

Orbital complications have been observed more commonly among males (62.5%). Although male involvement was predominant, gender was not considered as a risk factor for development of specific type of orbital complications as it was statistically not significant.

Nageswaran et al. [9], Huang et al. [10] and Sultész et al. [11] also showed male predominance (73%, 65.63% and 54.8% respectively) without statistical significance. In a study done by Goytia et al. [12], the male gender has been identified as a risk factor in post-septal infections.

The mean age of presentation was 8.9 years. Both pre- and postseptal infections occurred more frequently in patients above the age of 7 years (69%), however, there was no statistical significance to consider the age as a risk factor predisposing to a certain type of orbital complications. In a study done by Smith et al. [13], the mean age was 6.5 years and age over 9 was considered as a risk factor for developing post-septal infection ($p < 0.01$). Huang et al. [10] found in their study that the mean age was 6.95 years and the age of 6 years or less as a risk factor for post-septal infection ($p = 0.023$). Botting et al. [14] obtained a statistically significant difference between pre- and post-septal groups with regards to age (3.9 vs. 7.5 years, $p < 0.001$). From the previous discussion we could note that no specific pediatric age could be obtained as a risk factor for developing either pre- or post-septal orbital infections.

CT scan is considered the gold standard for sinus imaging because it can better depict the anatomy of the globe, retro-orbital tissues, sinuses, and cranium, and provides information for surgical planning. Huang et al. [10] have used CT scan in all patients in their study to prove the presence of concomitant sinusitis. Ho et al [15] in a retrospective study of 80 patients with orbital cellulitis used CT scan to classify their patients. Clary et al. [16] in a retrospective review of 19 patients (with orbital complications who underwent surgical exploration within 24 hours of their CT scan) found that 15 of the 19 CT scan interpretations indicating the outcome of orbital complications of acute RS in children 9 abscesses that were verified intraoperatively, 2 patients had negative surgical exploration despite CT interpretations predicting abscesses, an abscess was also surgically documented in 1 of 2 patients whose preoperative scans indicated cellulitis alone. They concluded that the

correlation between radiologic and operative findings, although not absolute, does substantiate the use of CT scanning as a therapeutic guide in children presenting with orbital disease secondary to paranasal sinusitis. Despite these findings, the use of CT scan as a routine investigation in the management of pediatric complicated rhinosinusitis is not well documented, as it is not always necessary to expose them to a high dose of radiation especially those presented with mild orbital manifestation of pre-septal cellulitis, in addition, CT scan is not always available in all medical facilities and the nearest one may be far away from the local facility. Howe and Jones [17] developed criteria for indication of CT of the sinuses and the orbit in the following situations: central neurological signs; inability to perform adequate ophthalmological examination; no improvement in orbital signs or general clinical condition after 24 hours of appropriate intravenous antibiotics and deterioration of orbital signs (proptosis, ophthalmoplegia, altered color vision, or visual acuity).

The most common type of orbital complications of rhinosinusitis was preseptal cellulitis (62.5%). Ozkurt et al. [18], Sobol et al. [19] and Ailal. et al. [20] also found that pre-septal cellulitis was the commonest orbital complication and the percentages were 62.3%, 72% and 73% respectively. The fact beyond this result is that orbital septum lacks lymphatic channels and thus, forms a barrier limiting infections from passing directly through the eyelids into the orbit [1]. Specific orbital manifestations including impaired visual acuity, impaired extraocular movement and proptosis were significantly associated with postseptal infections. Ho et al. [15] found that patients with postseptal involvement had a significantly higher rate of proptosis and limited extraocular motility. Sobol et al. [19] concluded that postseptal complications of sinusitis can be diagnosed by the presence of ophthalmoplegia and proptosis. Botting et al. [14] found that ophthalmological examination identified diplopia ($P < 0.001$), ophthalmoplegia ($P < 0.001$), and proptosis ($P < 0.001$) as significant features of a postseptal infection. These features developed mostly due to abscess collection at the medial aspect of the orbit between orbital periosteum and lamina papyracea (subperiosteal abscess) that displaces the orbital contents and globe downward and laterally with impaired ocular mobility, or collection of abscess within the orbital tissues (orbital abscess) displacing orbital contents forward with resultant severe exophthalmos, complete ophthalmoplegia and visual impairment due to pressure effect on optic nerve with a risk for progression to irreversible blindness [5]. The great majority (90 percent) of pre-septal cellulitis were successfully and effectively managed by medical treatment alone. Sobol et al. [19] and Moubayed et al. [21] also found that preseptal disease was resolved with antibiotics. In our study, 1 patient with preseptal cellulitis and another 1 with orbital cellulitis showed progression of signs and symptoms within 24 hours and required surgical intervention. Ho et al. [15] found that 2 out of 34 patients with outcome of orbital complications of acute RS in children, 10 preseptal cellulitis and 3 out of 6 patients with orbital cellulitis required surgical intervention for management of their orbital infection.

All patients in our study, who presented with abscess collection and those with cellulitis who failed to respond to medical treatment, required surgical intervention. In general, surgical intervention has been recommended in cases when there is CT evidence of abscess formation, 20/60 or worse visual acuity is observed on initial evaluation, progression of orbital signs and symptoms occurs despite medical treatment, or lack of improvement is seen within 48 hours despite aggressive medical treatment [1]. There are 3 surgical approaches that were used for treating these complications: external ethmoidectomy, endoscopic sinus surgery and combined surgical approach. The location of the abscess will dictate if an endoscopic approach is feasible. Orbital cellulitis is mainly secondary to ethmoid rhinosinusitis, the position of any abscess is likely to be medial to the globe. Some surgeons suggest that endoscopic ethmoidectomy together with removal of the

lamina papyracea and perinasal drainage of the orbital abscess is sufficient treatment. Endoscopic drainage avoids external scars and results in rapid resolution of periorbital inflammation [22]. Migirov et al. [23] found that there were no postoperative sequelae in children treated endoscopically, in contrast to facial scarring, delayed healing, stitch abscess, unresolved diplopia, or recurrent periorbital cellulitis with or without abscess following external drainage. They recommended exclusive use of an endoscopic approach for treating medial orbital subperiosteal abscess and saving external ethmoidectomy for drainage of superior orbital abscesses. However, the anatomical limits of the pediatric nose and the vascular mucosa make it technically difficult and unless the surgeon is extremely familiar with endoscopic nasal surgery, it is probably easier and wiser to use an external approach [24]. Using both external and endoscopic techniques in the management of subperiosteal and orbital abscess has been shown to achieve the best results regarding the adequacy of abscess drainage and surgical access and minimizing the possibility of iatrogenic complications.

Rubin et al. [25] found that the percentage of surgical failures requiring additional drainage was 25% in endoscopic approach and 14% in external approach but this difference was not significant, there were no failures in the combined approach group. They concluded that combined approach seems to be a viable surgical option combining the advantages of both endoscopic and external approaches. Dewan et al. [26] concluded that combined approach was associated with improved treatment outcome, demonstrated by absence of abscess re-accumulation compared with the other 2 approaches.

With early and adequate management, the prognosis of orbital complications of rhinosinusitis is usually good. Same findings were obtained by Sultész et al. [11], Ho et al. [15] and Moubayed et al. [21].

In conclusions, orbital manifestations are strongly correlated with CT findings in distinguishing between pre- and postseptal infections. However, there are certain situations in which CT scan is mandatory.

Medical treatment is very effective for pre-septal infection. The choice of surgical approach for managing postseptal infection will depend upon the expertise of the surgeon, the site and extension of the abscess and the state of the nasal cavity. Early and accurate management can reduce morbidity and mortality.

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Conflict of interest

Author declare no conflict of interest.

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The Using of Multiplex RT-qPCR for Pooled Samples to Detect Hepatitis B Virus, Hepatitis C Virus, Human Immunodeficiency Virus within Iraqi Blood Donors

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Abstract

Background: The blood of blood donors is screening for hepatitis B virus (HBV), hepatitis C virus (HCV), and human immunodeficiency virus (HIV) infection by enzyme immunoassay (EIA) in the National Center of Blood Bank in Baghdad. The residual risk of EIA negative samples is not estimated till now in Iraq.

Objective: To detect HBV, HCV and HIV viruses within seronegative plasma of blood donors by a commercially available multiplex nucleic acid amplification tests (NAT) with mini-pooling system.

Methods: One thousand (1000) blood donors were screened by EIA revealed negative results then NAT was performed on pools of samples, each pool contain ten seronegative plasmas (MP10) i.e., 100 minipools.

Results: The detected positive minipools 10 seronegative plasmas by NAT for HBV, HCV and HIV were 3 MP10.

Conclusion: The use of NAT appeared to be sensitive and reliable to detect occult HBV and overcome the seroconversion problem related with HCV and HIV within seronegative plasmas of blood donors. Therefore, the implementation of NAT with mini-pooling in addition to serological tests for routine blood donor screening will improve and ensure the safety of blood transfusion in Iraq.

Keywords: Blood transfusion, widow period, multiplexes PCR

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List of abbreviations: EIA = Enzyme immune assay, ELISA = Enzyme-linked immunosorbent assay, MP = Minippools, NAT = Nucleic acid amplification technique, PCR = Polymerase chain reaction, vDWP= Viral diagnostic window period

Introduction

Serological tests (detection of antigens or antibodies) had historically been the foundation of blood screening for transfusion transmitted infections [(1)], but it remains limited and problematic because of 1) viral pre-seroconversion window period (PWP) i.e., the time period that needed for specific antibodies development and become detectable in the blood after a recent virus infection, 2) donors infected with genetic and immune variants viral strains and 3) cases of immunosilent infections (carriers), while the serological tests only provide positive results once the donor's immune system reacts against the respective pathogens [(2)].

Over two decades ago, advanced and newer tests like nucleic acid amplification tests (NAT) have helped in shorting the viral "window

period" [1]. NAT is a molecular technique with high sensitivity and specificity. It is an amplification technique of targeted regions of viral RNA or DNA and could detect the presence of viruses earlier than the serological screening methods, so it will narrow the window period of hepatitis B virus (HBV), hepatitis C virus (HCV), and human immunodeficiency virus (HIV) infection [3].

International survey on NAT testing of blood donations implemented from 1999-2009 to screen blood donations for HCV and HIV-1 on more than 300 million blood donors and about 100 million blood donors screened for HBV. The results revealed there were over 2000 NAT-reactive with serology negative donations that would otherwise have been transfused [4] also the results have shown that all over infection rates for HCV 1:447000, for HIV 1:111000 and for HBV 1:66000 [5]. But NATs are expensive technique with about 5-10-fold greater than that of the most expensive enzyme immunoassay. To overcome the cost problem that related to NATs, two strategies have been suggested, the using of pooled plasma samples so that fewer tests are required to screen large numbers of samples with using of multiplex polymerase chain reaction (PCR) assays that can detect simultaneously several viruses in one reaction tube [6]. World health organization (WHO) mentioned in their guidelines in 2017 that the length of viral diagnostic window period (vDWP) for different assays categories is clearly different, the using of mini-pooling of 16 samples with multiplex NAT test is shortening the vDWP 7-11 days, 27-37 days, 5-7 days for HIV, HBV and HCV respectively while the enzyme immunoassay (EIA) is shortening the vDWP 16-21 days, 42 days, 60 days for HIV, HBV and HCV respectively [7].

In Iraq, till now serological tests are the reliable standard methods for screening donated blood. The question here, are these tests enough to ensure the safety of blood or plasma for transfusion? Therefore, this study was designed as an attempt to make insight on many important points related to blood transfusion in Iraq.

The study aimed to determine the presence of positive HBV, HCV and HIV in seronegative donated blood within Iraqi blood donors, also to elucidate sensitivity and specificity of the currently used serological tests in blood screening in comparison with NAT assay, and the rate of transmitted HBV, HCV, and HIV during the seroconversion window.

Methods

Subjects and samples collection

Blood volunteers attended to National Center of Blood Bank in Bab-AlMuadham, Baghdad, during the period from July 2018 to January 2019.

The serological assays were performed on plasma samples by using most recent serological kits to detect the HBsAg by enzyme-linked immunosorbent assay (ELISA) (Lot no. Bs-1904-4, fortress, UK), Advanced diagnostic (Lot no. 201704120, USA); advanced kit for detection of antibody to hepatitis C virus by ELISA and detect the presence of HIV-1/2 antibodies and/ or HIV-1 p24 antigen in plasma by EIA through Fourth generation (Lot no. 2017091201, Advanced USA) kit.

One thousand (1000) blood donors who revealed seronegative results for HBV, HCV, and HIV were enrolled in this study and about 1.5 ml of seronegative plasmas were collected in microcentrifuge tube then stored at -80°C until use. The samples were categorized into 500 of plasma samples collected after 6 h from blood withdrawal and the rest (another 500) were collected after 12 h from blood withdrawal (according to the blood bank system).

Pooling of plasma samples

Pooling of seronegative plasma from blood donors for nucleic acid extraction and NAT was carried out as shown in table (1); pre-estimated positive samples 10^3 copies/ml for HBV and HCV and 10^4 copies/ml for HIV were used for qualifying the pooling system to obtain the most appropriate numbers and volume of

samples that could be used in pooling. The pooling validation was done by mixing positive samples with negative samples, for example: One HIV positive plasma (150 µl) + one HCV positive plasma (150 µl) + one HBV positive plasma (150 µl) + 150 µl from 7 individual negative plasma samples resulting in totally 1500 µl pool for 10 individual samples i.e., mini pool 10 (MP10).

Table 1. Pooling system validation used in this study

Sample's volume	No. of pooling	Results for HIV, HCV and HBV
100 µl from each sample	5 samples pooled	Failure (+-+)
100 µl from each sample	6 samples pooled	Failure (--+)
100 µl from each sample	10 samples pooled	Failure (---)
150 µl from each sample	5 samples pooled	Success (+++)
150 µl from each sample	6 samples pooled	Success (+++)
150 µl from each sample	10 samples pooled	Success (+++)

Nucleic acid extraction (RNA and DNA)

QIAamp® MinElute® Virus Spin kit 50 (Lot no. 163029117 Qiagen/Germany), was used according to the manufacturer's instruction for simultaneous extraction and purification of viral RNA and DNA from mini- pooled 10 seronegative plasmas of blood donors. The concentration and the purity of all extracted samples were measured using Quantus Florometer (promega/USA); then, DNA extracts (eluent) were stored at -20°C.

Detection of HBV, HCV, and HIV in minipooled plasmas of seronegative blood donors using multiplex real-time RT PCR

The HCV/HBV/HIV Real-TM (Lot no.10H18H705 sacace /Italy) [8] kit was used; it is a qualitative Real-Time RT PCR test for detection of HIV RNA, HCV RNA and HBV DNA in human plasma. The extracted RNA/DNA from plasma is amplified by using RT-amplification then detection of fluorescent reporter dye probes specific for HCV, HBV, HIV and internal control (IC). This kit is intended for use for individual donors or could be used to test pools comprised of equal aliquots. The recommended samples numbers in one pool must be not more than 5-10 (100-200 µl of the plasma for each sample). The extracted RNA/DNA sample (15 µl) were added to tube with prepared Reaction Mix according to manufacturer's instructions and mixed. The tubes (Samples, positive and negative controls) were transferred to real-time PCR thermalcycler ABI® 7500 (Applied Biosystem /USA). Thermo-cycling profile on Real-time instrument (plate type) was as follows: 1 cycle at 50°C for 20 min, 1 cycle for 15 min at 95°C, 4 cycles for 20 sec at 95°C, 40 sec at 46°C, then 42 cycles for 5 sec at 95°C, 40 sec at 60°C, 40 sec at 45°C. Results were accepted if the positive amplification and negative amplification controls along with negative and positive controls of extraction are passed. Sample was considered to be positive for HCV or HIV or HBV if the value of Ct was lower than 33 according to the manufacturer's instructions [8].

Statistical analysis

The data were processed using SPSS version 16.0.0, Microsoft Excel 2010, and Graphpad Prism version 7.04. The data of the current study were scrutinized carefully in terms of being parametric or non-parametric using normality tests. Accordingly, the proper statistical tests were used. Student t-test and ANOVA test were used for parametric data and Mann-Whitney u test was used for non-parametric data to measure the significance of

difference in means taking into account whether variables of analysis sharing different or equal variance.

Results

The collected 1000 seronegative plasma samples from blood donors were pooled in 100 mini-pools (MP10). The pooled samples were screened by NAT tests with commercial multiplex PCR kit for HBV, HCV and HIV detection (Figures 1 and 2). The results of multiplex PCR revealed that about 3/100 of MP10 seronegative plasmas were HBV, HCV, and HIV positive, as shown in figure 3.

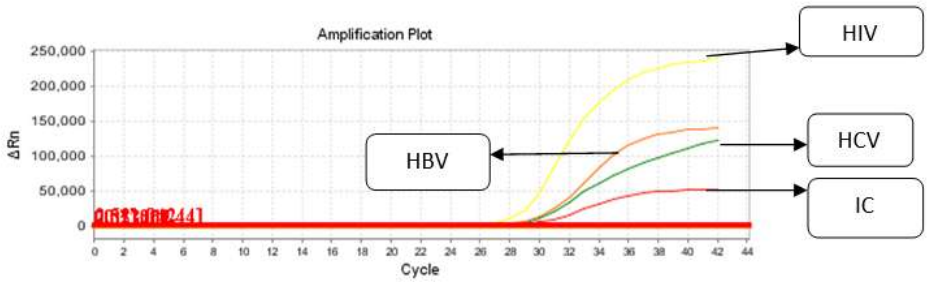

Figure 1. Demonstrate the simultaneous detection of HBV, HCV and HIV with internal control (IC) by multiplexRT- qpcr. on the Fam (Green) channel HCV cDNA was detected, while the Joe (Yellow)/HEX/TET/Cy3 channel detected the HIV cDNA, on the Rox (Orange)/TexasRed channel the HBV DNA was detected and IC on the Cy5 (Red) channel

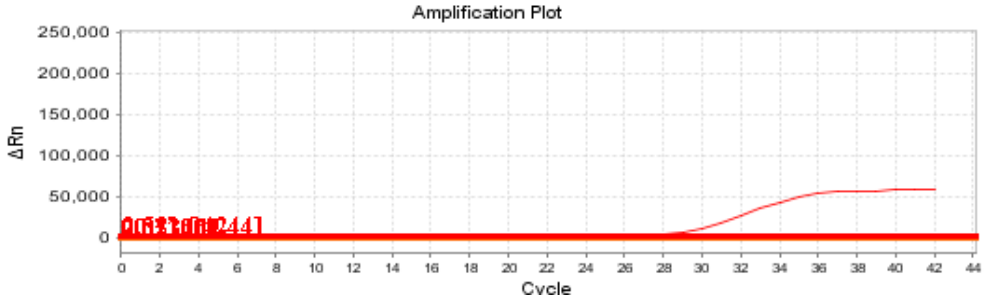

Figure 2. Represent the NC (negative control of extraction) of multiplex RT-qPCR, when the IC only is amplified

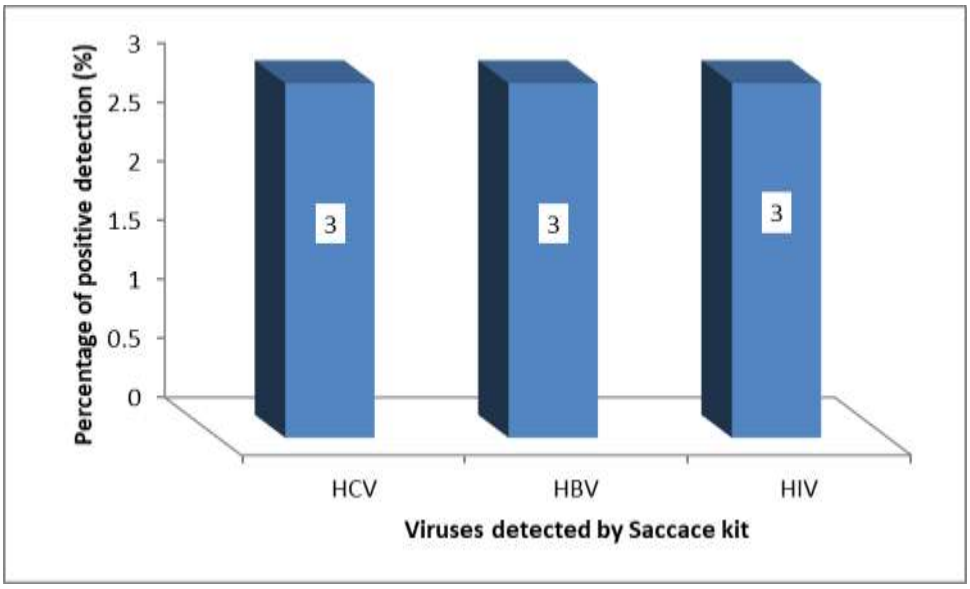

Figure 3. The percentage of positive mini- pooled 10 seronegative plasma samples of blood donors that detected by Sacace commercial kit multiplex PCR for HBV, HCV, and HIV

The detection rate of HBV, HCV, and HIV within 6 h versus 12 h after being withdrawn was shown to be borderline different for HIV and HCV. All the three positive minipools, 3/50 (6%) samples for HIV RNA and HCV RNA were found in plasma from the group of 6 h blood after withdrawal while zero positive sample was found in 12 h group (P=0.06), as shown in figure 4. On the other hand, the positive HBV DNA was found in 2/50 (4%) in 12 h blood group versus 1/50 (2%) in 6 h blood group; however, there was no significant difference in the rate of positive HBV DNA between 6h and 12h blood groups (P>0.05), as shown in figure 4.

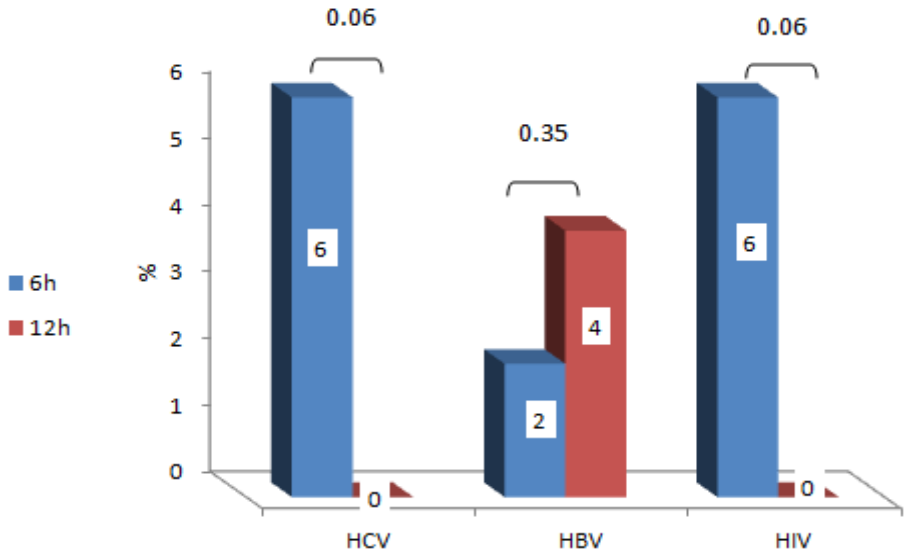

Figure 4. Percentage of positive detection of HCV, HBV, and HIV by Saccace commercial kit within the first 6h versus 12h h from withdrawing blood. The difference in detection rate of HCV and HIV is borderline significantly better in 6h compared to 12h withdrawal of blood. (P value < 0.05)

Discussion

In Iraq, serological screening for blood donors is a standard dependable method for blood screening assay in absence of NAT. There is no previous study found to estimate the residual risk of HIV, HCV, and HBV transmission through blood transfusion during viral seroconversion. The current study estimated the rate of positive HBV, HCV, and HIV within blood donor's seronegative plasma by using multiplex real-time RT-PCR, for mini-pooling samples. Hence, 100 mini-pools (MP10) of seronegative plasma were screened by multiplex PCR revealed that 3 of MP10 were positive for HBV, 3 for HCV, and 3 for HIV. Accordingly, if each positive MP10 contained at minimum one reactive virus, the percentage of transmitted HBV, HCV and HIV during seroconversion will be 0.3% or slightly more. The current research findings are comparable to the results of the research done by donor's database of Dubai Blood Donation Centre (DBDC) from 2008-2009; after introducing of multiplex assay in UAE through 2 years (19%) of blood donors were HBV NAT positive and HBsAg negative. A study applied on 59,283 samples, the potential HBV-positive donors were 187 screened by NAT and serologic assays. Up to 50 HCV-infected donors (12.3%) were reactive for HCV RNA by NAT with negative anti-HCV but only two HIV-infected donors who they were HIV RNA and anti-HIV reactive [(9)].

Another study that was conducted in Turkey in 2017, screening of 3000 seronegative donors by NAT was performed on pools of six blood sera, 9 HBV (0.3%) and 1 HCV (0.03%) and 1 HIV (0.03%) were detected and revealed positive results by NAT [(10)]. In 2019 Iraqi study by Al Sharifi et al. was conducted on 100 multitransfused thalassemic patients to estimate the prevalence of hepatitis B and C viruses by EIA and PCR in thalassemic patients and its relation with blood transfusion, they didn't have any previous HBV and HCV infection and didn't have vaccine, (12%) of patients had a positive HBcAb, while 3 (3%) had positive HBsAg, and higher percentage of HCV infected patients (91%) who regularly received blood transfusion every month and finally the Iraqi study concluded that the sensitive and reliable screening tests required for blood transfusion improvement [(11)]. According to WHO guidelines for blood transfusion in 2017, NAT screening method reduces the window period 4-7 days for HIV, 3-5 days for HCV and 17-27 days for HBV while the viral window periods were 14-28 days for HIV, 9-80 days for HCV and 42-55 days for HBV with serological tests [(7)].

The pooling of 6 to 16 specimens that termed minipool nucleic acid testing (MP-NAT) is done in United State, while in some other countries individual donation testing is performed [(12)]. At the first time, the majority of countries performed the NAT testing in minipools of 96–16 pooled samples; but recently there was a direction towards smaller pools of 6 to individual donations (ID) in order to increase testing sensitivity [(13)].

The current study estimated the impact of the time from blood collection to screening assay and observed that the detection of HCV and HIV was higher after 6 h than 12 h without such observation for HBV. Almeida et al. evaluated the HBV DNA in plasma samples stability when it stored at 4°C for up to 7 days and then at -7°C (frozen), their results revealed insignificant decrease in viral load [(14)]. Gessoni et al. used samples containing different titers of HCV, HIV-1, and HBV to study the stability of viral genomes; they noticed that HCV and HIV-1 RNA can be stored at 4°C for 72h; HBV DNA can be stored until 168 h or about 7 days without reducing in the viral titer [(15)]. The stability of HBV DNA for longer period than HCV and HIV RNA related to the principle that DNA is more stable than RNA and, hence, more resistant to the effects of storage conditions [(16)].

Finally, in spite of NAT has the ability to detect the transfusion-transmissible viruses during the window periods or seroconversion too earlier than serological screening assay, hence the blood screening should be done by serological and NAT assay because both NAT and serological assays can complement each other [(17)].

This study concluded that was an estimated risk of HCV, HIV and HBV transmission through blood transfusion in already-tested seronegative donated blood samples. This can

originate from the sereconversion of HBV, HCV, and HIV and it is obviously that NAT screening assay was sensitive and reliable screening assay in the detection of transfusion-transmissible viruses. Moreover, the use of multiplex qPCR along with mini-pooling of samples was cost effective, time-saving and reduced the cross-contamination problems.

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Author contribution
Dr. Majid: Collection of samples, performing minipooling and NAT assay. Both Dr. Abdulamir and Dr. Ahmed supervised the study. Aufi: Results interpretation and analysis of NAT assay. Abdullah: Performing the serological assay for all collecting plasma samples.

Conflict of interest
Authors declares that there is no conflict of interest.

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Controlled Randomized Clinical Trial on Using Ivermectin with Doxycycline for Treating COVID-19 Patients in Baghdad, Iraq

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Abstract

COVID-19 patients suffer from the lack of curative therapy. Hence, there is an urgent need to try repurposed old drugs on COVID-19. Randomized controlled study on 70 COVID-19 patients (48 mild-moderate, 11 severe, and 11 critical patients) treated with 200 µg/kg PO of Ivermectin per day for 2-3 days along with 100 mg PO doxycycline twice per day for 5-10 days plus standard therapy; the second arm is 70 COVID-19 patients (48 mild-moderate and 22 severe and zero critical patients) on standard therapy, which is (vitamin C, D, and zinc, azithromycin, dexamethasone and oxygen supply if needed). The time to recovery, the progression of the disease, and the mortality rate were the outcome-assessing parameters. Among all patients and among severe patients, 3/70 (4.28%) and 1/11 (9%), respectively progressed to a more advanced stage of the disease in the Ivermectin-Doxycycline group versus 7/70 (10%) and 7/22 (31.81%), respectively in the control group (P>0.05). The mortality rate was 0/48 (0%), 0/11 (0%), and 2/11 (18.2%) in mild-moderate, severe, and critical COVID-19 patients, respectively in Ivermectin-Doxycycline group versus 0/48 (0%), and 6/22 (27.27%) in mild-moderate and severe COVID-19 patients, respectively in standard therapy group (p=0.052). Moreover, the mean time to recovery was 6.34, 20.27, and 24.13 days in mild-moderate, severe, and critical COVID-19 patients, respectively in Ivermectin-Doxycycline group versus 13.66 and 24.25 days in mild-moderate and severe COVID-19 patients, respectively in standard therapy group (P<0.01). It is concluded that Ivermectin with doxycycline reduced the time to recovery, the percentage of patients progress to more advanced stage of disease, and reduced mortality rate in severe patients from 22.72% to 0%.

Keywords Ivermectin, Doxycycline, COVID-19, Coronavirus, SARS-CoV-2

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List of abbreviations: COVID-19 = Coronavirus disease-19, CT = Computerized tomography, FDA = United States Food and drug administration, PCR = Polymerase chain reaction, SARS-CoV-2 = severe acute respiratory syndrome-coronavirus-2, WHO = World Health Organization

Introduction

Since December 2019, the world has been facing unprecedented health caused by global spread of a novel coronavirus, SARS-CoV-2, which causes respiratory, and multi-organ viral infection, called COVID-19. The pandemic of COVID-19 resulted in 35 million infections and more than one million deaths till the date of writing this article [(1)]. Almost all patients start as mild-moderate disease; however, about 15% of them progress within 5-14 days post infection to a more advanced stage of the disease, being severe and critical patients [(2)]. The highest risk patients have been shown to be elderly, obese, diabetic, immunosuppressed, or those with cardiovascular diseases [(3,4)].

The main problem of COVID-19, there is no curative therapy till now; and the vaccine

development takes time. Even after developing a successful vaccine, SARS-CoV-2 seems to induce a short-lasting humeral immunity, 3-12 months only. Therefore, in the coming years when COVID-19 will be converted to seasonal endemic viral infection, a reliable therapy is still needed along with working vaccines [5].

From December 2019 till September 2020, no single drug was found to be a silver bullet for COVID-19. COVID-19 starts as respiratory viral infection but, in some patients, progress to severe viral pneumonia and very dangerous immune deregulation condition called cytokine storm, which if it is not promptly treated, it might result in acute respiratory distress syndrome (ARDS), multi-organ failure, lethal coagulopathies and death [2,4,6]. Therefore, potent antiviral therapy and immunomodulatory therapy for COVID-19 patients are desperately needed.

Ivermectin reduced viral load of SARS-CoV-2 in vitro by 5000 folds within 48 h [7]. Moreover, several previous reports revealed antiviral activity of Ivermectin on Dengue HIV, Yellow fever, West Nile, Hendra, Newcastle, and Zika viruses [8-10]. Furthermore, several observational studies and real-world clinical practice showed that Ivermectin is effective in treating COVID-19 patients at both mild-moderate and severe phases of the disease [11-14]; accordingly, it is thought that Ivermectin might possess antiviral as well as immunomodulatory activity [12-14]. Ivermectin is most probably a host-specific antiviral drug and it acts as a specific inhibitor of importin α/β-mediated nuclear import inhibiting replication of several viruses such as HIV-1, Zika and dengue viruses [10]. It is thought that Ivermectin might inhibit SARS-CoV-2 using the same mechanism [7]. In addition, there have been several reports revealed that Ivermectin acts as anti-inflammatory and immunomodulatory agent and it can curb over-reacting innate and cellular immune responses [8,15]. This explains how Ivermectin could alleviate symptoms of COVID-19 patients at viral replication phase (the first 7-10 days of infection) as well as the later hyperinflammatory phase [7-10].

Doxycycline is a broad-spectrum antibiotic with reported antiviral activities on several viruses including SARS-CoV-2 [16-18]. The mechanism of the antiviral effects of tetracycline derivatives might be due to transcriptional upregulation of intracellular zinc finger antiviral protein, which serves for encoding genes in host cells [19]. Doxycycline acts as an ionophore for zinc facilitating zinc entry into human cells and increasing cytoplasmic zinc concentration; high intracellular zinc levels are inhibitory to the replication of RNA viruses in cytoplasm of the cells by inhibiting RNA-dependent RNA polymerase enzyme [20]. Moreover, doxycycline has immune dampening effect making it useful to ease over-reacting immune systems [21].

In an attempt to find an effective therapy to COVID-19 patients, the current clinical trial was set up to test the combinational therapy of Ivermectin and Doxycycline in treating COVID-19 patients at different stages of the disease.

Methods

Patients

One hundred forty (140) COVID-19 patients at different stages of the disease were included in this study. Half of them (70 patients) received Ivermectin with Doxycycline and standard care while the other half (70 patients) received the standard care only. The patients were recruited in Alkarkh and Alforat hospitals in Baghdad city in the duration from July 1st to September 30th. The recruited patients were either outpatients or inpatients, according to the severity of the disease. Mild-moderate patients were outpatients while severe and critical patients were all inpatients. All of the recruited COVID-19 patients were diagnosed by clinical, radiological and laboratory polymerase chain reaction (PCR) testing. Alike, recovery of COVID-19 patients was based on the disappearance of symptoms, clearance of radiological chest x-ray or Computerized tomography (CT) scans, and getting negative PCR results.

The classification of COVID-19 patients to mild-moderate, severe, and critical was carried out according to the World Health Organization (WHO) guidelines. Ivermectin-Doxycycline group consisted of 48 mild-moderate, 11 severe and 11 critical patients while the control group consisted of 48 mild-moderate and 22 severe patients. For ethical basis, no critical patient recruited in this study was allocated to the control group; all of critical patients were allocated to the Ivermectin-Doxycycline group. The classification of the recruited patients was based on the stage of the disease at the 1st day of recruitment; the designated therapy of the current study was initiated at the 1st day of recruitment. Inclusion criteria of the patients enrolled in the clinical trial were those who were symptomatic for no more than three days for mild-moderate cases, no more than two days after being severe cases, and no more than one day after being critical cases. The purpose behind this was to assess Ivermectin-Doxycycline therapy versus standard care therapy at the beginning of each stage of the disease. The recruited patients were monitored till recovery or death.

The present study was approved by the Ethical and Scientific Committee in Baghdad-Alkarkh General Directorate of under the approval number BKH-CT-016.

Randomization of patients

COVID-19 patients were randomly allocated to one of the study groups depending on a simple method. Patients recruited at dates with odd number were allocated to Ivermectin-Doxycycline group while other patients were allocated to the control group. Inside each group, maximal limit of 48 mild-moderate patients and 22 severe and/or critical patients were allowed. The randomization process as well as the patients records for disease progression, recovery, and clinical or laboratory testing were supervised by the health authority of Alkarkh Health General Directorate in Baghdad city.

Protocols of therapy

Ivermectin-Doxycycline group

Ivermectin 200 µg/kg PO per day for two days, and in some patients who needed more time to recover, a third dose 200 µg/kg PO per day was given 7 days after the first dose. Doxycycline 100 mg capsule PO every 12 h per day was given for 5-10 days, based on the clinical improvement of patients. In addition, standard care was given to the patients of Ivermectin-Doxycycline group based on the clinical condition of each patient.

Control group

The patients in this group received only standard care which included all or some of the following, according to the clinical condition of each patient.

Standard care

- Acetaminophen 500 mg on need
- Vitamin C 1000 mg twice/ day
- Zinc 75-125 mg/day
- Vitamin D3 5000 IU/day
- Azithromycin 250 mg/day for 5 days
- Oxygen therapy/ C-Pap if needed
- Dexamethasone 6 mg/day or methylprednisolone 40 mg twice per day, if needed
- Mechanical ventilation, if needed

Outcome-assessing parameters of the disease progression or recovery

Three parameters used in the present study were to assess the disease progression or recovery in COVID-19 patients who received standard care only compared to patients who received standard care with Ivermectin and Doxycycline therapy. These three parameters are:

1. Time to recovery, if any. It is the time between taking therapy till recovery.
2. Percentage of patients who progress to a more advanced stage of the disease after at least 3 days of giving therapy. For example, a patient was recruited as mild-moderate; after 3 days from starting therapy, the patient progressed to severe stage; such patient is considered a progressing patient

even though she or he was under treatment.
3. Mortality rate among mild-moderate, severe, or critical patients in Ivermectin-Doxycycline group versus those in control group.

Statistical analysis

Data was processed according to the normality tests results; parametric data were represented with mean values and non-parametric data were represented with median values. Percentage of mortality rate was calculated for each group of the study. Odds ratio and chi-square were used to test the strength and significance of association. P values less than 0.05 was considered significant.

Results

Patients' characteristics

Mean age of the recruited patients was 48.7±8.6 year with range 16 to 86 year; patients in Ivermectin-Doxycycline and control groups were age- and sex- matched. Mean age of Ivermectin-Doxycycline group was 50.1±9.3 year with 53% males and 47% females while mean age of control group patients was 47.2±7.8 year with 51% male and 49% females (P>0.05). In both groups, the median post-infection day for starting therapy was 3 days in mild-moderate, 7 days in severe, and 8.5 days in critical cases. The mean weight of Ivermectin-Doxycycline and control patients was 79.6±13.2 kg and 71.5±11.9 Kg, respectively (P>0.05).

Time to recovery

The time to recovery was shown to be significantly reduced in the Ivermectin-Doxycycline compared to the control group; mean recovery time in Ivermectin-Doxycycline group was 10.61±5.3 days versus mean recovery time in control group, 17.9±6.8 days (P<0.05). Hence, using Ivermectin along with Doxycycline reduced mean time to recovery up to 7 days. By analyzing the mean time to recovery in mild-moderate, severe, or critical patients in each group, it was shown that the mean time to recovery in Ivermectin-Doxycycline group was 6.34±2.4, 20.27±7.8, 19.77±9.2 days, respectively versus 13.66±6.4, 24.25±9.5 days, in control group, respectively (P<0.01). Accordingly, Ivermectin-Doxycycline reduced recovery time about 7.32 days in mild-moderate, and 3.98 or roughly 4 days in severe patients (Table 1, Figure 1).

Progression of the disease

The rate of progression of the disease, or the deterioration of the clinical condition of the patients, despite of taking standard care with/without Ivermectin-doxycycline therapy, was shown to be varied between the two groups studied. At beginning, no single mild-moderate patient in both groups progressed to a more advanced stage of the disease. For severe COVID-19 patients, 1/11 (9%) in Ivermectin-Doxycycline group versus 7/22 (31.81%) in control group progressed to more advanced stage of the disease, namely being classified as critical cases (P>0.05). Thus, Ivermectin-Doxycycline protocol was shown to lower progression of the disease in severe patients if given within the first two days of the severe stage of the disease. For critical patients, ethically, critical patients were not included in the control group as critical patients need to receive all possible medications for saving their lives; hence, it was not possible to compare the rate of progression of the disease in critical patients between the two studied groups (Table 1, Figure 2).

Mortality rate

The mortality rate was shown to be 0/48 (0%) in mild-moderate patients in both groups. Nevertheless, the mortality rate was diminished to 0/11 (0%) in Ivermectin-Doxycycline group compared to 6/22 (27.27%) (P=0.052). For critical patients, Ivermectin-Doxycycline did not prevent death in those patients as mortality rate was shown to be 2/11 (18.2%), (Table 1, Figure 3). No critical patients were included in the control group to compare with; however, it is obvious that 18.2% of death in critical COVID-19 patients

received Ivermectin-Doxycycline is much lower than the death rate in critical cases of COVID-19 in Iraq that might reach above 50% (based on real-world data not official published data).

Table 1. Parameters of the study outcomes between Ivermectin-Doxycycline and control groups

Outcome parameter	Subgroup of patients	Ivermectin-Doxycycline	Control	P value
Time to recovery (day)	Total	10.61± 5.3	17.9±6.8	<0.0001
	Mild-moderate	6.34±2.4	13.66±6.4	<0.0001
	Severe	20.27±7.8	24.25±9.5	0.29
	Critical	19.77±9.2	/	
Rate of progression of disease number/total (%)	Total	3/70 (4.28%)	7/70 (10%)	0.19 (OR*=0.4, P=0.2)
	Mild-moderate	0/48 (0%)	0/48 (0%)	1
	Severe	1/11 (9%)	7/22 (31.81%)	0.15 (OR=0.21, P=0.17)
	Critical	2/11 (18.2%)	/	
Mortality rate	Total	2/70 (2.85%)	6/70 (7.14)	0.14 (OR=0.31, P=0.16)
	Mild-moderate	0/48 (0%)	0/48 (0%)	1
	Severe	0/11 (0%)	6/22 (27.27%)	0.052 (OR=0.11, P=0.14)
	Critical	2/11 (18.2%)	/	

OR*: Odds ratio

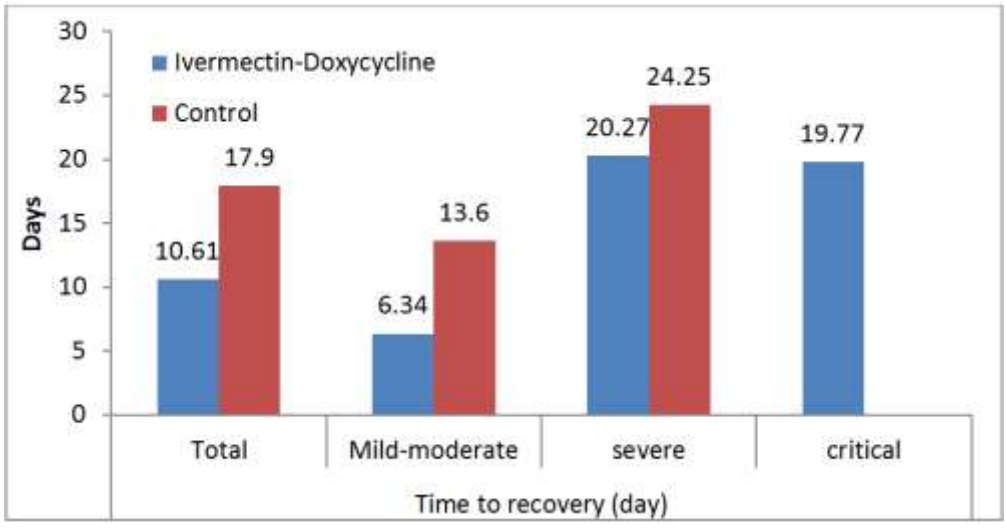

Figure 1. Time to recovery in days between Ivermectin-Doxycycline and control groups

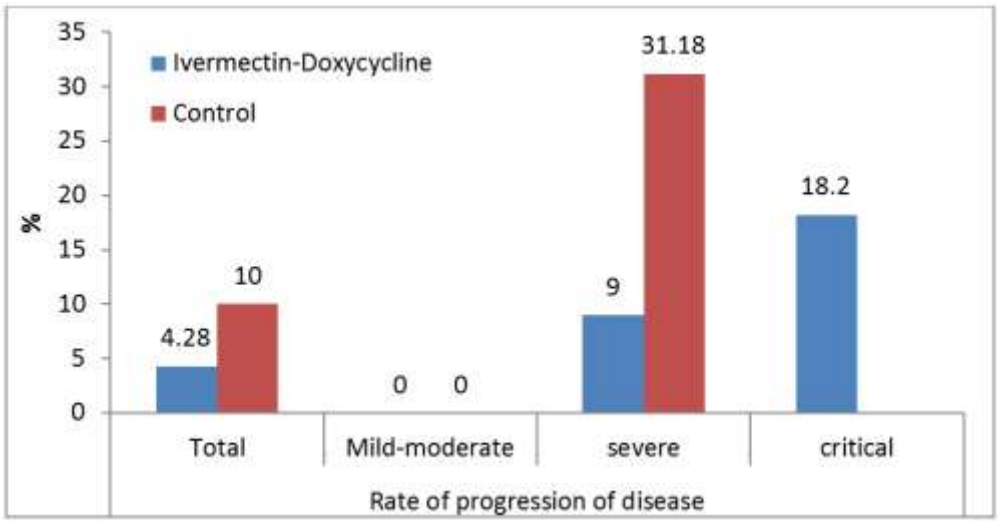

Figure 2. Percentage of patients who showed progression of COVID-19 disease between Ivermectin-Doxycycline and control groups

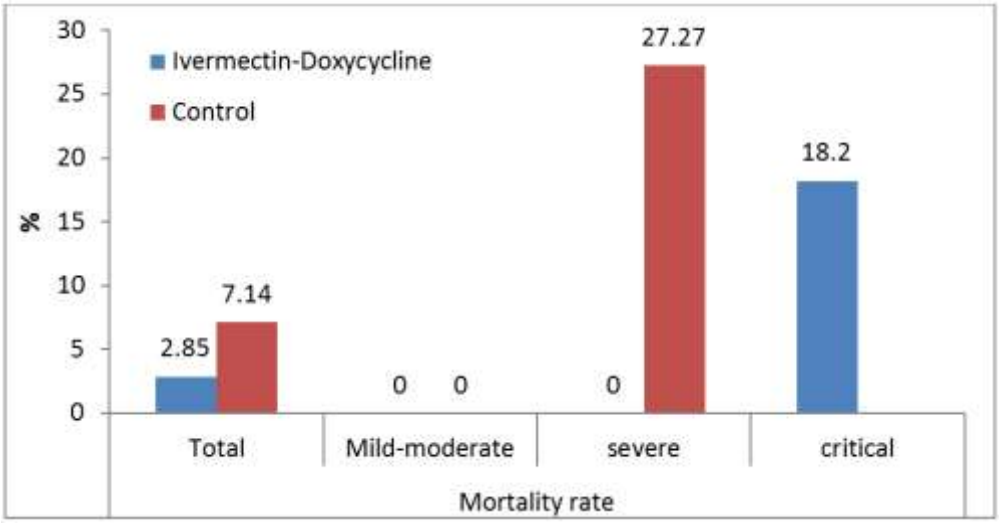

Figure 3. Percentage of patients died in Ivermectin-Doxycycline group versus control group

Discussion

Finding an effective therapy for COVID-19 is an ultimate goal for health bodies all over the world. The problem of the standard care for COVID-19 patient is not curative; however, the current situation is much better than the first months of the pandemic, after introducing steroid therapy for severe/critical patients and high doses of vitamin D3, vitamin C and Zinc for mild-moderate cases [22]. COVID-19 is a multiphasic disease starting with virus replicative phase lasting for 7-10 days then, in some patients, is followed with hyperinflammatory phase and cytokine storm where the most fatalities occur [2,4]. If the viral and hyperinflammatory phases of the disease were not addressed early and properly, the patient might progress to ARDS, which is almost fatal [2,6]. Hence, antiviral, anti-inflammatory, and immunomodulatory medications are necessary to stop the vicious progression of COVID-19 from mild-moderate to severe and to stop the clinical deterioration of the already severe patients.

Accordingly, Ivermectin and Doxycycline were used in this study because both drugs have shown antiviral and immunomodulatory activities [8-15]. Moreover, Doxycycline is a broad-spectrum antibiotic, which tackles the problem of secondary bacterial infection in COVID-19 patients [16-20]. Both drugs are United States Food and Drug Administration (FDA) approved and have a high historical safety record [8,10,16,19]; moreover, no interaction is known between Ivermectin and Doxycycline or between Ivermectin-Doxycycline and any of the medications given in the standard care. By contrary, for example, Azithromycin with Hydroxychloroquine are known to interact adversely for prolonging QT interval of cardiogram which might lead to serious complications [23].

Using FDA approved and safe antiviral and immunomodulatory medications for COVID-19 is scientifically justified for untreatable disease like COVID-19. It has been found that most of COVID-19 patients who progress to severe/critical disease have high viral load of SARS-CoV-2 and over-reacting immune response [24]. Therefore, reducing the viral load and dampening the immune response and inflammatory cytokines are necessary to save patients' lives.

The findings of the current trial showed that Ivermectin-Doxycycline reduced the mean time to recovery from 17.9 to 10.61 days in the recruited COVID-19 patients. Alike, for mild moderate patients, Ivermectin-Doxycycline reduced mean time to recovery from 13.66 to just 6.34 days with reduction in time up to 7.32 days. Nevertheless, Ivermectin-Doxycycline reduced the mean time to recovery in severe patients only 4 days, from 24 to 20 days. Based on these findings, Ivermectin and Doxycycline protocol proves to be effective in speeding up recovery in both mild-moderate outpatients and severe inpatients. This has a tremendous effect on lowering the burden of the disease, minimizing chances of developing immune deregulation, and freeing as quickly as possible hospital beds to other patients. This adds further evidence that Ivermectin-Doxycycline could exert both antiviral and immunomodulatory actives. Several observational studies showed that Ivermectin with/without Doxycycline shortens the time needed to recover COVID-19 patients and Ivermectin is beneficial for mild-moderate as well as severe patients [8-15].

In the current study, Ivermectin-Doxycycline arm lowered the rate of progression of the severe patients from 31.81% to as low as 9%. More interestingly Ivermectin-Doxycycline abolished death in severe patients, 0% mortality rate, compared to control arm, 27.27%. It is noteworthy to mention that the non-progression of the disease and the zero mortality in mild-moderate patients in both arms of the study might be attributed to the early diagnosis and therapy; moreover, the current standard care has become more effective than that used in the early months of the pandemic. However, larger study population is required to trace differences in the disease progression or the mortality rate of mild-moderate patients of COVID-19 taking Ivermectin-Doxycycline compared to patients taking standard care.

The present study reveals that Ivermectin-Doxycycline might stop disease progression and reduce death rate in severe patients of COVID-19. An observational preprint study conducted in Florida showed that Ivermectin cuts mortality rate of severe COVID-19 patients from 80.7% to 38.8% [25]. Interestingly, both Ivermectin and Doxycycline concentrations in the tissue of the lung have been estimated 2 times more than that in plasma [26,27]. Therefore, their antiviral and anti-inflammatory effect on pulmonary tissues is expected to be prominent. These findings provide evidence that Ivermectin might be a potent immunomodulatory in addition to being antiviral agent. Nevertheless, these observational findings still need confirmation by a large randomized controlled study.

This clinical trial concluded that Ivermectin with Doxycycline reduced the time to recovery, the percentage of patients progress to more advanced stage of disease, and reduced mortality rate in severe patients from 22.72% to 0%. Taken together, the earlier administered Ivermectin with doxycycline, the higher rate of successful therapy. Moreover, the observed

benefits of Ivermectin-Doxycycline on the enrolled COVID-19 patients cannot be separated from the effect of the standard care, including Vitamin D and C, Zinc, and steroids, which was given concomitantly. Therefore, giving Ivermectin-Doxycycline along with Zinc, Vitamins D and C, and steroids at the viral and/or the hyperinflammatory phase of the disease seems clinically of benefit. This might shape the future most-fit combinational therapy of COVID-19 patients to minimize as could as possible the death rate and to decrease the duration and the progression of the disease.

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Author contribution

Dr. Hashim and Dr. Maulood applied the intervention on patients and collected data. Dr. Abdulamir, Dr. Ali, Rasheed, Fatak, and Kabah designed the clinical trial and the study. Dr. Ahmed S. Abdulamir wrote the manuscript.

Conflict of interest

Authors confirm that there is no conflict of interest concerning this research and the written manuscript.

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Fibroblast Growth Factor Receptor 3 (FGFR3) Gene Amplification in Patients with Urothelial Carcinoma of the Urinary Bladder

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Abstract

Background The fibroblast growth factor receptors (FGFRs) family includes five tyrosine kinase receptors, which play an important role in many cellular mechanisms including proliferation, migration and survival. Deregulations in the genes that coding for these receptors, mainly the FGFR3 gene, had been reported in patients with urothelial carcinoma of the urinary bladder.

Objective To estimate the frequency of FGFR3 gene amplification in patients with urothelial carcinoma of the urinary bladder and its relation to some clinico-pathological parameters of the tumor.

Methods The present retrospective study included 30 paraffin blocks of urothelial carcinoma tissue (trans-urethral resection of bladder tumor (TURBT)), and 10 samples of normal bladder tissue (control group). Sections were taken from each paraffin block for studying FGFR3 gene amplification by fluorescent in situ hybridization (FISH) technique.

Results FGFR3 gene was shown to be amplified in 4 (13.3%) out of 30 urothelial carcinoma cases. There was significant relation between FGFR3 gene amplification and tumor's pathological stage (p=0.037). FGFR3 gene wasn't statistically correlated with the patient's age, gender, tumor's grade or the lympho-vascular permeation.

Conclusion FGFR3 gene amplification was detected in 13.3% of urothelial carcinoma of the urinary bladder cases, which may reflect its role in carcinogenesis process. This amplification was statically correlated to tumor's pathological stage suggesting its relation to tumor progression.

Keywords FGFR3, FISH, TURBT, urothelial carcinoma, gene amplification

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List of abbreviations: FFPE = Formalin-fixed paraffin-embedded, FGFR3 = Fibroblast growth factor receptors 3, FISH = Fluorescent in situ hybridization, H&E = Hematoxylin and eosin, TURBT = Trans-urethral resection of bladder tumor

Introduction

Bladder cancer is the tenth most common cancer worldwide and the fourth most common cancer in Iraq. Male predominance is highly observed (Male: Female about 4:1) [(1)].

Urothelial (transitional cell) carcinoma account for 90% of all bladder cancers. Other rare types include squamous cell carcinoma, adenocarcinoma and small cell carcinoma account for 9% of bladder tumors. It is worth to mention that although squamous carcinoma is a rare type, it accounts for 81% of bladder cancer in area where schistosomiasis is endemic [(2)].

At molecular level, recent studies show that urothelial carcinoma follow two different pathways of gene deregulation. Fibroblast growth factor receptors 3 (FGFR3), phosphoinositide 3-kinases/ protein kinase B (PI3K/AKT) pathway and Rat Sarcoma (RAS)

pathway are usually associated with low grade, non-invasive tumors, while high grade, muscle invasive tumors usually follow different pathway include deregulation in tumor suppressor gene P53, p16 and RB [3].

Fibroblast growth factor (FGF) pathway plays an important role in various cellular functions, including cellular differentiation, proliferation, apoptosis and migration. Also, the fibroblast growth factor/fibroblast growth factor receptor (FGF/FGFR) signaling pathway have role in embryogenesis, angiogenesis, tissue homeostasis and wound healing process. Deregulation of FGF/FGFR signaling pathway shown to be play important role in carcinogenesis. The mechanisms by which this pathway can be deregulated include mutation, translocation or gene amplification [4].

Deregulations in FGF/FGFR signaling pathway has been reported to promote the development of different type of cancer through oncogenesis, neoangiogenesis and anti-cancer drug resistant mechanisms [5].

Alteration in FGFRs genes, especially FGFR3 gene, had been reported in patients with urothelial carcinoma of the bladder. These alterations could be in form of activating mutation, gene amplification or chromosomal rearrangement. These finding make the FGF/FGFR signaling pathway a potential therapeutic target for many types of cancers [6].

Many clinical trials had been conducted to evaluate the efficacy of FGFR target therapy in urothelial tumors that harbor specific alteration in FGFR3 gene [7,8].

Recently, the Food and Drug Administration (FDA) grants an accelerated approval for the pan-FGFR inhibitor Erdafitinib, a selective tyrosine kinase inhibitor for patients with urothelial carcinoma whom have alteration in FGFRs genes [7].

So, the aim of the study to estimate the frequency of FGFR3 gene amplification among patients with urothelial carcinoma in order to identify those who will get benefit from recently released, FDA approved target therapy.

Methods

Sample collection

The present retrospective study included 30 formalin-fixed paraffin embedded (FFPE) tissue blocks of urinary bladder biopsies from patients whom had been diagnosed with urothelial carcinoma of the urinary bladder during 2017 and 2018, were collected from Al-Basra Teaching Hospital Laboratory, Al-Mawanea General Hospital Laboratory and Doctor Sawsan Al-Haroon Private Laboratory.

Also, 10 samples of normal bladder tissue (control group) collected from autopsy cases from Forensic Medicine Unit in Basra and were processed as FFPE blocks in the Department of Pathology and Forensic Medicine, College of Medicine/ Al-Nahrain University.

Clinical and pathological information were collected from patient admission case sheets and pathology reports.

Sectioning

From each FFPE tissue block, two sections of 5 µm thickness were obtained. One section was processed and stained with hematoxylin and eosin (H&E) stain for revision of the diagnosis, and the other section were applied onto positively charged slides and submitted to a two days procedure of fluorescent in situ hybridization (FISH) study for FGFR3 gene.

Cytogenetic study procedure

Sections which applied onto positively charged slides were processed through two days procedure for FISH study of FGFR3 gene using ZytoVision FGFR3/4p11 dual-color probe (Germany).

In day one, slides were incubated at 70°C for 10 minutes then incubated in xylene solution twice for 10 minutes each time. Slides then rehydrated by series of descending concentrations of ethyl alcohol (100%, 100%, 90% and 70%) for 5 minutes each time. The slides then washed twice with distilled water for 2 minutes each time, and incubated in pre-warmed Heat Pretreatment Solution Citric at 98°C for 15 minutes duration. Furthermore, the slides washed two times in distilled water for two minutes each time, the allowed to dry.

Pepsin drops then applied over the specimen and the slides then incubated in humidity chamber at 37°C for 25 minutes. The slides then washed with Wash Buffer saline-sodium citrate (SSC) for 5 minutes and then washed with distilled water for one minute. Slides then dehydrated by series of ascending concentrations of ethyl alcohol (70%, 90% and 100%) for one minute each time and then allowed to dry. Ten µl of ZytoLight SPEC FGFR3/4p11 dual color probe was applied onto each specimen and cover-slip was applied and sealed with rubber cement. Denaturation was allowed for 10 minutes at 75°C then slides were transferred to humidity chamber to hybridize overnight at 37°C.

Next day, slides were removed from humidity chamber, rubber cement was removed carefully and the slides were submerged in pre-prepared 1X Wash Buffer A for 3 minutes at 37°C to remove the cover slips, and then washed twice with the 1X Wash Buffer A twice for 5 minutes duration each time, followed by incubation in a series of ascending concentration of ethyl alcohol (70%, 90% and 100%) for one minute each time. Slides then allowed drying in dark area and 25 µl of diamidino-2-phenylindole (DAPI) solution was applied followed by cover slip. Finally, slides incubated for 15 minutes in dark area then transferred to the refrigerator at 6°C and stored till the time of examination.

Evaluation of FGFR3 gene had been carried out using Zeiss Axio Imager Z2 fluorescent microscope with 64X oil immersion subjective lens. Three filters had been used Texas Red, DAPI and fluorescein isothiocyanate (FITC) filter for specimen evaluation.

Image capturing had been done using CoolCube 1 Digital High-resolution Camera and photo analyzed by fluorescence imaging system by MetaSystem Company.

The FGFR3/4p11 Dual color probe composed of green-labeled poly-nucleotides (ZyGreen) that target FGFR3 gene region and orange-labeled poly-nucleotides (ZyOrange) that target a specific sequence in chromosome 4 in the chromosomal region 4p11.

The number of FGFR3 and 4p11 signals was counted in 50 nuclei and identified to be amplified if has one of the following criteria: [6,9]

1. FGFR3/4p11 ratio is ≥ 2.
2. The average number of FGFR3 signals per tumor cell is ≥ 6.
3. Ten percent of the tumor cells or more contain ≥ 15 FGFR3 signals or large clusters.
4. Fifty percent of the tumor cell or more contain ≥ 5 FGFR3 signals.

The statistical analysis of this study was performed with the statistical package for social science (SPSS Version 25) and Microsoft Excel 2013. Numeric variables were described as mean, median and standard deviation. Independent-samples ttest was used to estimate the statistical difference between them. Categorical data were formulated as numbers and percentages. Chi-square test was used to measure the association between categorical variables. P-value was considered to be significant if it was less than 0.05.

Results

Out of total 30 urothelial carcinoma cases, 24 (80%) patients were males and only 6 (20%) patients were females with a male to female ratio were 4:1.

The overall mean age was 65.73±8.38 years (range: 51-77 years).

Eighteen patients (60.0%) out of 30 cases with urothelial carcinoma had high grade tumors (Figure 2); while low grade tumors had been recorded in the remaining 12 cases (40.0%) (Figure 1) with no case had met the criteria for papillary urothelial neoplasm of low malignant potential (PUNLMP) category.

Fourteen cases (46.67%) out of 30 shown to have muscle invasion features (T2) (Figure 3), while the remaining 16 cases (53.33%) where have lamina propria invasion only (T1). No cases with a feature of Tis or Ta subcategories had been recorded.

Lymphovascular permeation was observed in 18 (60%) out of 30 urothelial carcinoma cases (Figure 4), while the remaining 12 cases (40%) were didn't have lymphovascular permeation.

Four (13.3%) cases out of 30 urothelial carcinoma cases show feature of FGFR3 gene amplification (Figure 5), while the remaining 26 (87.7%) cases didn't meet any feature of FGFR3 gene amplification (Figure 6).

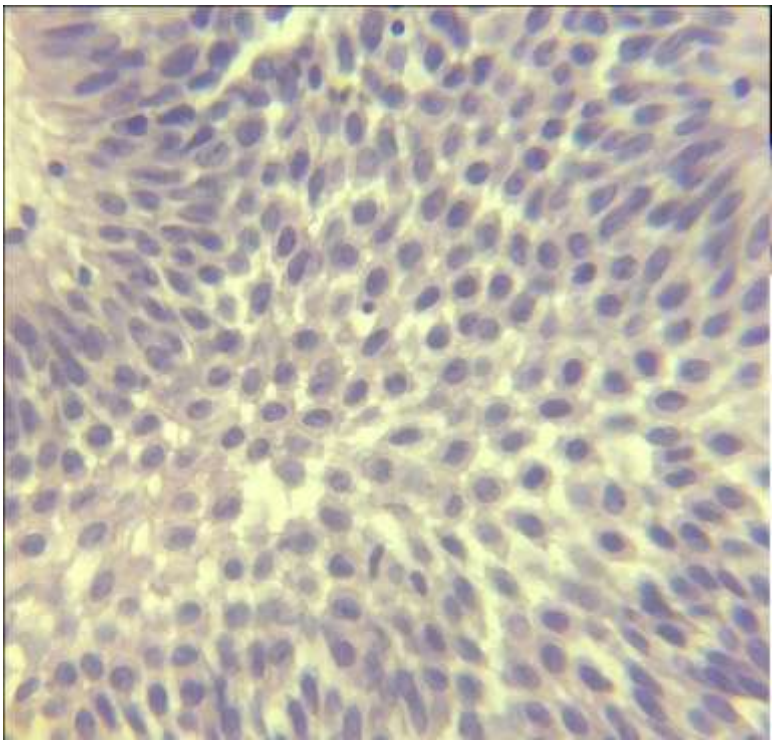

Figure 1. Low grade, papillary urothelial carcinoma of the urinary bladder shows minimum cytological atypia, minimum nuclear pleomorphism with infrequent mitosis. (40X)

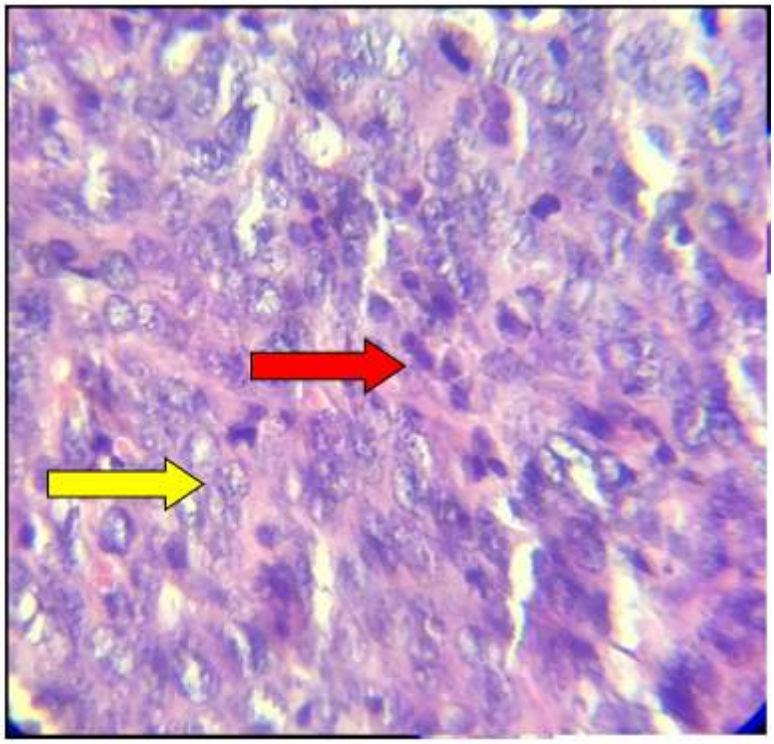

Figure 2. High grade urothelial carcinoma of the urinary bladder shows marked cytological atypia, loss of polarity, nuclear pleomorphism, prominent nucleoli (yellow arrow) and increased mitotic figure (red arrow). (40X)

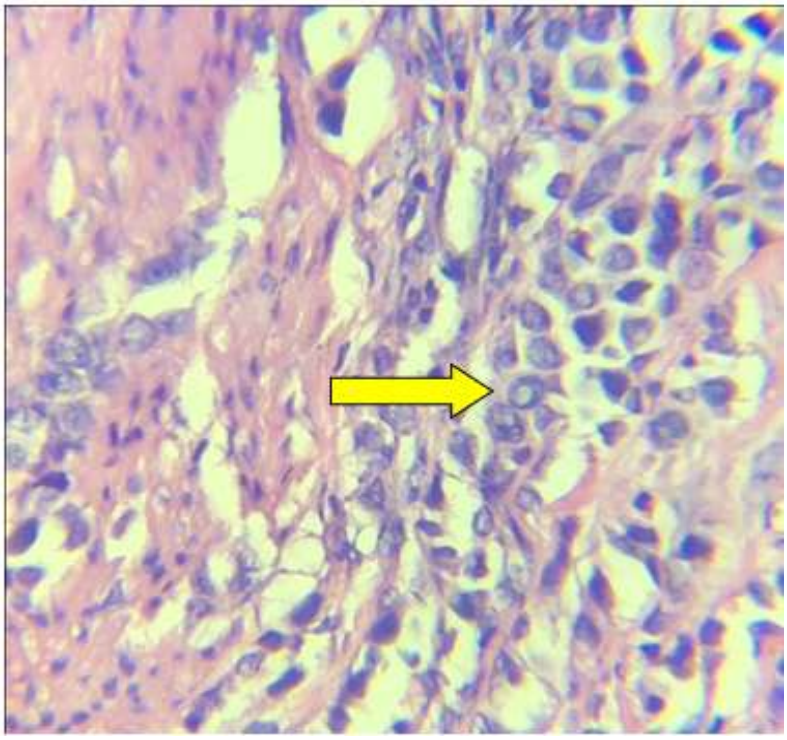

Figure 3. High grade urothelial carcinoma, pathological stage T2. Cluster of tumor cells with marked pleomorphism, hyperchromasia and prominent nucleoli invading muscular layer (Arrow). (40X)

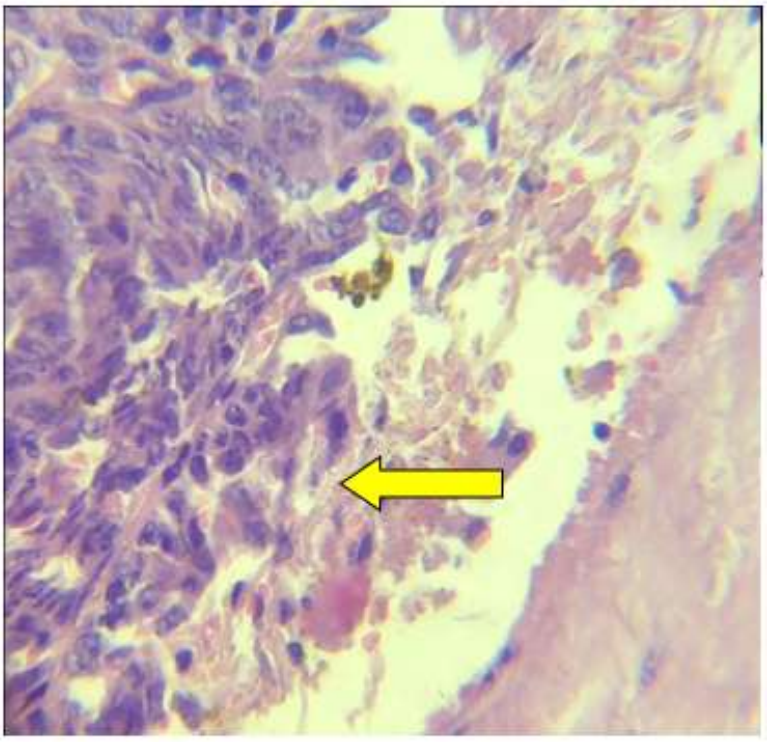

Figure 4. High grade urothelial carcinoma with lymphovascular invasion, shows nest of tumor cells with large pleomorphic nuclei and prominent nucleoli invading vessel wall (Arrow). (40X)

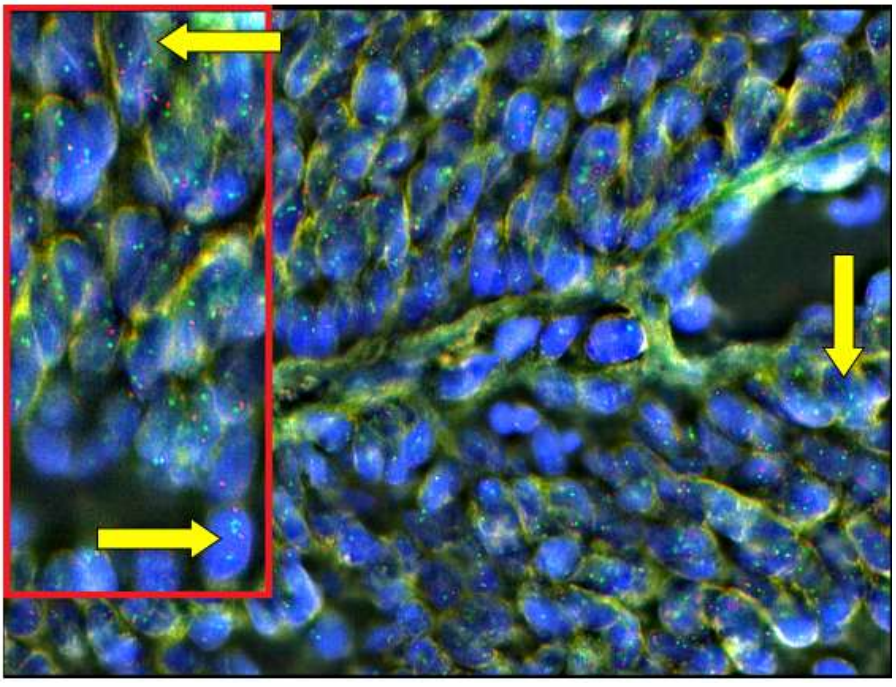

Figure 5. High grade urothelial carcinoma of the urinary bladder with FGFR3 gene amplification. FGFR3 dual color probe hybridize to malignant cells showing FGFR3 gene amplification as indicated by two chromosome 4 (Red) signals and >2 FGFR3 (Green) signals in the nucleoli (Arrows). (64X)

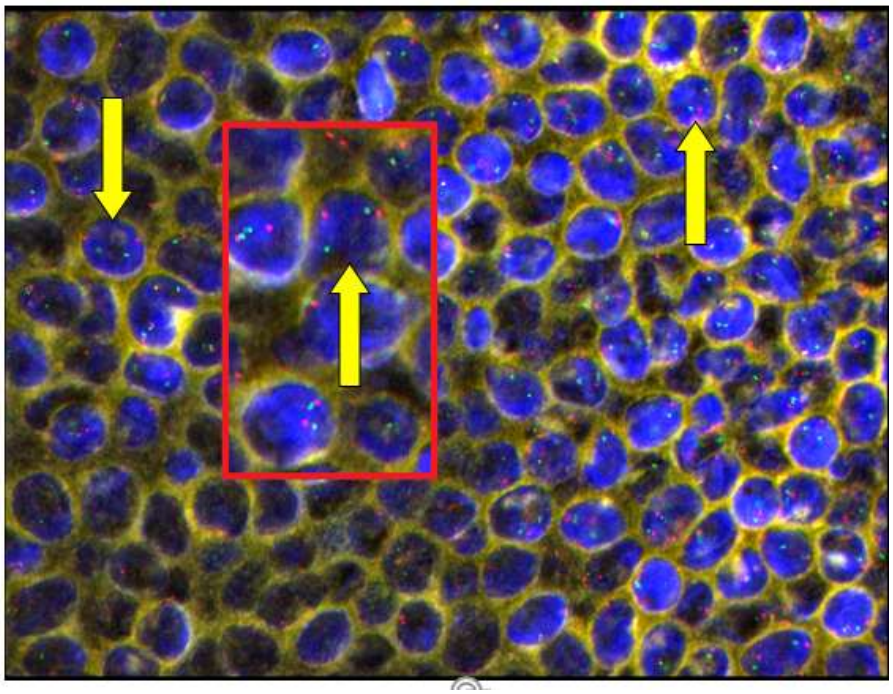

Figure 6. Low grade urothelial carcinoma of the urinary bladder with no amplification in FGFR3 gene. FGFR3 dual color probe hybridize to malignant cells showing no amplification in FGFR3 gene as indicated by two chromosome 4 (Red) signals and two FGFR3 (Green) signals in the nucleoli (Arrows). (64X)

Mean age of FGFR3 gene amplified group was 65.0±5.77 in years, while the mean age of FGFR3 gene non-amplified group was 65.85±5.79 in years. There was no statistically significant difference in mean age between FGFR3 amplified and FGFR3 gene non-amplified groups (P value=0.855).

Two (33.3%) out of the total 6 female patients and 2 (8.3%) out of total 24 male patients had an amplified FGFR3 gene (P value=0.169). FGFR3 gene was shown to be amplified in 2 (16.67%) out of 12 cases of low-grade urothelial carcinoma and in 2 (11.11%) out of total 18 high grade cases (P value=1.00). FGFR3 gene were shown to be amplified in 4 (22.2%) out of 18 cases with lymphovascular permeation, while all 12 non-lymphovascular invasive cases were associated with no amplification in FGFR3 gene. (P value =0.13), (Table 1).

Four (28.57%) case out of 14 muscle invasive (T2) cases, were shown to have amplified FGFR3 gene, while all the 16 lamina propria invasive (T1) cases shown to have no amplification in FGFR3 gene. There is a significant correlation between pathological stage and FGFR3 gene amplification (P value=0.037), (Table 1).

Table 1. Relation between FGFR3 gene amplification to patient's clinico-pathological parameters

Parameter		No. of patients	FGFR3 gene amplified	P value
Gender	Male	24	2	0.169
	Female	6	2	
Grade	High	18	2	1.00
	Low	12	2	
Stage	pT1	16	0	0.037
	pT2	14	4	
LVI*	Absent	12	0	0.13
	Present	18	4	

* LVI: Lympho-vascular invasion

Discussion

FGFR3 is a tyrosine kinase receptor that is encoded by FGFR3 gene, which located in 4p 16.3 region [10]. Its signaling pathway is involved in many cellular physiological processes, including cellular proliferation, differentiation, migration and survival. Alterations in FGFR3 gene will lead to aberrations in this signaling pathway and play an important role in carcinogenesis process, tumor progression and acquired resistance to anti-cancer therapies in many types of human cancers. These alterations could be in form of activating mutation in FGFR3 gene, chromosomal rearrangement or gene amplification [5].

Alterations in FGFR3 gene had been recorded frequently among patients with urothelial carcinoma of the urinary bladder. This may reflect that FGFR3 may have role in the carcinogenesis process of these tumors and suggest that the FGFR3 may be a potential therapeutic target for urothelial tumors [11].

Among these alterations, the activating mutation in FGFR3 gene have been found to be the most common alteration that may affect the FGFR3 gene among patients with urothelial carcinoma and associated mainly with low grade, non-invasive tumors [6]. Mostly, these mutations involve the extracellular and transmembrane domain and lead to constitutive activation and ligand-independent dimerization of this receptor [12].

Additionally, chromosomal rearrangement involving FGFR3 gene, which forms a constitutively activated fusion gene had been reported in about 6% of invasive urothelial tumors [12]. The transforming acid coiled coil 3 (TACC3) gene, which located within 48kb of FGFR3 on 4p16.3 region, had been found to be the most common fusion partner [13].

The present study was conducted to estimate the frequency of FGFR3 gene amplification among patients with urothelial carcinoma of the urinary bladder and to assess its relation to other clinical and pathological parameters.

In this study, FGFR3 gene was shown to be amplified in 13.3% of patients with urothelial carcinoma of the bladder. This result was higher than the result of a German study by Fischbach et al. which reported that FGFR3 gene was found to be amplified in 3.4% of bladder cancer cases [6].

Other study conducted in USA by Helsten et al. [11] reported that FGFR3 gene was amplified in 2% of cases of urothelial carcinoma.

In Egypt, a study by Hammam et al. [14] shows that the amplification of FGFR3 gene was documented in 88.2 % of cases with urothelial carcinoma of the urinary bladder, mostly cases of bilharzias associated carcinoma. This may explain the high percentage of reported FGFR3

gene amplification in this study due to endemicity of bilharzias in this region.

Present study revealed that there is significant correlation between tumor pathological stage and FGFR3 gene amplification that is parallel to the Egyptian study done by Hammam et al. 14 but, disagree with the German study by Fischbach et al. [6]. This correlation could be explained by that FGFR3 gene amplification is a late event in the development of urothelial carcinoma.

The present study also shows no significant correlation between FGFR3 gene amplification and tumor grade which is parallel to the German study by Fischbach et al. [6], which found that FGFR3 gene amplification were almost equally distributed in low- and high-grade tumors.

Furthermore, no significant correlation between FGFR3 gene amplification and lymphovascular invasion was found in the present study.

In conclusions, FGFR3 gene amplification was detected in 13.3% of urothelial carcinoma of the urinary bladder cases which may reflect its role in carcinogenesis process. This amplification was statically correlated to tumor's pathological stage suggesting its relation to tumor progression.

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Author contribution

Al-Marashi: Collection of samples, perform the laboratory procedures, and analyze the result statistically. Dr. Qasim is the supervisor of this study. She contributed by slide revision, FGFR3 gene amplification scoring revision and assesses the results of this study. Both authors contributed in writing the manuscripts.

Conflict of interest

The authors declare no conflict of interest.

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The Effect of Low Level Laser Therapy on Early Onset Rheumatoid Arthritis Patients

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Abstract

Background Rheumatoid arthritis (RA) is a systemic chronic, inflammatory disease that may affect many tissues and organs.

Objective To investigate effectiveness and safety of Low-level-laser-therapy (LLLT) in management of early onset RA compared to symptomatic non-steroidal anti-inflammatory drugs (NSAIDS) therapy.

Methods A convenient selection 3 arms single blinded trial conducted in Al-Saraj Center for Rheumatoid Diseases in Baghdad during period between January-May 2017. Thirty-four patients with RA onset below one year were recruited. Disease activity score (DAS28) formula with American College of Rheumatology criteria (ACR20), erythrocyte sedimentation rate (ESR), visual analogue scale (VAS), complete blood count (CBC), C-reactive protein (CRP), rheumatoid factor (RF) were measured. Patients were divided into three groups: group 1 (n=12) received LLLT, group 2 (n=12) received placebo laser and naproxen and group 3 (n=10) received only naproxen. Primary outcomes measured were disease activity using DAS28 score, clinical improvement using ACR20 and pain assessment using VAS. Secondary outcomes measured were remission ACR50 and 70 and inflammatory indicators.

Results LLLT group has shown significant decrease of DAS28 (P=0.02), morning stiffness duration (p=0.05), number of tender joints (p=0.03), number of swelling joints (p=0.04), and VAS (p=0.01) compared to baseline whereas placebo laser group with naproxen and naproxen only group showed only significant reduction in duration of morning stiffness(P=0.04) and (p=0.048) respectively. There was marginal lowering of ESR (P=0.06) in LLLT group but no changes in CRP, RF. There were no reported side effects of LLLT use.

Conclusion Laser therapy is better than NSAIDS in controlling RA symptoms with no associated side effects. Therefore, it is recommended as first-line therapy in early onset RA.

Keywords laser, Low level laser therapy, rheumatoid arthritis, NSAIDS, ESR, Nerve root

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List of abbreviations: ACPAs = Anti-citrullinated protein antibodies, ACR = American College of Rheumatology criteria, AlGaAs = Gallium aluminum arsenide diode laser, CBC = Complete blood count, CRP = C-reactive protein, DAS28 = Disease activity score, DMARDs = Disease-modifying antirheumatic drugs, ESR = Erythrocyte sedimentation rate, LLLT = Low-level-laser-therapy, M±SD = mean±standard deviation, mW = milli watt, NIPH = National Institute of Public Health, RA = Rheumatoid arthritis, RF = Rheumatoid factor, SJN = Swollen joint number, TJN = Tender joint number, VAS = Visual analogue scale

Introduction

Rheumatoid arthritis (RA) is a systemic chronic, inflammatory disease that may affect many tissues and organs, but primarily affects joints causing an inflammatory synovitis that frequently contributes to articular cartilage damage and joint ankyloses

[1-3]. RA may also cause systemic inflammation of the lungs, pericardium, and sclera, as well as nodular lesions, which are most prevalent in skin. While etiology of RA is not fully understood, autoimmunity plays a key role in its chronicity and progression [4,5]. Worldwide, approximately 1% of the population suffers at some point of their life from RA. Females are three times more affected than men with onset most prevalent between 40 and 50 years old, yet can influence any age. RA can be an impaired and debilitating disease as a result of severe progressive loss of control and mobility [6].

The primary diagnosis starts with history and physical examination in association with laboratory tests especially rheumatoid and anti-citrullinated protein antibodies (ACPAs) and X-rays [7]. Different therapeutic protocols are available. Physical exercise, brace, splint, and occupational therapy are used with non-pharmacological care. The goal of therapy is firstly to achieve pain relief, and secondly to avoid potential joint damage and the resultant impairment if the condition continues untreated. These two goals do not necessarily coincide: while pain killers do accomplish the first goal, the long-term outcomes are not affected [8]. Analgesia and anti-inflammatory medications, including steroids, are used to relieve the symptoms, while anti-rheumatic disease-modifying medicines (DMARDs) are also used to slow or interrupt the underlying immune response, produce long-lasting symptomatic remissions [9] and avoid long-term damage. DMARDs influence biological measures such as levels of erythrocyte sedimentation rate (ESR) and hemoglobin and autoantibody and reduce the risk of bone and cartilage harm. The new generation of biologics has recently expanded therapeutic choices [10].

Low-level light therapy (LLLT) also known as cold laser, low power laser, bio-stimulation, photo-biomodulation, is a medical technique in which the low-level laser is used to stimulate or inhibit the cellular function. LLLT precipitates a complex series of cellular-level physiological interactions that reduce acute inflammation, reduce pain and speed up tissue healing [11] and effectiveness in treating chronic and acute pain associated many inflammatory diseases has been reported [12]. The role of LLLT in RA has been investigated, however, results are contradicting [13-15]. This study aimed to investigate the effectiveness and safety of LLLT in the management of early onset RA in a sample of Iraqi patients, applying different ways to alleviate disease activity and improve pain including acupuncture points, trigger points, nerve supply and referring pain points.

Methods

Study design

This is a convenient randomized clinical trial conducted at Al-Saraj Center for Rheumatoid Diseases during the period between January-May 2017. The trial was registered at National Institute of Public Health (NIPH) Clinical Trials of Japan (rctportal.niph.go.jp), with a Unique ID number (UMIN000042632). The study protocol was approved by the Scientific and Ethical Committee in Al-Kindy College of Medicine, University of Baghdad.

Patients and groups

Thirty-four patients diagnosed with RA according to revised criteria for RA classification in 1987 [16] when they showed at least four of these seven criteria: 1) morning stiffness; 2) arthritis of three or more joint area; 3) arthritis of hand joints; 4) symmetrical arthritis; 5) rheumatoid nodules; 6) serum rheumatoid factor; 7) radiographic changes, given that these criteria have been present for at least 6 weeks and not exceeding one year. Patients with 2 clinical diagnoses were excluded. None of the participants had extra articular involvement such as rheumatoid nodules or Felty syndrome or skin or cardiovascular.

Fifteen (44.1%) of patients were classified as moderately active disease DAS28 (3.2-5.1) and 19 (55.9%) had severely active disease DAS28 (>5.1). Patients were conveniently randomized to three groups so that each group contain

approximately equal rate of disease severity as shown in Table 1; first, group1 (n=12) received LLLT, second, group 2 (n=12) received placebo laser and naproxen and the third, group 3 (n= 10 patients) who received only naproxen.

Table 1. Distribution of patients in three groups according to disease activity score DAS28

Activity DAS28	Group 1	Group 2	Group 3	Total
Moderate (3.2-5.1)	5 (14.7%)	5 (14.7%)	5 (14.7%)	15 (44.1%)
Severe (>5.1)	7 (20.6%)	7 (20.6%)	5 (14.7%)	19 (55.9%)
Total	12 (35.3%)	12 (35.3%)	10 (29.4%)	34 (100%)

Intervention

Patients in group 1 received 20 sessions of LLLT, in the form of 2 courses separated by 10 days free, each course contains 10 sessions of LLLT divided as 3 sessions per week. Each session included four steps: 1) Irradiate the point of pain, which the patient complains from the most for 15 min. 2) Irradiate the acupuncture points according to (HAND laser acupuncture treatment protocol) for 15 min [(17)]. 3) Irradiate the site that might be the origin of the referring pain to the figures and wrist according to anatomical map of trigger points for 15 min [(18)]. 4) Lastly irradiate the site of C7, C6 and C5 at the side of the pain for one minute each.

Two laser apparatuses were used for therapy in this study; Gallium aluminum arsenide diode laser (AlGaAs), 830 nm, maximum output power 300 mW used for the first 3 steps [(15)] and Helium-Neon Laser of wave length 632,8 nm, continuous emission, output power 7.3 mW applied in step four of a session [(19)].

Patients in group 2 received deactivated placebo laser courses (electrical transcutaneous simulator) with naproxen 1000 mg/day in two divided doses.

Patients in group 3 received naproxen 1000 mg/day in two divided doses.

Outcome's measurement

The main three outcomes measured were disease activity using DAS28 and clinical improvement using ACR20% and pain assessment using visual analogue scale (VAS). DAS28 formula which is a composite score derived from the assessment of 4 measures [(20)]: 1) count the number of swollen joints (out of the 28), 2) count the number of tender joints (out of the 28), 3) ESR and 4) patients' global assessment of health by a questionnaire. The final score is calculated by a special formula, a DAS28 of greater than 5.1 implies active disease, less than 3.2 low disease activity, and less than 2.6 remission [(20)].

Clinical improvement was further measured according to criteria of ACR20% (20) by assessing 2 parameters; 1) equal or more than 20% improvement in tender joint number (TJN); 2) equal or more than 20% improvement in swollen joint number (SJN). A positive ACR20% should have fulfill both above parameters in addition to ≥20% in three of the following five parameters: patient pain assessment, patient global assessment, physical global assessment, patient self-assessment disability and acute phase reactant ESR and C-reactive protein (CRP).

VAS is estimated using a scale between 0 and 10 cm, for which 0 represents no pain and 10 represents maximum pain (unbearable) using a questionnaire filled by the patient [(21)].

Secondary outcomes included ACR remission criteria measured by fulfilling five or more of the following criteria for at least 2 consecutive months: morning stiffness <15 min, no fatigue, no joint pain, no joint tenderness or pain on motion, no soft tissue swelling, ESR <30 mm/hr in females and <20 mm/hr in males. Complete blood count (CBC), ESR, CRP, liver function test,

renal function test, rheumatoid factor (RF) and X ray for affected joint were obtained for each patient at baseline and after 10 weeks [20].

Statistical analysis

Statistical analysis was done using IBM SPSS version 20 (IBM, Armonk, NY, USA). The results were presented in mean± standard deviation or range as necessary. Comparison between means was calculated using Student-t test and ANOVA test with post-hoc test as indicates implementing $p<0.05$ as a significance level.

Results

The mean age of participants was 49.6 ranged between 28 and 69 years, most of them were women (73.5%) as shown in table 2 and figure 1, female:male ratio was 2.77:1.

Table 2. Age and gender distribution of study groups

Variable		No.	%
Age categories	20-40	7	20.6
	41-60	22	64.6
	>60	5	14.7
Gender	Male	9	26.5
	Female	25	73.5
Total		34	100

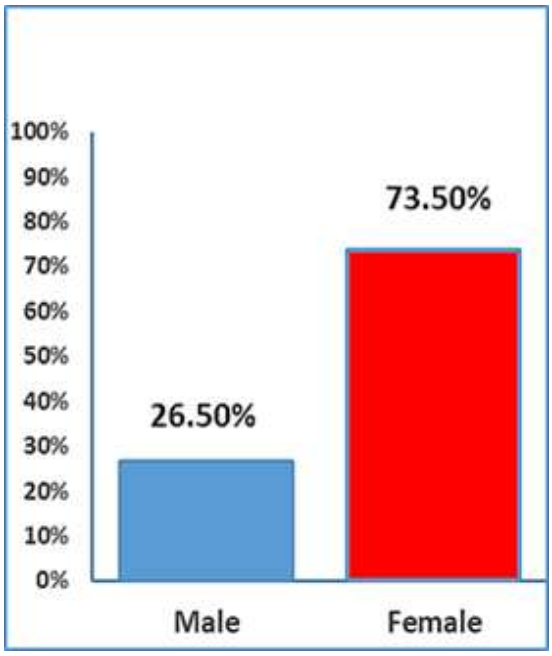

Figure 1. Distribution of patients according to gender

At base line assessment, DAS28, ESR, morning stiffness, TJN, SJN and VAS were not significantly different between study groups. RF was positive in only 56% of the patients shown in figure 2.

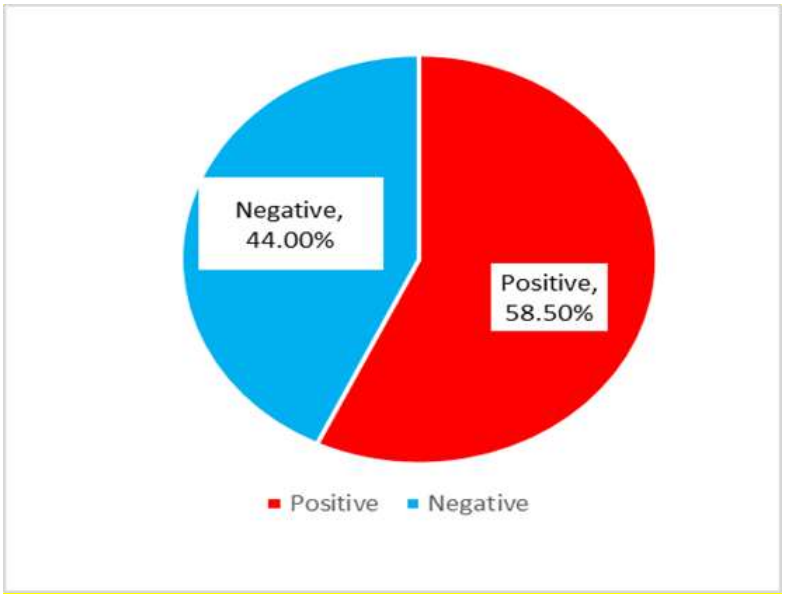

Figure 2. Distribution of patients according to presence of rheumatoid factor

Patients in group 1 showed a significant reduction of DAS28 score after completing the LLLT courses (P=0.02) as well as all the clinical parameters, morning stiffness (P=0.05), number of tender joints (P=0.03), number of swelling joints (SJN) (P=0.04) and VAS (P=0.01) (Table 3), whereas the improvement in patients of group 2 and 3 was limited to the duration of morning stiffness P=0.04, 0.048 respectively.

Marked positive dynamics of clinical parameters were observed in group 1 patients; there was 27% reduction in TJN, 23% reduction in SJN and 26% lower VAS compared to reduction by 11% in TJN, 11% in SJN and 11% in VAS observed in group 2 and 16%, 10% and 13% in group 3. Similarly, there DAS28 score reduced by 12% compared to 3% and 7% in group 2 and 3 respectively.

Table 3. Changes in clinical parameters before and after treatment in study groups (M±SD)

Parameter	Group 1 (n=12)		Group 2 (n=12)		Group 3 (n=10)	
	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
DAS28	5.65±0.57	4.98±0.57*	5.62±0.7	5.47±0.7	5.57±0.6	5.2±0.5
ESR	43.8±n8.83	36±7.5	44.5±9.25	40.1±7.9	44.07±9.9	40.5±10.3
Morning stiffness	120.7±8.2	97.6±7.6*	122±8.7	105±7.4*	119.5±8	107.1±5.8*
TJN	8.0±2.0	5.83±1.9*	7.66±2.0	6.83±2.2	7.8±2.0	6.4±2.0
SJN	8.16±1.9	6.33±1.7*	8.33±1.83	7.42±1.95	7.8±1.8	7.0±1.8
VAS (mm)	47.1±10.7	34.7±11.4*	49.16±9.3	43.75±9.7	48.5±9.5	42±12.4

*Significant differences before and after treatment in p<0.05. Abbreviations: disease activity score (DAS); tender joint number (TJN); swelling joint number (SJN), visual analogue scale (VAS).

ESR on the other hand reduced by 17% in group 1 (P=0.06), 10% in group 2 and 8% in group 3. It is clear that LLLT group showed the highest resection rate, however, the change was not significant. In terms of CRP and RF, there was no significant change in in all groups. Furthermore, 70% percent of patients received LLLT showed positive ACR20 whereas none in the other two group achieved ACR20; however, none of the study groups achieved remission according to criteria of ACR.

Most importantly, no side effect has been reported by group 1 patients during and after treatment whereas 25 % and 20% of group 2 and 3 respectively complained of gastrointestinal symptoms at some point during the study, (Figure 3).

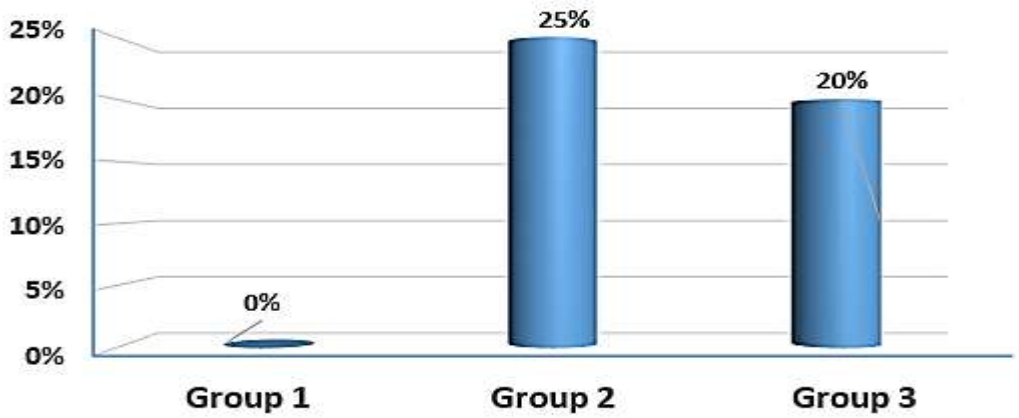

Figure 5. Side effects reported in patients during and after therapy (in percentages)

Discussion

RA is a progressive autoimmune inflammatory disease associating with pain and movement limitation. LLLT has been suggested as an alternative noninvasive therapeutic procedure in RA about 20 years ago [13] and was a focus of research for couple of decades. To our knowledge, this is the first study in Iraq to investigate the effect of LLLT on recent onset RA using four different steps targeting affected joints and relevant nerve root and including several assessment scores and parameters that have not been investigated before such as DAS 28 and ACR20.

In our cohort, the female:male ratio was 2.77:1 which lies within the reported range 2-2.5:1 [22]. We have demonstrated that the intervention group exhibited significant improvement of disease activity (17% reduction in DAS28 score, P=0.02) and clinical parameters including morning stiffness, joint swelling, tenderness and pain, whereas the other 2 groups have not. A metanalysis conducted by Brosseau et al. pooled data form five placebo-controlled trials with a total of 222 patients, with 130 randomized to laser therapy concluded that LLLT reduced pain by 1.10 points (95% CI: 1.82, 0.39) on VAS relative to placebo, reduced morning stiffness for duration by 27.5 minutes (95%CI: 2.9 to 52 minutes) and increased tip to palm flexibility by 1.3 cm (95% CI: 0.8 to 1.7). Other outcomes such as functional assessment, range of motion and local swelling in aforementioned metanalysis were not different between groups [14]. Further, studies used other limbs, as control depicted no significant difference in stiffness duration, or pain RR 13.00 (95% CI: 0.79 to 214.06). Unlike all previous studies, for each patient in the intervention group we targeted four sites including nerve roots, which might explain the significant improvement we illustrated in clinical aspects.

LLLT is non pharmacological medical technique works through generating extremely pure light with no evident side effects. The effect of LLLT is related to photochemical reactions in the cells rather thermal [14]. Light can simultaneously target many cascades of immune system activation in comparison with drugs, so photo-biomodulation can modulate cellular dysfunctions by initiating self-organization phenomena and finally and subsequent healing [12]. ESR is a phase reactant and serve as an indicator of disease activity and patient follow up [20]. We have reported 17% reduction in ESR and 12% reduction in DAS28 after completing the LLLT therapeutic plan suggesting inflammatory modulation effect beside the pain relief. Although the ACR20% was achieved by 70% of LLLT treated patients, the effect was not enough to induce remission and DMARDS remained an important aspect of RA management.

We have not reported any side effect in patient received LLLT. By contrast, 25% of patients in group 2 and 20% of those in group 3 complained of gastrointestinal side effect of NSAID suggesting LLLT as an alternative symptomatic and therapeutic substitute for patients with NSAID contraindication such as those with bronchial asthma, hypertension, or heart diseases.

In conclusion, laser therapy has a positive role in lowering parameters DAS28 and can promote a modest improvement of symptoms and signs according to ACR20. Laser therapy is better than non-steroidal anti-inflammatory drugs in improving clinical features of RA with no side effects.

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Author contribution

Study design and execution conducted by Dr. Al-Saraj and Dr. Al-Attar. Dr. Al-Attar and Dr. Al-Ethary generated the data and drafted the first manuscript. Dr. Al-Attar revised the manuscript.

Conflict of interest

Authors declare no conflict of interest.

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