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Editorial

Getting Your Work Published: Personal Reflections

Time Restricted Feeding: Implications to Healthy Well-Being

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Abstract:

Circadian rhythmicity optimizes the health and physiology by coordinating temporally the cellular functions, tissue functions, and behavior. These endogenously generated rhythms gets dampen with age and therefore compromise the temporal coordination. The pattern of fasting and feeding sets an external cue that profoundly influences the robustness of daily circadian rhythms. Irregular eating patterns can change the temporal coordination of physiology and metabolism leading to chronic diseases. Robust sustaining of fasting and feeding cycle, without altering nutrition quality, can reverse or prevent the chronic diseases as already depicted in an animal model. However, in humans, studies have shown that erratic pattern of eating can elevate the risk of diseases, whereas sustained fasting and feeding cycle, or prolonged overnight fasting, is correlated with protection from different cancers. Therefore, by just optimizing the external cues

timing well defined eating patterns, can thus sustain a robust circadian clock, which may result in preventing many diseases and can improve the prognosis. Time-restricted feeding (TRF) is a form of intermittent fasting, comprising a longer daily fasting period. Preliminary studies report that TRF improves cardiometabolic diseases, diabetes and cancer in rodents and humans.

KEYWORDS: circadian rhythm, lifespan, time-restricted feeding, diseases

"For everything there is a season, and time for every matter under heaven:
a time to be born, and a time to die;
a time to plant, and a time to pluck up what is planted;
a time to weep, and a time to laugh, God has made everything beautiful in its time"
Ecclesiastes 3:1

Nearly all living organisms present on Earth, ranging from archaea to mammals displays the circadian rhythms. Circadian (circa – approximately; dian – day) rhythms are approximately 24 hour oscillations that can be found at the molecular, physiological, and behavioral level in all living beings. (1) The circadian rhythms regulates the sleep and activity cycle and the associated rhythms in metabolic states emerging from a complex interplay of endogenous autonomous circadian oscillators, including daily exposure to light and darkness, and daily patterns of feeding and fasting. These daily behavioral rhythms easily oscillates or cues the functions of almost all organ systems: including metabolic organs, digestive system, immune system, reproductive system, endocrine systems, cardiovascular system, and several brain systems. The cells circadian oscillator in mammals is based on interlocked transcription-translation feedback loops. The circadian molecular clock regulates the cell's internal environment including redox state, NAD+ levels, Ca2+ levels and energy state (ATP/AMP ratio) (2). The invention of electric light, override the natural cycle of mechanism of diurnal rhythm by self-selecting a sleep-wake pattern that is according to the working schedule, which resulted to associated alterations in the fasting and feeding cycle, the biggest culprit in human health. Such a chronic disruption of circadian rhythms can therefore compromise the health and wellbeing through multiple discrete mechanisms. Reduced sleep thus disrupted the metabolic homeostasis by mechanisms that are yet to be fully understood with (3,4). Lights during the night time suppress sleep and promote extended wakefulness time, thus allowing the disturbed and injested behavior to continue late into the night. This extended eating period may contribute to elevated caloric intake that often correlates with modern human lifestyle. Therefore, eating at sub-optimal time of the 24 hours day can promote excessive energy storage, leading to obesity and metabolic syndrome. Quality

of nutrition can also impact hunger, satiety, and hedonic drive for intake of food and thereby affecting the daily pattern of eating, leading to impact the robustness of circadian oscillators in various organs. Chronic disruption in circadian rhythm due to erratic lifestyle or shift work compromises health and wellbeing and results in the increase of several chronic diseases that are associated with aging (5,6). Conversely, recent research has shown that maintaining a defined daily feeding-fasting rhythm, as in time-restricted feeding (TRF), can prevent or attenuate several chronic diseases. TRF is well defined as eating within a ≤10 hour period and fasting for at least 14 hours per day. TRF has a broad class of interventions that have alternate eating periods and extended fasting time. In animal models, TRF, has reported to improve cardiometabolic health, slow tumor growth, reduce cancer incidence, regenerate organs by increasing stem cell production, and increase lifespan (7,8). In humans, TRF related data is little but it suggests that it lowers the body weight, insulin levels, blood pressure, inflammation, and appetite, and by improving the insulin sensitivity and lipid profiles (9,10). These clinical features are driven by a reduction in insulin levels; improved insulin signaling; a reduction in oxidative stress; an increase in antioxidant defenses and autophagy; a reprogramming of aging-related pathways and hormones such as sirtuin 1 (SIRT1), brain-derived neurotrophic factor (BDNF), mechanistic target of rapamycin (mTOR), and insulin-like growth factor (IGF-1) and other mechanisms (11). Although TRF can also be included in the venture of Ramadan fasting, several studies in rodents model reported that TRF has reduced the body weight, improved glycemic index, lowered the insulin levels, reduced the blood pressure, prevented hyperlipidemia, decreased hepatic fat, improved the inflammatory markers, slowed down the tumor growth, and increased the lifespan, even when food intake is matched to the control group. (12,13,14) Several studies on TRF have already been conducted in Humans, Interestingly, they resulted in weight loss and improvements in cardiometabolic events like insulin levels, insulin sensitivity, and blood pressure when participants ate early or in the middle of the day (15,16,17) but worsened cardio-metabolic health when they ate late in the day (18,19).

These endogenously generated circadian rhythm system may greatly explain these effects on health. The circadian system orchestrates, approximately 24-hour circadian period in a day, it therefore changes the normal rhythm in metabolism, physiology, and behavior of an individual. It produces these rhythms through coordinated transcriptional–translational feedback loops involving circadian genes such as *BMAL1*, *CLOCK*, *PER1/2*, and *CRY1/2*, which causes oscillations in downstream targets. For example, sensitivity in insulin and the thermic food effect

exhibits a 24-hour rhythm that peaks in the morning (20). A large number of plasma lipids and age-related hormones such as cortisol, insulin, and growth hormone also vary across the 24-hour day. Metabolic and hormonal rhythms peak in the morning and are downregulated in the evening time, thus implicating morning time as optimal for food intake time (20). Therefore, eating in sync with these rhythms may improve cardiometabolic health and overall health of an individual. In contrast, when eating in the circadian misalignment for example eating late in the day, worsens several cardiometabolic endpoints, particularly glucose tolerance and disturbs the stomach enzymes rhythmic pattern of digestion (21,22) Therefore, TRF interventions where food intake is limited to early in the day time may be particularly effective in improving cardiometabolic health and overall wellbeing.

TRF also improves several facets of health through both circadian and mechanisms related to fasting. It improves glycemic index by lowering 24-hour glucose levels, reducing glycemic excursions, and potentially by improving signaling of insulin. Importantly, these improvements in glycemic index may be driven not only by eating earlier in the day time but also by having a short meal time interval, suggesting that TRF interventions with longer inter-meal intervals may be less effective at improving glucose levels. TRF also alters the diurnal patterns in fasting cholesterol, ketones, cortisol, and diurnal clock genes; particularly, it modestly elevates ketone levels in the morning time and improves the amplitude of the cortisol rhythmicity. Furthermore, TRF also affects hormones and genes related to longevity and autophagy. These important findings demonstrate that TRF improves cardiometabolic health, alters diurnal rhythms, and may also have anti-aging effects.

Conflict of Interest: None declared

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ORIGINAL ARTICLES

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Impact of Internet and Social Media on Academic Performance, Social Interaction, and Mental Health among a Sample of Iraqi University Students

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ABSTRACT

Background: *Social media is an online communication tool that is now a necessary component of our everyday existence. It enables people to communicate, exchange data, and produce web content.*

Aim of the study: *The study aims to display the effect of social media on academic performance, social interaction, and mental health among a sample of Iraqi university students.*

Patients and methods: *The study was conducted on 440 Iraqi students from different colleges, and the data were collected through a self-administered questionnaire.*

Results: *The majority of the sample (98.4%) mentioned they currently use social media, and the mobile phone was the most used device (95.9%) for viewing social media. The study found that social media has both positive and negative effects on social interaction and academic performance. Most of the participants (60.7%) and (21.6%) agreed and strongly agreed respectively that the use of social media is improving connection and information exchange with others. However, (30.9%) strongly agreed that social media may lead them to limit their time with friends and family members. Regarding academic performance, most of the sample (44.5%) strongly agreed that the use of social media may lead to wasting time specified for studying, and 26.8% of them strongly agreed that it distracted them from studying. More than half of those students (53.4%) agreed and strongly agreed that social media harms their academic performance in general.*

Conclusions: *The study concluded that social media has a significant impact on the academic performance, social interaction, and mental health of university students, and it is important to spread awareness among them to reduce excessive and bad use of social media.*

Keywords: University Students, Academic Performance, Mental Health, Social Relationships, Communication, Social Media.

Article Information

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INTRUDUCTION

Social media (SM) is defined as "any electronic-based communication tool, such as websites or applications for social networking, through which users can create online communities to share information, ideas, and connect with people" [(1)]. Social networking sites are one of the latest technologies, at present, that have changed the course of communications significantly. So, access to information has become available and various social networking sites have emerged like Facebook, WhatsApp, Messenger, Instagram... etc. to connect people all over the world and in all areas of life. In

addition, social networking sites and networks are characterized by ease of use and speed of spread which make the individuals spend a long time using it without feeling the passage of time. However, it is, like any technology, has those who support and those who oppose its use [2]. Some see positive effects in that use as it may transcend time and space, provide information for all, and give anyone the opportunity to see the ideas and opinions of others and to dialogue with them without restrictions. Others believe that it has negative effects on the individuals who may prefer to communicate through it and distance from direct relationships with others from the family, relatives, and friends, which leads to the impact and contraction of realistic social relations [3].

The availability of smartphones, tablets, laptops, and other smart devices increased students' every day heavy reliance on social media. The student's life, social interaction, academic achievement, and even self-concept is all affected by this addiction (4,5). It was found that the extreme use of social media may have a negative effect on the user's personal and professional life (6,7). One undesirable effect is that it can easily lead clients to be habituated to it (8,9). A previous study demonstrated social media addiction and declining levels of self-esteem are significantly correlated (10). Students who spend more time on social media may have psychological and mental problems like depression and anxiety due to negative interactions in their communication with friends and families. University students are using social media for different reasons like reaching the scientific information that helps in their study and the availability of sites and groups for discussion makes them more liable for addiction. So, it is crucial to look at the functions that social networking sites serve for those valuable groups of society (11). The effect of social media use and academic success are highly reliant on the individual student; while some use it to raise their academic performance, others find it more distracting (12).

In Iraq, one study was conducted on a sample of Kufa University students and found that students from the College of Medicine suffered from internet addiction with (54.6%) of them having moderate internet addiction [13]. Another study was conducted to assess the impact of social media on academic performance, political views, and the economic situation of the students [14]. Few studies aimed to assess the benefit of the Internet and social media to improve the interaction between students and lecturers, in addition to improving their English language [15,16]. Even though a lot of studies examined the effect of social media on academic performance among university students, no recent study assessed the effect on their face-to-face social interaction with others and the effect on their mental health for different colleges. This study was conducted on students from Wasit University / Iraq to give a description of the impact of social media on students' educational achievement. In addition to its effect on communication skills and their mental health. It will be beneficial to spread awareness among them to reduce excessive and bad use of social media.

PATIENTS AND METHODS

The study population was Wasit University students from different colleges (scientific and humanitarian). The minimum required sample size for this study was 361 students according to the sample size equation calculation for cross-sectional studies [17]. Considering that social media can affect 37.5% of students' academic performance from a previous study [18]. Inclusion criteria include all students from colleges of Wasit University who agreed to participate and fill out the questionnaire aged above 18 years old. Samples were selected randomly from students' name lists by a computer program. The questionnaires

were distributed to the selected students by hand and were filled by the student himself/ herself. A total of 440 filled questionnaires were returned to the researcher for the final analysis.

The Study Design

This is designed as a descriptive cross-sectional study conducted during the period from 1st May till the end of July 2023 in Wasit province/ Iraq.

Subjects

This study included 440 Iraqi population was Wasit University students from different colleges (scientific and humanitarian).

Exclusion criteria

Those who were engaged within the lecture rooms during the data collection time or those who had exams were excluded.

Data collection:

The study compares how frequently students use the following social media sites: Facebook, Twitter, Instagram, Viber, YouTube, and WhatsApp. Three sections make up this questionnaire. The first demographic profile like age, gender, social status, place of living, type of college, stage, and grade in the last academic year. The second section included data about using social media, duration of usage, and the used instrument for that purpose. The third part includes questions related to the effect of social media on academic performance, social interaction, and mental health. Section three questions were answered by using structured items based on five Likert scale items to measure the impact of SM on the study variables starting with strongly agree, agree, neutral, disagree, and strongly disagree.

The instrument was designed depending on previously published articles [19-21] after some modification and translation from English to the Arabic language by an expert translator. The validity was done by the researcher's specialist consultant in community medicine, and his comments and suggestions were incorporated into the final draft. The questionnaire was also pretested on 7 selected students for suitability of time and understandability of terms.

STATISTICAL ANALYSIS

The data were entered and analyzed by the Statistical Package for Social Sciences software program (SPSS) version 26. Frequency and percentage were used to describe the categorical data while mean and standard deviation (SD) were used for quantitative continuous data.

RESULTS

In this study, the data were collected from 440 Iraqi students with a mean± SD age (23.01±2.49) years old ranging from 18 to 31 years. Table 1 shows the socio-demographic features of the study sample. Females represented about (71.8%) of the total sample, and about (63.2%) lived in city centers. Most of the participants (81.4%) were from scientific colleges, and more than one-quarter (25.7%) of the participants were in the sixth stage of college. The highest percentage of the students (38%) passed the last academic year with 'good' grade.

Table 1: Socio-demographic features of the study sample (n=440).

Variable	Category	Frequency	Percentage
Sex	Male	124	28.2
	Female	316	71.8
Social status	Single	371	84.3
	Married	69	15.7
Place of living	City center	278	63.2
	District or subdistrict	129	29.3
	Village or countryside	33	7.5
Type of college	Scientific	358	81.4
	Humanitarian	82	18.6
Stage of college	First	16	3.6
	Second	37	8.4
	Third	69	15.7
	Fourth	100	22.7
	Fifth	105	23.9
	Sixth	113	25.7
Student grade last year	Fail	2	0.5
	Pass	48	10.9
	Average	116	26.4
	Good	167	38.0
	Very good	87	19.8
	Excellent	20	4.5

The majority of the sample (98.4%) mentioned that they currently use social media. The highest percentage (40%) among them used it for a period (6-10) years followed by (37%) from 1-5 years. The mobile phone was the aperture used by most of them (95.9%) for viewing these media for a period reached more than 3 hours per day in about 58.9% of them as shown in Table 2.

Table 2: History of social media use among the study samples (n=440).

Variable	Category	Frequency	Percentage
Current use of social media	Yes	433	98.4
	No	7	1.6
Number of years of social media use	Never or less than 1 year	16	3.7
	1-5 years	166	37.7
	6-10 years	176	40.0
	More than 10 years	82	18.6
Number of hours of social media use per day	Never or less than 30 minutes	7	1.6
	30 minutes-1 hour	18	4.1
	1-2 hours	54	12.3
	2-3 hours	102	23.2
	More than 3 hours	259	58.9
Tools used for social media	Mobile phone	422	95.9
	PC or I-pad	18	4.1

Table 3 shows the effect of social media on social interaction. Most of the participants (60.7%) and (21.6%) agreed and strongly agreed that the use of social media is improving connection and information exchange with others. While (30.9%) were strongly agreed that social media may lead them to limit their time with friends and family members. Half of them (50.7%) also agreed about that. So, about (41.8%) and (22.5%) were agreed and strongly agreed that using SM may lead to social isolation. Most of the sample (56.8%) thought it may lead to a violation of personal privacy.

Table 4 shows students' responses to questions related to the effect of social media on academic performance. Most of the sample (44.5%) strongly agreed that the use of SM may lead to wasting time specified for studying. And 26.8% of them strongly agreed that it distracted them from studying. So, more than half of those students (53.4%) strongly agreed and agreed that social media harms their academic performance in general. Even though, 73.2% strongly agreed and agreed with the positive effect of SM on academic performance.

Table 3: Frequency distribution of participant response to the effect of social media on social factors (n=440).

Social factors	Strongly agree	Agree	Neutral	Disagree	Strongly disagree
Improve connection and information exchange with friends and family	95(21.6%)	267(60.7%)	65(14.8%)	10(2.3%)	3(0.7%)
Limit or reduce the time you spend with family and friends	136(30.9%)	223(50.7%)	60(13.6%)	15(3.4%)	6(1.4%)
This can lead to social isolation	99(22.5%)	184(41.8%)	105(23.9%)	49(11.1%)	3(0.7%)
This leads to a violation of privacy	59(13.4%)	191(43.4%)	111(25.2%)	76(17.3%)	3(0.7%)
Improve social connection in general	36(8.2%)	180(40.9%)	136(30.9%)	82(18.6%)	6(1.4%)

Table 4: Students' responses to questions related to the effect of social media on academic performance.

Academic performance	Strongly agree	Agree	Neutral	Disagree	Strongly disagree
Lead to wasting studying time	169(44.5%)	158(35.9%)	57(13%)	25(5.7%)	4(0.9)
Distract from studying	118(26.8%)	151(34.3%)	86(19.5%)	85(19.3%)	0(0%)
Had a positive effect on academic performance	105(23.9%)	217(49.3%)	74(16.8%)	37(8.4%)	7(1.6%)
Had a negative effect on academic performance	93(21.1%)	142(32.3%)	134(30.5%)	66(15%)	5(1.1%)

When students were asked about the magnitude in which SM affects their regular studying time, around (44.6%) mentioned a higher effect as shown in figure 1. Only 4.8% said it was not affecting and 5.9% mentioned a very little effect.

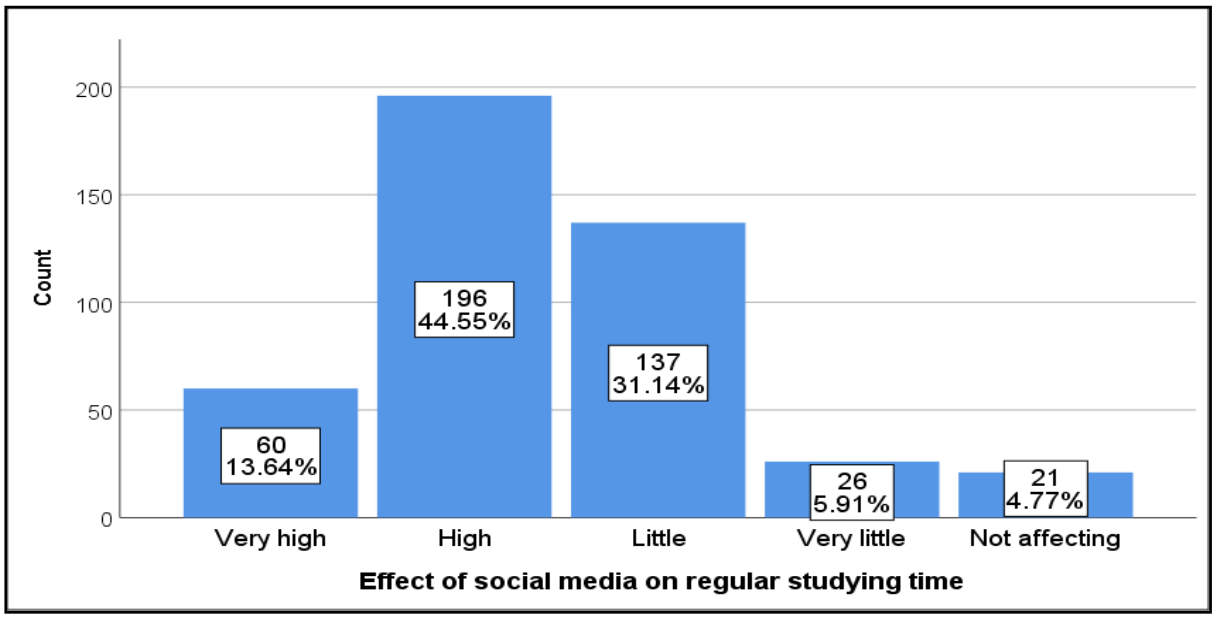

Figure 1: Effect of social media on regular studying time.

In Table 5, the effect of social media on psychological and mental health was demonstrated. More than one-third of the participants (33.2 %) agreed and strongly agreed that the SM may cause hostility, and about (45%) agreed and strongly agreed that the SM causes loss of self-esteem. Sleeping disturbances can also arise from SM as most of the samples (72.3%) mentioned. Leading to a reduced concentration and feelings of tiredness as 65% of the study participants agreed and strongly agreed with this.

Table 5: Effect of social media on psychological and mental health of the study samples.

Psychological and mental effects	Strongly agree	Agree	Neutral	Disagree	Strongly disagree
Causing hostility	33(7.5%)	113(25.7%)	137(31.1%)	157(35.7%)	0(0%)
Sleeping disturbance	107(24.3%)	211(48%)	46(10.5%)	66(15%)	10(2.3%)
Loss of self-esteem and passion	66(15%)	136(30%)	109(24.8%)	107(24.3%)	22(5%)
Feeling tired, low energy, and reduced concentration	88(20%)	198(45%)	80(18.2%)	65(14.8%)	9(2%)

Figure 2 shows the effect of social media on sleeping time. The highest percentage of the sample (40%) mentioned a little effect on their sleeping time by social media followed by (32.06%) who represented a high effect on their sleeping time by use of social media.

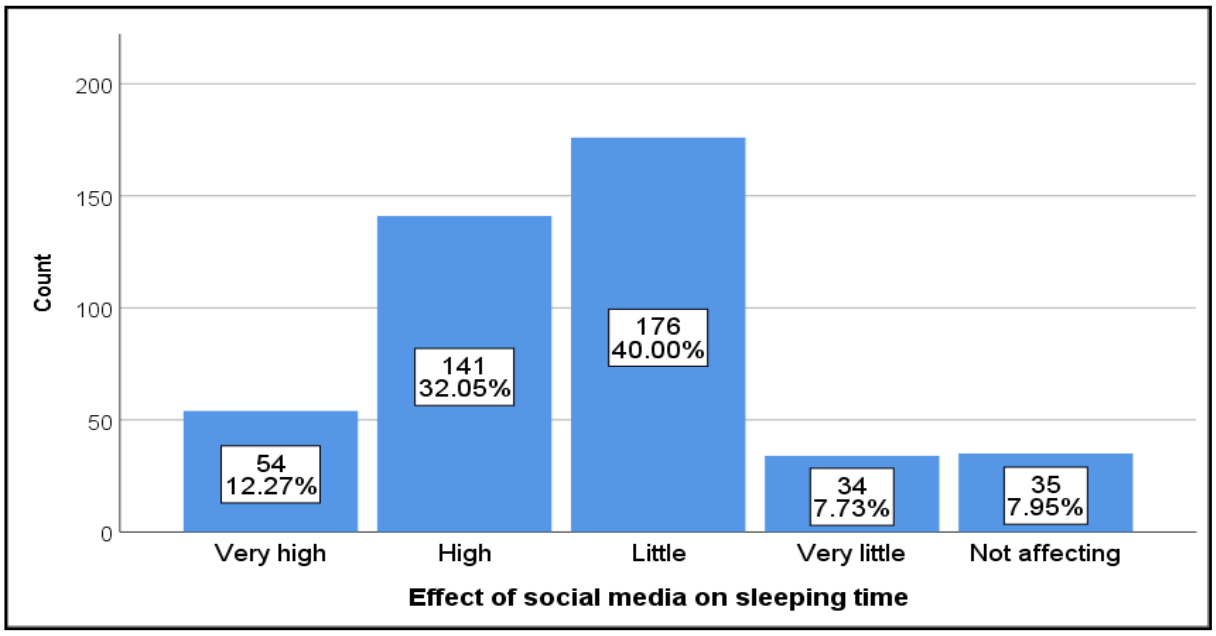

Figure 2: The effect of social media on sleeping time among the study sample.

DISCUSSION

Social media usage is currently one of the most common activities commonly distributed among children, adolescents, and adults approximately in all societies. Social media provides an easy way of entertainment and communication, in addition to accessing up-to-date information and news [22]. This study included 440 Iraqi students, from both scientific and humanities colleges. In this study, the prevalence of using social media among university students was 98.4%. This finding coincides with Perrin's study [23] which stated that the use of social media among American adults aged 18-29 years, who represent a high percentage of university students, rose from 12% in 2005 to 90% in 2015. Also, a 93.4% prevalence was reported among medical students in Turkey. The reported high prevalence rate is attributed to the integrated use of social media into the lifestyle of the younger generation, as well as the current popular use of social media as a means of communication between college administration and students [24].

Regarding the time spent on social media, this study shows more than 3 hours per day spent on social media, this finding agreed with the findings of a previous study [25] in which students reported that they spent a lot of time reached to 6-8 hours daily on social media. These findings also agreed with the findings of Ajewole and Fasola [26] who reported that students spend 6-7 hours daily on social media. The finding of the study disagreed with Chaffeys' report in 2016, which found that students spend only 2 hours daily on social media.

In the current study, students mentioned that social media has both positive and negative effects on social interaction. The majority of participants agreed and strongly agreed that social media improves connection and information exchange with others. Another recently published study found a positive relationship between using SM with social interaction among youth and this positive effect is perhaps decided by the level of trust present inside online communities [27]. It is important to note that the relationship between social media use and social interaction is complex and may depend on various factors, such as the type of social media use and the individual's personality and social context [28]. However, a significant

proportion of participants (30.9%) strongly agreed that social media may lead them to limit their time with friends and family members, and half of them (50.7%) agreed with this statement. The majority agreed that social isolation situations may occur due to excessive use of SM and depending on hours spent using it. The same result was shown among Saudi Arabian students who mentioned negative effects on their relations with friends and families in about 59% [29].

Additionally, most of the sample (56.8%) thought that social media use may lead to a violation of personal privacy. These findings are consistent with the concerns expressed in the search results about social media's impact on privacy. Social media platforms are designed to get users to share personal information willingly, and this can lead to breaches of privacy [30,31]. Overall, the results suggest that social media has both positive and negative effects on social interaction and privacy. So, it is recommended to be aware of these effects and to use social media responsibly and mindfully. Social media users should take steps to protect their privacy, such as adjusting their privacy settings and being cautious about sharing personal information online.

The results indicate that while a majority of students strongly agreed and agreed with the positive effects of social media on academic performance, a significant portion of the sample strongly agreed that the use of social media may lead to wasting time specified for studying, and more than one-quarter of them strongly agreed that it distracted them from studying. This suggests that social media can have both positive and negative impacts on students' academic performance. The positive impact of social media on academic performance is also found in the findings of Kabilan et al. [32] who reported that students who use social media learn correct spelling, sentences, pronunciation, and essay writing by Googling the words into nets. Besides, corroborated by Mehmood and Taswir [11] who also found that social media is useful in improving correct spelling and writing for students. A lot of positive effects of social media on academic performance were reported like enhancement of online learning and improved Creative Element [33]. On the other hand, harming academic performance by SM was shown in Ezeji's [34] study which revealed that the rate of social media usage among students was very high and harmed students in general grade. Another study among medical students in China found that students thought SM caused wasted time and distracted them from studying [35]. The impact of social media on academic performance can vary depending on factors such as personality traits, time spent on social media, and the purpose of use [36].

A recently published study indicated that social media can cause mental disorders in students such as sleepiness, isolation, violent action, depression, insomnia, and anxiety [37]. In this study, more than one-third of participants agreed and strongly agreed that SM causes hostility, nearly half agreed and strongly agreed that SM causes loss of self-esteem, and most of them suffered from sleepiness. This finding corroborated Zubair et al. study that shows social media causes sleepiness, isolation, and mental disorders [38]. Hostility which can be caused by social media, is associated with an increased risk of adverse cardiac events and poor health outcomes. It may promote poor health behaviors such as smoking, physical inactivity, and medication nonadherence It is important to be aware of the potential negative effects of social media and to take steps to manage stress and promote healthy behaviors [39]. Studies have found diverse results about the effect of social media on mental health. Some found it may help enhance connection, increase self-esteem, and improve a sense of belonging. But others found that it can lead to tremendous stress, pressure to compare oneself to others , and increased sadness and isolation [40]. Sleep

problems that arise due to SM can also affect the academic performance and physical function of the body [41-43]. Even some mentioned no difference between those with high and low-grade academic performance with their sleep style [44].

CONCLUSIONS

Social media can be considered a double-edged sword for the whole population and especially for university students. It can both positively and negatively affect their social communication with friends and society, their academic performance, and even their mental health and psychology.

RECOMMENDATIONS

It is recommended to guide those students to use SM in a way that makes them get the maximum benefits for improving their communication skills, academic results, and mentality. So, awareness should be distributed in addition to more research on different aspects of social media. It's so important to make another study for smaller age groups like primary and kindergarten.

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Histomorphometric and Histological Evaluation of Renal Cortex in Response to Sleep Disturbance in Adult Male Rat

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ABSTRACT

Background: *Sleep disturbance affects kidney structure reflected in functional derangement causing renal diseases; this occurs through sympathetic system activation and inflammation. Changes in the renal vascular bed affect the renal corpuscle-related structures such as the glomerular area, and urine space, in addition to the kidney tubular apparatus. Sleep deprivation or sleep interruption differ in their effect on systolic blood pressure causing renal tissue changes that predispose to chronic kidney disease (CKD).* ***Aim of the study:*** *The study aims to evaluate the effect of sleep disturbance on histological changes of renal tissue in control and experiment groups.*

Patients and methods: *An experimental study on a sample of thirty adult male albino Rats, was divided into three groups (10 animals per group). The control group had a normal sleep rhythm which was 12 hours in the dark and 12 hours in light. Group A: at 12 hours light and 12 hours dark with the production of a flashlight at three-time intervals, every 2 hours, during their sleep period, while Group B includes rats that were exposed to a reduction in sleep time by continuous flashlight stimulation for 7 hours per day, during their sleep period. Then, the kidneys were dissected and prepared for histological evaluation and quantification. The experiment lasted for 14 days for all groups, and the study was performed during the period between the 1st of January 2023 to the 1st of August 2023 in the anatomy department in Al- Nahrain Medical College.*

Results: *This study showed the effect of sleep disturbance patterns (sleep reduction, and sleep interruption) by light stimulation in adult male rats on cortical renal tubules and cortical vessels. A prominent dilatation in cortical renal tubules with the presence of cortical hemorrhagic areas and cortical necrosis with inflammatory cell infiltration was seen to be associated with sleep deprivation prominently. Histological changes of renal corpuscular areas, glomerular tuft area, and renal space area showed significant variations in sleep disturbance groups, in a p-value ≤ 0.05.*

Conclusions: *Changes in sleep patterns indicate the importance of sleep in maintaining renal cortical tissue structural integrity by its effect on local hemodynamics of cortical vessels that ultimately affect the structure and area of the renal corpuscles. Sleep deprivation represents a powerful factor for renal cortical changes that lead to corpuscular and tubular damage.*

Keywords: Blood Pressure, Kidney, Light Stimulation, Sleep Disturbance.

Article Information

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INTRUDUCTION

Sleep is the most significant peripheral factor that sustains a circadian rhythm. Sleep issues may have a role in the renal system function and diseases (1). Since the physical function of the kidneys has a diurnal rhythm (2), the volume of the urine excreted, the renal blood flow, and the glomerular filtration rate, some chemical compounds filtration including sodium (3), calcium, potassium, and phosphorus (4), and several other metabolites, are dependent on the circadian pattern of the kidney. The typical circadian rhythm is mainly derived from a self-sufficient mechanism which is named the circadian clock system which is composed of a central part called the circadian clock positioned in the suprachiasmatic nucleus (SCN) in the anterior part of the hypothalamus, and a peripheral clock primarily located in the liver, kidneys, heart, lungs, skeletal muscles, adipose tissue, and other tissues. These components respond to and are derived from upstream signals from the circadian setup in the SCN (5). The central biological clock depends upon the light stimulus which is transformed from optical signals to electrical signals on the retina; these signals are then transmitted to the SCN via the retinal hypothalamic tract and have a role in the regulations of the expression of core clock gene in the brain, as well as having a direct effect on the expression of peripheral genes in various organs. This is all done to regulate the functions of these organs (6). Additionally, the timing mechanism of the central and peripheral nervous system can be disrupted when exposed to light at night, which impairs the transmission of internal rhythm to the external environment, and dysfunctions of normal and ideal 24-hour physiological and behavioral habits of the individuals. Though the direct impact of artificial light at night (ALAN) on the human circadian clock has not been studied directly by human researchers, the data collected from human correlative studies and various data from nocturnal and periodic studies on animals, suggest that the circadian rhythm is disordered, resulting in nocturnal and diurnal similar result found in mammals (7). Sleep disorder could activate the sympathetic nervous system (8), or activate systemic inflammation which leads to damaging glomerular endothelium and proteinuria (9), however, both sympathetic nervous system activation (10) and systemic inflammation (11) lead to the progression of chronic kidney disease. A growing number of studies have demonstrated that sleep disturbance affects the evolution of kidney disease, probably via the increased inflammation and sympathetic activity present at the renal vascular bed, these effects negatively affect the glomerular basement membrane and the tubular kidney apparatus (12,13). The renal corpuscle, is the component of the kidney that is responsible for filtration, is composed of the glomerulus and Bowman's capsule (14). A glomerulus is made by a bunch of capillaries through which blood flows to produce urine filtrate through a blood-urine barrier (15). The kidneys are vital organs that participate in the control of blood pressure, and previous research has demonstrated that the kidneys are involved in the body's periodical adjustment of blood pressure. For example, Bankir et al. (2008) (16) showed that the diurnal pattern of renal excreted sodium is crucial to the nocturnal drop in blood pressure. Studies of adults have proved a link between sleep duration and increased blood pressure (17). Other studies have demonstrated that individuals with short sleep duration have a higher probability of experiencing a decrease in eGFR as demonstrated by research on the connection between short sleep time and the rapid decrease in kidney function (18). The present study aims to evaluate the effect of sleep disturbance on histological changes of renal tissue in control and experiment groups.

PATIENTS AND METHODS

An experimental study was performed during the period between the 1st of January 2023 to the 1st of August 2023 and conducted at the Anatomy Department in Al-Nahrain Medical College

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Subjects

A sample of thirty adult male albino rats (Rattus Norvagigus) was selected and maintained at a temperature of 22 ± 2 °C. All animals in this experiment are kept and used according to the National Institute of Health (NIH) guidelines, in the Anatomy Department at Al Nahrain College of Medicine. The rats were divided into three groups of 10 animals per group.

Controls

At normal sleep rhythm which is 12 hours in the dark and 12 hours in light; Group A: at 12 hours in light and 12 hours dark with the production of a flashlight at three-time intervals of every 2 hours, during their sleep period; and Group B: rats were exposed to a reduction in sleep time by continuous flashlight stimulation for 7 hours per day, during their sleep period.

Method

Animals were euthanized in a chloroform closed chamber, and the kidneys were dissected, and fixed by formaldehyde (10%), for paraffin block preparation. Then, a thin series of sections of (5 µm) thickness were cut and placed on a regular glass slide for H&E staining according to (Suvarna et al., 2013) (19), and the slide was examined under a light microscope (LEICA DM750, Germany), for histological evaluation and quantification, the field captured by using Digital Microscope Camera, Image J analysis was done using the software Image J (Java8–based image processing program developed at the National Institutes of Health, USA) for morphometric study and measuring the areas of the renal corpuscle, which are the capsule, glomerulus, and renal space.

STATISTICAL ANALYSIS

An SPSS version 25 was used for statistical analysis by using an unpaired t-test to compare between groups, a significant P value ≤ 0.05 was selected as statistically significant.

RESULTS

The cortex is characterized by the presence of renal corpuscle and convoluted tubules; proximal convoluted tubules (PCT) are lined by simple cuboidal to low columnar epithelium with a characteristic brush border, while the distal convoluted tubule (DCT) is lined with simple cuboidal epithelium with smaller and flattened cells, that lake of brush border.

The renal corpuscle appeared as a rounded structure that is surrounded by Bowman capsules which are lined by simple squamous epithelium with the presence of a tuft of capillaries (glomerulus) in the center, and the glomerulus fills the space of renal corpuscles. Fig. 1 (a & b).

The mean total capsular area measured by image j was 5036.33; the mean glomerular area was 4343.98, and the mean renal space was 692.35. Tab. 1

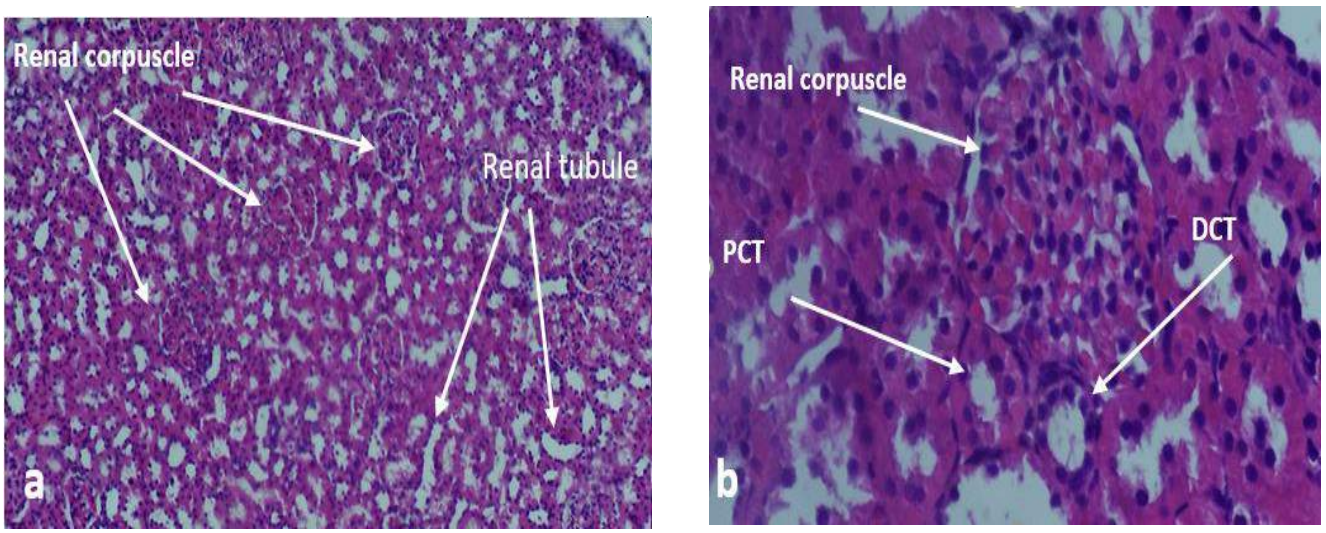


Figure 1: The cross-section in the kidney cortex of the control group shows: a) renal corpuscles and convoluted tubules (10X H&E stain), b) proximal and distal convoluted tubule and renal corpuscle (40x H&E stain).

Table 1: The difference in the capsule, space, and glomerular area between group A and the control group by unpaired t-test.

Parameter	Control group N=35 / Mean±SD	Group A N=35 / Mean±SD	P value*
Capsule	5036.33±916.47	4518.19±1152.52	0.041
Space	692.35±209.22	827.72±435.31	0.104
Glomerulus	4343.98±817.45	3690.48±1007.58	0.004

Significant P-value ≤ 0.05

Vascular structures seen in the cortex are small interlobular arteries that are distributed evenly within the cortex in between the glomeruli, the interlobular arteries give to the afferent arteriole that enters the renal corpuscle and exits as an efferent arteriole.

At the vascular pole, there is a row of closely packed nuclei that densely stained part of the distal convoluted tubule which is considered part of the juxtaglomerular (Jg) apparatus called Macula densa. Fig. 2 (a & b).

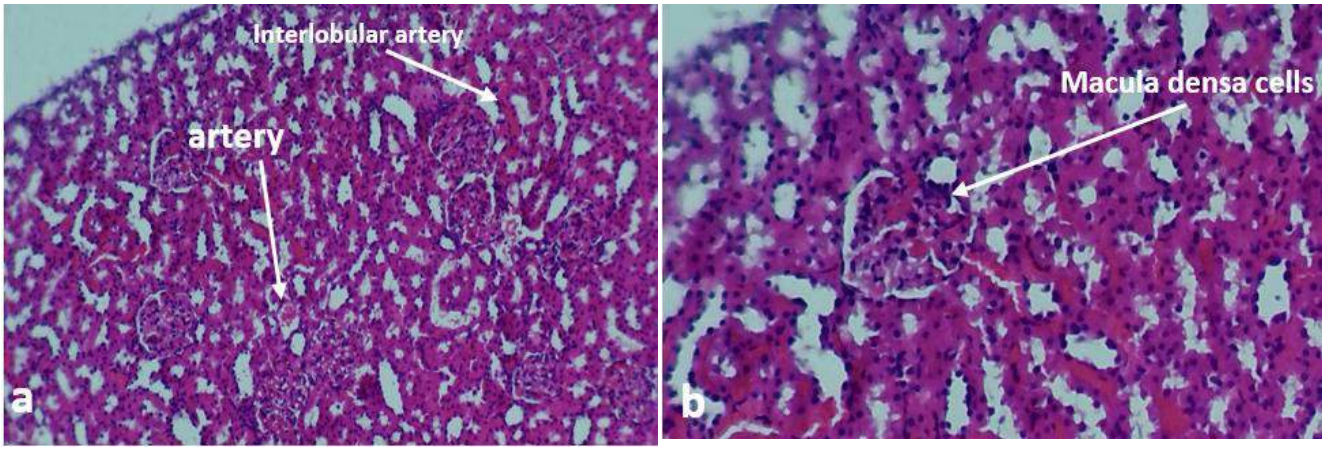


Figure 2: The cross-section in the renal cortex of the control group: a) demonstrates the distribution of cortical vasculature. / b) Macula densa at the vascular pole of the renal corpuscle (40x) H&E stain.

Renal cortex in sleep-interrupted group (A):

This group showed specific variations including widening of the renal tubules at the outer cortex and dilatation of cortical vessels with the presence of small hemorrhagic areas Fig. 3 (a & b).

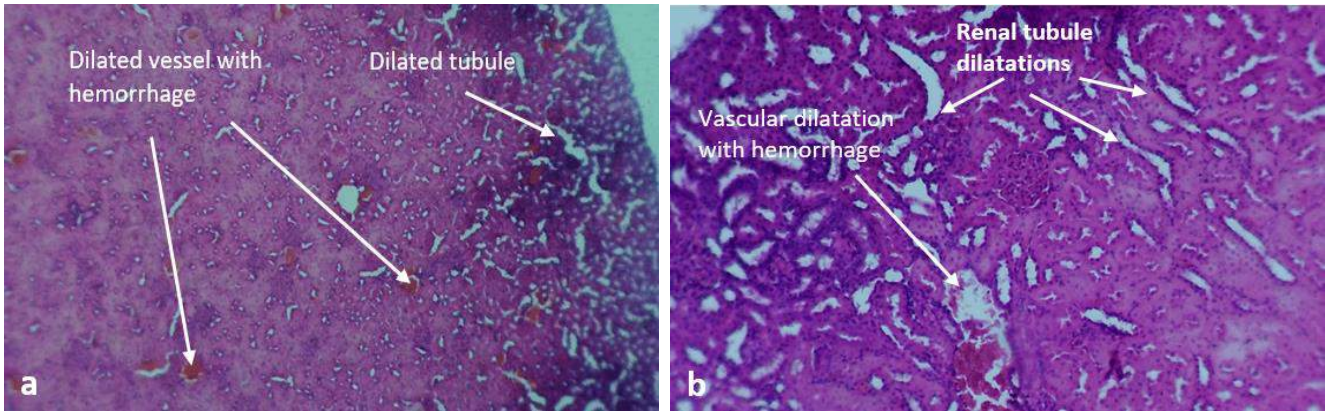


Figure 3: Cross-section in the kidney of group A: shows the dilatations of the renal tubules and vascular dilatation with hemorrhage. a: (4x), b: (10x) H&E stain.

The renal corpuscle seems to be irregular in shape and showed a significant reduction in both the capsular area and glomerular area with P-values 0.041 and 0.004 respectively, with non-significant enlargement in capsular space (Tab 1) Fig. 4

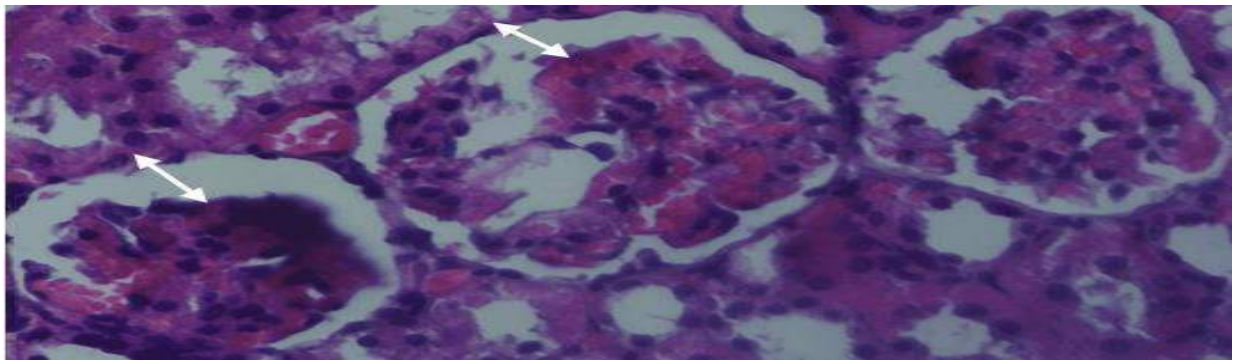


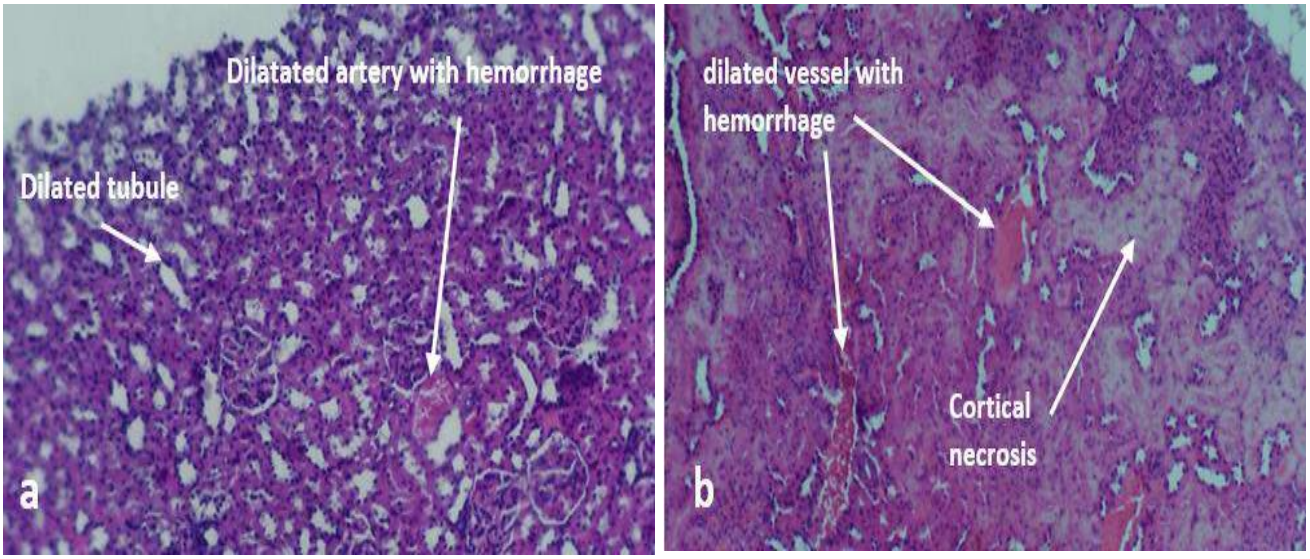
Figure 4: Cross-section in the renal cortex of group A: shows the enlargement of renal space(H&E,40x).

The comparison between the sleep-interrupted group and the control group shows significant changes in the renal capsule and glomerulus at $p \leq 0.05$, but in renal space, there is no significance difference between group A and the control group. Tab 1.

Renal cortex in sleep deprived group (B).

Changes in this group showed multiple variations including marked dilatation of renal tubules of the cortex and blood vessels and the presence of large areas of hemorrhage distributed among renal corpuscles. There is also a cortical necrosis (coagulative necrosis) with inflammatory cell infiltration in which the cell's outline and structure are somewhat preserved but the cells are in fact dead without any visibly stained nuclei and appear more acidophilic than the surrounding tissue.

There is a significant increase in the capsular area of group B compared to the control with a P-value of 0.034 and a highly significant increase in the capsular space with a P-value >0.001 and a non-significant change in the glomerular area. Fig. 5 (a, b, c, d) Tab 2.



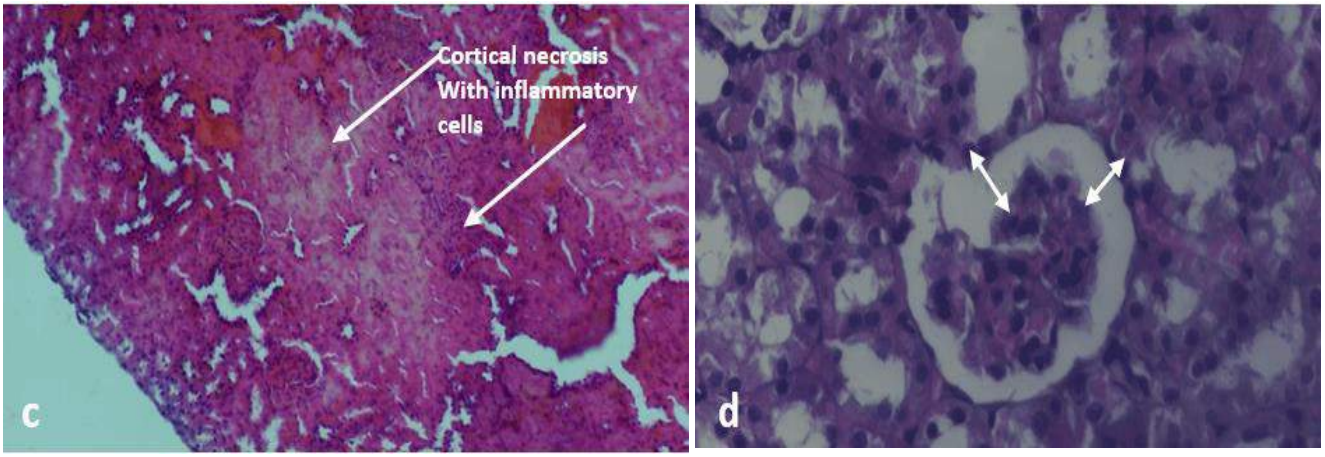


Figure 5: A cross-section of the renal cortex of group B shows: - a) dilated tubules and dilated vessels with hemorrhage. b) dilated vessel with hemorrhage and cortical necrosis (H&E stain,10x). c) ghostly appearance of renal tissue or cortical necrosis. d) enlargement of renal space (H&E,40x).

Table 2: Comparison of capsule, space, and glomerular area between group B and control group by unpaired t-test.

Parameter	Control group N=35 Mean±SD	Group B N=35 Mean±SD	P value*
Capsule	5036.33±916.47	5565.43±1121.77	0.034
Space	692.35±209.22	1081.13±409.84	<0.001
Glomerulus	4343.98±817.45	4484.3±999.82	0.523

Significant P-value ≤ 0.05

Table 3: Comparison of capsule, space, and the glomerular area between group A and group B by unpaired t-test

Parameter	Group A N=35; Mean±SD	Group B N=35; Mean±SD	P value
Capsule	4518.19±1152.52	5565.43±1121.77	<0.001
Space	827.72±435.31	1081.13±409.84	0.015
Glomerulus	3690.48±1007.58	4484.3±999.82	0.002

Significant P-value ≤ 0.05

DISCUSSION

Sleep disturbance has a pronounced effect on body health and life quality. There is a documented relationship between sleep disturbances and kidney diseases but limited knowledge of sleep disturbance and its effect on normal kidneys.

Light changes shift the endogenous oscillator in the suprachiasmatic nucleus (SCN), by the environmental day-night cycle. The setting of circadian rhythms originates in the eye and through an axonal pathway of retinal ganglion cells to the SCN (20). Animal data suggested circadian rhythm changes may induce kidney damage; however, human research on the link of sleep factors with incident kidney disease

needed to be evaluated. In animals, hereditary factors of circadian rhythms change lead to renal impairment such as an increase in serum creatinine, and fibrotic cortical changes with glomerular and tubular damage; these are preventable by strict adjustments of light-dark periodicity to maintain normal circadian rhythms (21).

The current results showed renal tubule dilatation which may be due to tubular injury mentioned previously, which Wang et al., (2011) (22) also mentioned in their study and they considered tubular dilatation, vascular changes, leukocyte infiltration, and edematous capillaries as a specific feature of renal tubular injury. Further, Jones et al., (2008) (23) showed that any elevation in serum creatinine level means a glomerular filtration rate was reduced. Sleep deprivations result in the disruption of circadian rhythm and the local circadian clock in peripheral organs such as the kidney (21).

Results of the present study in the sleep fragmentation group showed vascular dilatation in the renal cortex and discrete cortical hemorrhage while in the sleep reduction group, wider areas of vascular dilatation and hemorrhage were encountered in the cortical area. This may be related to the sleep disturbance effect on cortical blood flow which was explained by Spiegel et al., (2004)(24) who mentioned that sleep disorders and fragmentation lead to an increase in hypoxemia and sympathetic nervous system stimulation and decreased parasympathetic activity which results in a reduced fall in nocturnal blood pressure.

In this study, vascular changes in the experimental groups may related to the incident light on the retina that is connected to the SCN which leads to the activation of the sympathetic nervous system and changes in cortical vascular resistance. This was mentioned by Pepin et al . (2014)(25) who explained that the sleep disturbances were related to activations of the hypothalamic-pituitary-adrenal axis and sympathetic nervous system, as well as chronic inflammation, which may promote a non-dipping pattern, hypertension and subsequently alter renal function.

The present results showed significant histopathological changes in the capsular area, glomerular area, and renal space between experimental and control groups. This could be due to the effect of light stimulations on sympathetic activation which in turn affects cortical hemodynamics leading to changes in the histopathological parameter. This was explained by Qiu et al. (2019)(26) that hypertension alters peripheral resistance and blood flow pulsation leading to glomerular microvascular damage and alteration in glomerular filtration rate (27). Cottone et al., (2009) (28) linked changes in blood pressure during night shift work to endothelial dysfunction and symptoms of renal damage, specifically a decrease in GFR.

Blood pressure is closely linked to generalized endothelial dysfunction and subclinical atherosclerosis which may also be another pathophysiological mechanism of blood pressure-related early symptoms of renal damage (28). Therefore, blood pressure may be on the causal pathway between night shift work and decreased eGFR. Oberleithner., (2005) and Ishigaki et al., (2016) (29,30) mentioned that stress factors lead to sympathetic activation and a decrease in renal plasma flow and GFR, which affect renal function and activation of the renin-angiotensin-aldosterone system (RAAS) that lead to an increase in intraglomerular pressure and vascular endothelial cell damage.

The current results showed dilatations in cortical renal tubules specifically in the outer cortex of sleep disturbance groups and specifically in sleep reduction patterns. This may be due to changes in hemodynamics concerning circadian changes that affect GFR; this was mentioned by Martino et al. (2008) (21) as circadian disruption affects the proliferation of renal tubular epithelium and cell apoptosis. In addition, Kannan et al., (2014) and Sata et al., (2018) (31,32) mentioned that sympathetic activation leads to an increase in renin secretion and renal tubular sodium reabsorption and a decrease in renal blood flow.

The present results showed a prominent large area of cortical necrosis in the sleep reduction group (group B) which is related to sympathetic stimulation and parasympathetic suppression due to sleep reduction. This is supported by Emans et al. (2017) (33) as the oxygen content is more vulnerable to changes during the night, also Rabelink et al., (2007) (34) mentioned hypoxia induces endothelial dysfunction, leukocyte infiltrations and blocked blood flow leading to tissue loss of nephron. On

the other hand, McGettrick and O'Neill, (2020) (35) reported ischemia/hypoxia is one of the commonest causes of inflammation and inflammatory cell infiltration.
Moreover, (Sradnick et al., 2016) (36) reported selective epithelial injury in the tubular epithelial cells concerning a reduction in regional renal oxygen supply leading to inflammation, ischemia, necrosis, and reflecting the imbalance between arterial pressure and vascular resistance.
Furthermore, melatonin supplementation showed a beneficial effect in reducing blood pressure, inflammation, apoptosis, and promoting angiogenic properties, this was confirmed by (Nava et al., 2003) (37) as melatonin treatment reduces blood pressure, renal tissue inflammation, and oxidative stress.
Research has shown that melatonin regulates the biological circadian clock, and acts as an antioxidant, inhibiting sympathetic activity, and preservation of endothelial function (38-40). Its effects on the kidney have been proven by experimental data. Animal studies have demonstrated that melatonin treatment improves hypertension, and the mechanism of effect is associated with decreased renal inflammation resulting, in turn, from a reduction in local oxidative stress (37).

CONCLUSIONS

Sleep disturbance provides a stressful activation on the hypothalamic-pituitary axis, leading to local changes in peripheral organs including the kidneys mainly through an increase in blood pressure specifically by sympathetic over-activation. Kidney structural changes in response to changes in cortical hemodynamics including vasodilatation and hemorrhage were more prominent in the sleep reduction group. Glomerular structure change, including changes in capsular, glomerular, and renal space areas in response to sleep disturbance, is more pronounced in the sleep reduction group. High blood pressure due to sympathetic over activation can lead to damage of small blood vessels in the kidney over time and narrowing or blockage of these vessels can reduce blood flow to the renal cortex, leading to ischemia and then to necrosis of the affected region.

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Clinical and Epidemiological Characteristics Associated with the Severity of Bronchiolitis in Hospitalized Children in Iraq

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ABSTRACT

Background: Bronchiolitis is the most common acute infection of infants' lower respiratory tract especially in the first two years of age. Its clinical course is complex where symptoms vary in severity and may progress rapidly to respiratory failure. Some factors can predispose to severe bronchiolitis like inhalation of cigarette smoke, crowded living environment, congenital heart defects and lack of breastfeeding. **Aim of the study:** The study aims to study the clinical and sociodemographic factors that are related to the severity of acute bronchiolitis in the patients included in the study. **Patients and Methods**: A cross-sectional study achieved at Hevi pediatric teaching Children Hospital in Duhok north of Iraq in the period from 1st November 2022 to 1st February 2023. The study included all patients aged 1-24 months diagnosed to have bronchiolitis and treated as inpatient in the hospital during that period. The diagnosis was made according to the American Academy of Pediatrics (AAP) diagnostic criteria in 2014. The severity of bronchiolitis was classified according to the Modified Cincinnati Bronchiolitis Score (MCBS). For every participant, the clinical aspects and epidemiological factors associated with bronchiolitis were obtained through a questionnaire filled by direct interview with parents. The data were analyzed by using Statistical Package for Social Sciences (SPSS) software version 26; the clinical significance was considered when P value is less than 0.05. **Results:** The study included 148 patients with bronchiolitis. Most of them were of moderate severity and female were more common. The age group (3-6 months) was significantly associated with severity. Most of patients lived in urban areas and were delivered by Caesarean section. Exposure to tobacco smoke was significantly associated with the severity of the disease. The artificial feeding was the most common with significant association with the severity. Most of patients had siblings attending school and childcare without significant association with the severity and less than a quarter of them had a family history of asthma. Poor living conditions was significantly associated with severity. Local cradle (landik) was significantly associated with severity. Most chest X ray findings were normal in 126 patients (85.1%) with no significant association with severity. **Conclusion:** Age, exposure to tobacco smoke, type of feeding, poor living conditions and sleeping in the local cradle (landik) were significantly related to the bronchiolitis severity in Duhok.

Keywords: Bronchiolitis, Feeding, Infant, Severity, Smoke.

Article Information

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INTRUDUCTION

Bronchiolitis is the most common acute lower respiratory tract infection of infancy [(1,2)]. Yearly 150 million infants are diagnosed with bronchiolitis over the world, and 2–3% of them are hospitalized. There is evidence that bronchiolitis brings about wheezing bronchitis and bronchial asthma in the future [(3)]. Bronchiolitis results in increased expenditure both to families and healthcare systems [(4,5)]. Acute respiratory distress syndrome leads 2-3% of the pediatric patients to hospital admission

and 5% of them to intensive care units' admission. Severe bronchiolitis causes a mortality of 1-7% and is responsible for 30-40% of bronchopulmonary dysplasia in children, congenital heart defects, and preterm birth. Pediatric patients should have an early diagnosis and prompt treatment to minimize mortality [6].

When reaching the age of two years, most of children will have had at least one attack of bronchiolitis [7]. Some factors can predispose to severe bronchiolitis with longer hospitalization time and higher death rate like inhalation of cigarette smoke, crowded living environment, congenital heart defects and lack of breastfeeding [8].

To the best of the researcher's knowledge, lack of studies has been identified in the area in dealing with bronchiolitis despite its being a very common health problem. Knowing epidemiological factors, clinical findings and the risk factors of bronchiolitis are important aspects that help identify severe cases of bronchiolitis as an everyday practice clinical syndrome.

PATIENTS AND METHODS

A descriptive cross-sectional study was carried out at Hevi Pediatric Teaching Hospital in Duhok north of Iraq in the period from 1st November 2022 to 1st February 2023. Ethical approval for this study was obtained from the Council of Medical Ethics of the General Directorate of Health of Duhok, and an informed consent was also obtained from parents. The study has included all patients with age 1-24 months diagnosed to have bronchiolitis and admitted to the hospital during that period. The diagnosis of bronchiolitis was made according to the diagnostic criteria of the American Academy of Pediatrics (AAP) in 2014 , as follows[9]:

1. Upper respiratory tract inflammation: cough, fever, rhinorrhea and sneezing
2. Progression to chest indentation, tachypnea or intercostal muscle pull within 48 to 72 hours. Air stasis signs of were probably detected in chest X-ray or clinical examination
3. First or second attack of wheezing
4. Lung examination: crackles, hissing or rhonchi heard mainly during exhalation. There may be decreased vesicular murmur or no heard rale Diagnosing the bronchiolitis severity of depended on the Modified Cincinnati Bronchiolitis Score (MCBS) [6], Table 1. For every participant, the clinical aspects and epidemiological factors associated with bronchiolitis were obtained through a questionnaire filled by direct interview with parents.

The Study Design

It is a cross-sectional study.

Subjects

The study included 148 patients with bronchiolitis. Thirty-three (22.29%) had mild disease, 65 (43.91%) had a moderate disease and 50 (33.78%) had a severe disease.

Exclusion criteria

Children were to be excluded from the study if they have more than two attacks wheezing, were diagnosed to have bronchial asthma, if their ages were beyond the ages specified in this study, or the parents refused participation in the study.

STATISTICAL ANALYSIS

The data were analyzed by using the Statistical Package for Social Sciences (SPSS) software version 26. The association between categorical variables was analyzed by using Pearson's chi-square test. The data were presented as mean ± standard error of the mean Significance was considered when P value is less than 0.05.

RESULTS

The study included 148 patients with bronchiolitis. Thirty-three (22.29%) had mild disease, 65 (43.91%) had a moderate disease and 50 (33.78%) had a severe disease. As shown in Table 2, females were more common with no significant association with the severity. The largest age group was 3-6 months, with significant association with the severity. Their residence was most commonly in urban areas 84(56.8%). Only 8(5.4%) of the patients had a past medical history. Caesarean section was the most common mode of delivery but without any significant association with the severity of the disease. Exposure to tobacco smoke was significantly associated with the severity of the disease. The artificial feeding was the most common with significant association with the severity. Ninety (68.8%) had siblings attending school and child care without significant association with the severity. Thirty-four (23%) had a family history of asthma. Poor living conditions were significantly associated with severity. Local cradle (landik) was significantly associated with severity. Most chest X ray findings were normal in 126 patients (85.1%) with no significant association with severity.

Table 1: The Modified Cincinnati Bronchiolitis Score (MCBS).

Factors	0	1	2
Respiratory rate	Normal	>50/minute when not crying or agitated	
Muscles	Normal	Moderate retractions	Severe retraction
Air exchange	Normal	Localized decreased	Multi area decreased
Wheezes	None/End expiratory	Entire expiratory	Entire expiration and inhalation
Evaluation: Mild: 0-2 points; Moderate: 3-5 points; Severe: 6-7 points			

Table 2: Clinical, epidemiological and associated factors of bronchiolitis

Variables	**Mild No.(%)**	**Moderate No.(%)**	**Severe No.(%)**	**Total No.(%)**	**X^2**	**df**	**P-Value**
Age group							
> 3	9(27.3)	17(26.2)	13(26)	39(26.4)	11	6	0.04
3 to < 6 months	8(24.2)	16(24.6)	19(38)	43(29.1)			
6 to < 12 months	6(18.2)	15(23.1)	15(30)	36(24.3)			
1 to 2 years	10(30.3)	17(26.2)	3(6)	30(20.3)			
Gender							
Male	16(48.5)	32(49.2)	25(50)	73(49.3)	0.01	2	0
Female	17(51.5)	33(50.8)	25(50)	75(50.7)			9

Residency							
City	18(54.5)	39(60)	27(54)	84(56.8)	1.75	4	0.7
Rural	13(39.4)	19(29.2)	19(38)	51(34.5)			
Camp	2(6.1)	7(10.8)	4(8)	13(8.8)			
History of a previous attack							
YES	1(3)	3(4.6)	4(8)	8(5.4)	1.1	2	0.5
NO	32(97)	62(95.4)	46(92)	140(94.6)			
Mode of delivery							
VD	14(42.4)	26(40)	13(26)	53(35.8)	3.2	2	0.2
CS	19(57.6)	39(60)	37(74)	95(64.2)			
Premature birth							
YES	2(6.1)	8(12.3)	10(20)	20(13.5)	3.4	2	0.1
NO	31(93.9)	57(87.7)	40(80)	128(86.5)			
Chrinic heart or lung disease							
YES	0(0)	0(0)	2(4)	2(1.4)	3.9	2	0.1
NO	33(100)	65(100)	48(96)	146(98.6)			
Depressed immune system							
YES	0(0)	0(0)	1(2)	1(0.7)	1.9	2	0.3
NO	33(100)	65(100)	49(98)	147(99.3)			
Exposure to tobacco smoke							
YES	9(27.3)	32(49.2)	34(68)	75(50.7)	13.2	2	0.001
NO	24(72.7)	33(50.8)	16(32)	73(49.3)			
Feeding types							
Breast feeding	12(36.4)	21(32.3)	1(2)	34(23)	24	4	0.001
Artificial feeding	7(21.2)	25(38.5)	31(62)	63(42.6)			
Mixed	14(42.4)	19(29.2)	18(36)	51(34.5)			
Having siblings who attended School or daycare							
YES	16(48.5)	24(36.9)	18(36)	58(39.2)	1.5	2	0.4
NO	17(51.5)	41(63.1)	32(64)	90(60.8)			
Family History of Asthma							
YES	7(21.2)	18(27.7)	9(18)	34(23)	1.5	2	0.4
NO	26(78.8)	47(72.3)	41(82)	114(77)			
Poor living condition							
YES	12(36.4)	30(46.2)	36(72)	78(52.7)	12.1	2	0.002
NO	21(63.6)	35(53.8)	14(28)	70(47.3)			
Maternal education							

Illiterate	24(72.7)	41(63.1)	35(70)	100(67.6)	5.7	4	0.2
Primary & secondary school	3(9.1)	18(27.7)	9(18)	30(20.3)			
College & institute	6 (18.2)	6(9.2)	6(12)	18(12.2)			
Sleeping in local cradle (Landik)							
YES	24(72.7)	56(86.2)	47(94)	127(85.8)	7.4	2	0.02
NO	9(27.3)	9(13.8)	3(6)	21(14.2)			
X Ray details							
Irrelevant	28(84.8)	57(87.7)	41(82)	126(85.1)	2.3	4	0.6
Hyperinflation	5(15.2)	8(12.3)	8(16)	21(14.2)			
Collapse	0(0)	0(0)	1(2)	1(0.7)			
Total	33(100)	65(100)	50(100)	148(100)			

DISCUSSION

In this study, females were more common than males (50.7 vs 49.3). This result is in contrast to other studies that showed that males were more commonly affected at 69.2% 10; 63%14, male to female ratio of 1.42:115. The age of patients in this study shows that infants at age of less than 6 months are the most prevalent (55.5%) which is similar to what was found in another study: 57% were younger than six months [6]. There is a significant association between age and severity of bronchiolitis since the underdeveloped immune system at younger age and narrower caliber of bronchioles make them more likely to get infected and have a severe course of disease [10,11]. This is in line with other studies that found similar results [6,12,13].

Among the factors compared between severe and mild and moderate cases of bronchiolitis, exposure to tobacco smoke was significantly associated with severity of bronchiolitis. Similar results were also found by other studies [6,14-20]. This is because of the irritant effect of inhaled smoke on the respiratory tract epithelium. The present study revealed that the type of feeding is strongly associated with severity of bronchiolitis. Various immunologic factors present in breast milk exert a protective role against infections including bronchiolitis. This goes in line with studies done in different parts of the world [6,19-21]. Poor living conditions, especially living in an overcrowded house, are significantly associated with bronchiolitis severity in this study. This is in line with other studies that found that poor living conditions are related significantly to bronchiolitis severity [6,11,15,19,20]. This is because as the number of household members increases, the risk of exposure to respiratory secretions increases [13]. Sleeping in the local cradle (landik), where the infant is swaddled and tied to the cradle at a fixed supine posture, is significantly associated with severity of bronchiolitis. A study by Baudin et al showed that prone positioning in sleep for infants with bronchiolitis has a beneficial effect through decreasing respiratory effort and the metabolic cost of breathing [22].

In the current study, gender was not found to be associated significantly with severity of bronchiolitis in line with another study [6] but this in contrast to other studies that found a significant association [8,23]. Though living in urban areas was more common among infants with bronchiolitis, no significant association with severity was proved in this study. Air pollution in urban areas was found to a risk factor for bronchiolitis in other studies [19,20]. Delivery by Caesarean section was the most

common mode of delivery in most of the infants included in this study but no significant association was found with severity of presentation although it is known that delivery by Caesarean section impairs immunity thus increasing the risk of infection [6,11,24].

History of premature delivery was not found as a significantly associated factor with bronchiolitis severity in this study in contrast to other studies that found such a significant association [6,10,20,25,26]. This can be explained by smaller airway and suboptimal immune response in premature [26].

Chronic heart diseases were infrequent and not associated with severity of bronchiolitis. Different other studies found this a very significant associate with bronchiolitis severity [20,25]. The increased lung stiffness and hypoxemia associated with heart diseases intensifies the narrowing of airway and severity of presentation of bronchiolitis. Though having siblings attending school or day care is expected to an important way through which bronchiolitis is acquired by infants, the present study did not find a significant association with the severity of bronchiolitis in contradiction to other studies that found it significant [6,19].

Almost a quarter of the patients had a family history of asthma but this was not significantly associated, on the contrary to a study that found history of asthma in the mother a significant factor associated with severity of bronchiolitis [24]. The maternal education is not significantly associated with bronchiolitis severity in the current study although other studies found it significant [6].

CONCLUSIONS

This study shows that age, exposure to tobacco smoke, type of feeding, poor living conditions and sleeping in the local cradle (landik) were related significantly to severity of bronchiolitis in Duhok.

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Prevalence of Hashimoto's Thyroiditis among Rheumatoid Arthritis Patients

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ABSTRACT

Background: *Rheumatoid arthritis is an inflammatory illness that affects the entire body but its cause is unknown. There has been a considerable debate about the relation between thyroid gland and Rheumatoid arthritis.* ***Aim of the study:*** *To determine the frequency of Hashimoto's thyroiditis in patients with Rheumatoid Arthritis and determine whether patients with Rheumatoid arthritis are at higher risk of Hashimoto's thyroiditis.* ***Patients and methods:*** *This study is a cross-sectional observational study done in the governorate of Najaf from September 2022 to February 2023 involving 140 participants diagnosed with Rheumatoid arthritis, determined by rheumatologist doctors in line with ACR/EULAR 2010 Criteria and serological testing. Patients including 16 males and 124 females, ranging between the ages of 20 and 60. All participants underwent complete clinical and laboratory assessments. The data were collected during the direct patient interview and the information from the questionnaire, verbal approval has been received from the study participant.* ***Results:*** *The frequency of Hashimoto's disease in the selected patients of rheumatoid arthritis (N=140), was 45 (32.14%), While euthyroidism (Rheumatoid arthritis patients without Hashimoto's) was 95(67.9%). Hashimoto's disease included subclinical 9 (6.4%) and overt Hashimoto's thyroiditis 36(25.7%).* ***Conclusions:*** *HT is frequent among patients with RA. Therefore, there is a need of screening of thyroid hormone dysfunction as well as presence of Anti-TPO antibodies as markers of HT in RA patients particularly in young patients, females, and those with high disease activity. The association was a significance among RA patients with HT and ACCP, BMI, and ESR.*

Keywords: Rheumatoid arthritis, Hypothyroidism, Hashimoto's.

Article Information

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INTRODUCTION

Rheumatoid arthritis (RA) is a polyarticular symmetric illness that has an impact on a number of joints on both sides of the body. Pain as well as swelling, mainly in the hands and feet joints, are the most typical signs of RA. The most swollen joints are the wrists, metacarpophalangeal, metatarsophalangeal, and proximal interphalangeal (1).

One of the most important aspects of disease management is its focus on early detection and treatment (2). The increased occurrence of thyroid dysfunction in RA patients has been reported (3). Depending on the study population, geographic area, and definition of Autoimmune thyroid disease (AITD), the prevalence of AITD among RA patients has ranged from 3 to 30% (4).

A variety of studies have shown that hypothyroidism and its prevalent symptoms of discomfort, weariness, weight gain, and dyslipidemia can be easily missed in the early stages of rheumatoid arthritis since they are so similar to the initial signs of the disease (5).

AITD is an autoimmune attack on the thyroid gland caused by immune system dysfunction. It affects more people than any other autoimmune disorder (6). The etiology is complex, that involves genetic, environmental, and dietary variables . The most common form of AITD is Hashimoto's thyroiditis (HT) (7).

Women and the elderly are particularly at risk for developing hypothyroidism in RA patients (8). The exact mechanism through which RA and AITD are linked remains unknown, numerous studies have shown autoimmunity as a key player in the development of both conditions. Some genes, including STAT4, HLA-DRB1, and the vitamin D receptor, were also found to have important roles in the progression of both disorders (9). Further, environmental risk factors, including as infection and smoking, play important roles in the development of both RA and AITD (3)

The question of whether there are other links between RA and AITD, such as AITD having a causal effect on the onset of RA or sharing environmental triggers, remains unanswered. To better understand the nature of the link between RA and AITD, it may be helpful to evaluate the risk of acquiring AITD at different points in time for patients with RA (10). Several studies have linked hypothyroidism to a worsening of rheumatoid arthritis, particularly the destructive arthropathy that mostly affects the proximal interphalangeal joints(5).

The purpose of this study is to investigate the prevalence of HT among patients with RA.

PATIENTS AND METHODS

Participants in this research were Iraqi patients with RA who attended the Rheumatology department in Al-Sadr Medical City in Najaf.

The Study Design

It is a cross-sectional observational study.

Subjects

This research was carried out on a total of 140 participants diagnosed with RA, determined by rheumatologist doctors in line with ACR/ EULAR 2010 criteria and serological testing. The patients including 16 males and 124 females, ranging the ages of 20 and 60. Patients were asked about their name, gender, age, and any other items. Prior to the start of the study, the ethical committee of the Faculty of Medicine, University of Kufa, provided its permission. Individuals' informed agreement was also gained.

Exclusion criteria

Participants who have other rheumatologically diseases, prior thyroidectom, pregnant women, Evidence of malignancy, patients have chronic liver or renal diseases, or hyperthyroidism were excluded.

Data collection:

For data collection a questionnaire was designed to record the subject’s information.

Instruments

The equipment utilized in this study include gel serum tubes 5 & 10 ml, disposable sterile syringes, 5 &10 ml, disposable pipette tips, Eppendorf tubes, 1.5 & 2 ml, disposable ESR tubes, and disposable gloves. The instruments utilized in this study were a centrifuge, deep freeze, incubator, ELISA, Refrigerator, micro pipettes with different sizes.

Methods

The sample collected of venous blood was from five to ten ml. The blood sample was divided in half: 2 mL was placed in a disposable ESR tube for the Westergren method of measuring ESR, and 3–7 mL was placed in a sterile gel tube for further analysis. After the blood was drawn in its whole, it was left undisturbed at room temperature to clot within an average of about 10-20 minutes. Centrifugation at 3,000 to 3,000 rpm for 20 minutes was used to dislodge the clot. The sample needs to be centrifuged once more if precipitates form during the reservation. Each patient's sample was separated into three parts and stored in an Eppendorf tube at temperatures ranging from minus 20 to minus 45 ºC. Determination of Anti-CCP antibodies, anti-thyroid peroxidase (Ab-TPO) autoantibodies, Thyroid-stimulating hormone (TSH), and T4 in RA patients by ELISA kit.

STATISTICAL ANALYSIS

The SPSS program, version 26, was utilized to analyze the data. Descriptive statistics was done through calculation of frequency, percentage, mean, and standard error of mean of sociodemographic characteristics of study sample presented by tables. After testing the normality of data, inferential statistics were by application of independent T test and ANOVA to differentiate means of rheumatoid and autoimmune thyroiditis markers in addition to testing correlation and regression relationship of some numerical parameters. Chi square and Fishers exact probability tests were applied for categorical association. statistical significance was regarded as having a P value that must be equal to or less than 0.05.

RESULTS

Table 1 Show thyroid dysfunction definitions (Ralli et al., 2020).

Table 2 shows Frequency of Hashimoto's disease in the selected patients of rheumatoid arthritis (N=140), was 45 (32.14%), While euthyroidism, RA patients without HT, was 95(67.9%). HT disease included subclinical 9 (6.4%) and overt 36(25.7%).

Table 3 shows 36 (25.7%) patients had TSH levels >4 µIU/mL and FT4<0.8 ng/dl with elevated Anti-TPO (> 50 IU/mL); therefore, they were classified as having overt HT and 9 (6.4%) patients with TSH levels >4µIU/mL and normal FT4, with elevated Anti-TPO (> 35) as subclinical HA. There were no significant differences in regard to age, gender, residency, and duration of RA disease, between euthyroid patients and HT, (P-value >0.05). While Family history of RA and Treatment response have significant differences among three groups (euthyroid, overt HT and subclinical HT), (P-value <0.05). Family history of RA have (P-value=0.032) was more in euthyroid RA patients compared to RA patients with HT. Treatment response have (P-value=0.018), poor response was more in overt HT and subclinical HT compared to euthyroid RA patients the percent (55.6%,55.6%,30.5%) respectively.

Table 1: Thyroid dysfunction definitions.

Thyroid dysfunction	TSH (µIU/L)	Free T4 (ng/dl)	Anti-TPO (IU / ml)
Euthyroidism	0.3– 4 (normal)	(0.8–1.8)(normal)	< 50 (normal)
Subclinical HT	>4 (increased)	(0.8-1.8) (normal)	> 50 (increase)
Overt HT	>4 (increased)	<0.8 (decreased)	> 50 (increase)

Table 2: Number and percent of thyroid dysfunction among selected group (140).

Thyroid dysfunction	No.	(%).
Euthyroidism	95	67.9
Subclinical HT	9	6.4
Overt HT	36	25.7
Total	140	100

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Table 3: Comparison of socio and clinical characteristics between euthyroid patients and Hashimoto's patients.

Patient's characteristics			Study sample (N=140)			Total	P-value
			Subclinical HT; n=9	Overt HT n=36	Euthyroid n=95		
Age groups (Year)	≤ 40	(%)	2 (22.2%)	14 (38.9%)	26 (27.4%	42 30.0%	.382
	>40	(%)	7 (77.8%)	22 (61.1%)	69 (72.6%)	98 70.0%	
		Total	9 (100.0%)	36 (100.0%)	95 (100.0%)	140(100.0)	
Gender	Female	(%)	8 (88.9%)	35 (97.2%)	81 (85.3%)	124(88.6%)	.158
	Male	(%)	1 (11.1%)	1 (2.8%)	14 (14.7%)	16 (11.4%)	
		Total	9 (100.0%)	36(100.0%)	95(100.0%)	140(100.0%)	
Residency	Urban	(%)	7 (77.8%)	30 (83.3%)	74 (77.9%)	111(79.3%)	.785
	Rural	(%)	2 (22.2%)	6 (16.7%)	21 (22.1%)	29 (20.7%)	
		Total	9(100.0%)	36(100.0%)	95(100.0%)	140(100.0%)	
Marital status	Married	(%)	9 (100.0%)	35 (97.2%)	89 (93.7%)	133 (95.0%)	.550
	Single	(%)	0 (0.0%)	1(2.8%)	6 (6.3%)	7 (5.0%)	
		Total	9(100.0%)	36(100.0%)	95(100.0%)	140(100.0%)	
Occupation	Unemployed	(%)	8 (88.9%)	33 (91.7%)	88 (92.6%)	129 (92.1%)	.609
	Employed	(%)	1 (11.1%)	3 (8.3%)	4 (4.2%)	8 (5.7%)	
	Students	(%)	0 (0.0%)	0 (0.0%)	3 (3.2%)	3 (2.1%)	
		Total	9 (100.0%)	36(100.0%)	95 (100.0%	140 (100.0%)	
Duration of RA (Months)	≤ 12	(%)	3 (33.3%)	9 (25.0%)	35 (36.8%)	47 (33.6%)	.440
	> 12	(%)	6 (66.7%)	27 (75.0%)	60 (63.2%)	93 (66.4%)	

		Total	9 (100.0%)	36(100.0%)	95 (100.0%	140(100.0%)	
Family history of RA	Yes	(%)	2 (22.2%)	2 (5.6%)	25 (26.3%)	29(20.7%)	.032
	No		7 (77.8%)	34 (94.4%)	70(73.7%)	111(79.3%)	
		Total	9(100.0%)	36(100.0%)	95(100.0%)	140(100.0%)	
Family history of HT	Yes	(%)	0 (0.0%)	2 (5.6%)	6 (6.3%)	8 (5.7%)	.737
	No	(%)	9 (100.0%)	34 (94.4%)	89 (93.7%)	132 (94.3%)	
		Total	9 (100.0%)	36(100.0%)	95(100.0%)	140(100.0%)	
Treatment response	Good	(%)	4 (44.4%)	16 (44.4%)	66 (69.5%)	86 (61.4%)	.018
	Poor	(%)	5 (55.6%)	20 (55.6%)	29 (30.5%)	54 (38.6%)	
		Total	9 (100.0%)	36 (100.0%	95 (100.0%	140 (100.0%)	

Table 4 show that there was a higher mean concentration of ESR among patients with overt HT (38.05±.2.46 mm/h) and subclinical (43.88±6.39 mm/h), when compared to the mean concentration of the Euthyroid RA patients (30.33±1.96 mm/h); i. e., there was a significant difference (P-value = 0.019) among RA patients with HT and euthyroid RA patients regarding to the concentration of ESR. There was a higher mean concentration of Anti-CCP, Anti-TPO, TSH among patients with HT (overt and subclinical) in compared to euthyroid patients. The mean concentration of Anti-CCP, Anti-TPO, TSH in overt HT was (73.78±5.38 U/ml, 142.98±10.29 IU/ml, 5.87±.174 mlU/L) respectively, while in subclinical was (75.52±14.98 U/ml, 139.17±22.97 IU/ml, 5.940± 0.52 mlU/L) respectively and in euthyroid was (40.46±4.29 U/ml, 0.169±0.0057 IU/ml, 1.39±0.050 mlU/L) respectively.

Table 4: Comparison of study parameters according to clinical status of Hashimoto's disease

Laboratory Estimates (Mean ±SE)	Study sample (RA) (N=140) (Mean. ± Standard error)			P value
	Subclinical HT, n=9	Overt HT; n=36	Euthyroid; n=95	
ESR mm/h	43.88±6.39	38.05±0.2.46	30.33±1.96	0.019
Anti-CCP U/ml	75.52±14.98	73.78±5.38	40.46±4.29	0.0001
TSH mlU/L	5.940±0.52	5.87±0.174	1.39±0.050	0.0001
T4 ng/dl	1.257± 0.08	0.51± 0.034	1.36± 0.023	0.0001
Anti-TPO IU/ml	139.17± 22.97	142.98±10.29	0.169 ±0.0057	0.0001
BMI kg/m2	35.24±1.86	35.68±0.82	31.63±0.65	0.002

That means there were a highly significant differences (P-value = 0.0001) among RA patients with HT and euthyroid RA regarding to the concentration of Anti-CCP, Anti-TPO, TSH. Besides, T4 shows a highly significant difference (P-value = 0.0001) among RA patients with HT and euthyroid RA patients, the mean concentration in overt HT was (0.51± .034 ng/dl) and subclinical HT was (1.257± .08 ng/dl) compared to the mean concentration of the euthyroid RA patients (1.36± .023 ng/dl). BMI has significant differences (P-value = 0.002) among RA patients with HT and euthyroid RA patients, the mean concentration in overt HT was (35.68± .82 kg/m2) and subclinical HT was (35.24±. 1.86 kg/m2) compared to the mean concentration of the Euthyroid patients (31.63±. .65 kg/m2).

DISCUSSION

The current study involved 140 adult RA patients, 16 males (11.4%) and 124 (88.6%) females. HT was present in 45 (32.14%) RA patients including subclinical HT 9(6.4) and overt HT 36(25.7) which agrees with (11) that show 36 (24%) RA patients had overt hypothyroidism, while 6 (4%) had subclinical hypothyroidism; And near similar to (12) who found that 20 (38.4 %) patients had hypothyroidism. The correlation in our study was a significant among RA patients with HT and ACCP (P= 0.0001), BMI (P=0.002), and ESR (P= 0.019). This indicates that hypothyroid present in patients with high ESR, obese, and positive ACCP. These results are similar to (11), (12), and (5) who found that patients with RA and hypothyroidism had a statistically significant correlation with BMI, and ESR, (P< 0.05). In addition, the present study has found no significant association among RA patients with HT in regards to age that have P value= 0.382 and gender (P=0.158). This is compatible with (12), (11), and (5), who find no significant association of age and gender P value >0.05. Besides, (13) finds that the P value of age =0.32**,** and Gender =0.18 and disagrees with (8) who documented a rise in the occurrence of hypothyroidism as individuals age, along with a greater prevalence among females compared to males. A recent study shows a significant difference (p<0.05) between RA patients with HT and treatment response (P=0.018). HT is higher in patients with poor response compared to good response; this disagrees with (8) who found that there is no association between medication for RA and an increased risk of hypothyroidism.

In the recent study, no significant difference between RA patients with HT and disease duration (P=0.44); this agrees with an Egyptian study by (3) who found that the disease duration has no significant differences (p=0.49) and disagree with (11), (12) and (5). They reported a statistically significant correlation (P<0.05) between RA patients with HT and disease duration. In a recent study that compared the sociodemographic characteristics of the RA patients with HT and euthyroid RA patients according to residency, it has been found that there was no significant difference of p > 0.05. Family history of RA was more in RA patients without HT, as there was a significant difference P-value <0.05 compared to RA patients with HT

CONCLUSIONS

HT is frequent among patients with RA. Therefore, there is a need of screening of thyroid hormone dysfunction as well as presence of Anti-TPO antibodies as markers of HT in RA patients particularly in young

patients, females, and those with high disease activity**.** The association was a significance among RA patients with HT and ACCP, BMI, and ESR.

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Evaluating the Effect of Copper Oxide Nanoparticles after Added to the Maxillofacial Silicone on the Adherence of *Staphylococcus Epidermidis*

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ABSTRACT

Background: *Maxillofacial defects may arise from congenital, developmental, traumatic, or surgical procedures. The presence of such defects may adversely affect an individual's appearance and function, resulting in an inability to lead a typical life and impacting their psychological well-being. Surgical reconstruction is often regarded as the primary therapeutic option in such instances. However, it may not be feasible in numerous cases due to various unfavorable conditions. Consequently, the need for maxillofacial prosthesis reconstruction becomes obligatory.* ***Purpose****: This study assesses the antibacterial efficacy of copper oxide nanoparticles at different rates against Staphylococcus epidermidis after incorporation into maxillofacial silicone.* ***Methods****: A pilot study was first conducted in Iraq and copper oxide nanoparticles were added to VST50F silicone elastomer in different five percentages (0.01 wt%, 0.02 wt%, 0.03 wt%, 0.04 wt%, and 0.05 wt%). Thirty specimens were prepared and grouped into six groups: one control group and five experimental groups, and then the best effective two percentages (0.03 wt% and 0.04 wt%) were selected for the main study. Thirty specimens for the main study were divided into three groups: control group (A) and two experimental groups (B and C). A statistical analysis was done with an ANOVA and the Games-Howell multiple comparison test ($P < 0.05$). The data's normal distribution and homogeneity were assessed. Field emission scanning electron microscopy (FES-EM) and Fourier transform infrared spectroscopy (FTIR) were also conducted.* ***Results****: The statistical analysis showed a significant difference between all groups with $P < 0.05$. There was a significant difference between control group A and experimental groups B and C with $P < 0.05$, as well as between experimental groups B and C ($P < 0.05$). FESEM showed that nanoparticles were distributed well within the silicone matrix. FTIR spectra proved no chemical reaction to occurr between the copper oxide nanoparticles and VST50F silicone.* ***Conclusion****: Incorporating copper oxide nanoparticles into VST50F maxillofacial silicone improved their antibacterial efficacy against Staphylococcus epidermidis.*

Keywords: Maxillofacial Prothesis; Nanoparticles; Silicone Elastomers; *Staphylococcus Epidermidis*

Article Information

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INTRUDUCTION

Maxillofacial defects may arise from congenital, developmental, traumatic, or surgical procedures. The presence of such defects may adversely affect an individual's appearance and function, resulting in an inability to lead a typical life and impacting their psychological well-being (1). Surgical reconstruction is often regarded as the primary therapeutic option in such instances. However, it may not be feasible in numerous cases due to various unfavorable conditions. Consequently, the need for maxillofacial prosthesis reconstruction becomes obligatory (2). Polymeric materials are commonly employed in the fabrication of maxillofacial prostheses., the aforementioned materials encompass vinyl chloride polymers and co-polymers, acrylic types such as polymethyl methacrylate, and silicone elastomers, which can be classified into either high temperature vulcanization or room temperature vulcanization. Silicone elastomers have garnered a significant clinical significance due to their notable attributes such as heat and chemical inertness, durability, strength, elasticity, ease of manipulation, and esthetics (2,3).

However, it is worth noting that there is currently no single maxillofacial material, including silicone, that fully satisfies the criteria for an ideal prosthesis. These materials have encountered challenges such as mechanical deterioration, color instability, limited durability, and changes in properties. Various factors induce the issues, which require frequent replacement of the prosthesis (4). Thus, in order to overcome these issues, it is necessary to reinforce the silicone maxillofacial material. Due to the development of nanoscience and nanotechnology, the application of nanoparticles (NPs) in elastomers has been investigated as a means of improving their properties. Numerous NPs have undergone testing, and subsequent studies demonstrate the efficacy of NPs in enhancing the mechanical, physical, and antimicrobial characteristics of silicone elastomers [5,6]. Microbial biofilms have been identified as significant contributors to the degradation of maxillofacial prostheses Biofilms refer to organized communities of microorganisms consisting of a single or multiple species enclosed in a self-produced exopolysaccharide matrix that adheres to a biotic or abiotic surface (7,8). Candida albicans and Staphylococci spp, especially Staphylococcus epidermidis (S. epidermidis) and Staphylococcus aureus (S. aureus), are the essential causative agents of infections related to prosthetic devices. Mixed biofilms may develop from a variety of factors, including the porosity and roughness of the prostheses (9,10).

S. epidermidis, referred to as a gram-positive staphylococcus with coagulase-negative properties, is a highly prevalent bacterium that colonizes the skin of healthy individuals. It can be found in various skin microenvironments, such as dry, moist, sebaceous, and foot regions. It can either aid or harm the skin. S. epidermidis plays a beneficial role in maintaining the equilibrium of microorganisms on the epithelial surfaces of humans by controlling the proliferation of harmful pathogens, especially S. aureus. Conversely, S. epidermidis is recognized as a significant etiological agent responsible for nosocomial infections in humans, which can cause severe infection (11,12). S. epidermidis is commonly implicated in infections associated with the use of indwelling medical devices, implants, and prosthetic devices following adhesion to surfaces and the formation of micro-colonies (13). Coagulase-negative staphylococcal species exhibit a proclivity for invading the human body through prosthetic devices, wherein a restricted population of bacteria migrates along the prosthetic device and gains access to the bloodstream. Bacteria possess the capacity to form biofilms as a means of protecting themselves against immune responses from the host or antimicrobial agents (14). The conventional cleaning procedures employed in the regular maintenance of prostheses are insufficient to eliminate all microorganisms present on the surface (15). Commonly used disinfectants cause deterioration in color, hardening, and significant variations in the surface roughness of silicone prostheses. Therefore, there is a requirement for further developed silicone materials that exhibit a greater resistance to microbial proliferation (16).

The development of microbial resistance to traditional antimicrobial agents has become a prominent issue in contemporary times. The emergence of multidrug-resistant (MDR) infectious microorganisms presents a formidable obstacle to the healthcare system. The utilization of nanomaterials as a viable option for anti-infective purposes has gained credibility owing to their distinctive chemical properties and high surface area to volume ratio. Metal and metal oxide NPs with antimicrobial characteristics are a promising approach for suppressing MDR strains among nanomaterials (17,18).

Copper oxide (CuO) is a chemical compound comprised of copper and oxygen. CuO NPs have the appearance of a brownish-black powder. CuO NPs are photocatalytic, stable, and inexpensive, and they have the potential to be used as anti-infective agents due to their unique crystal morphologies and extremely large surface areas [19]. CuO NPs have a wide range of microbicidal activity against fungi, viruses, and bacteria, including MDR bacteria [20,21]. CuO NPs have been widely investigated in various biomaterials. Furthermore, several studies have provided evidence that the incorporation of CuO NPs into various dental materials yields a notable decrease in microbial biofilm formation and potentially enhances the materials' physical and chemical characteristics [22]. Nonetheless, there is a lack of data regarding their incorporation into maxillofacial silicone. The objective of this investigation was to assess the impact of CuO NPs on the adhesion of *S. epidermidis* biofilm subsequent to their incorporation into VST 50F maxillofacial silicone at different weight percentages.

METHODS

CuO NPS (40 nm) (Sky Spring Nanomaterials, USA) and VST 50F room temperature vulcanized maxillofacial silicone (FactorII Inc., USA) were used. A preliminary study was conducted to determine two optimal percentages to be added to VST 50F silicone elastomer in the main study. CuO NPs were added to VST 50F silicone elastomer in five different percentages (0.01 wt%, 0.02 wt%, 0.03 wt%, 0.04 wt%, and 0.05 wt%). Thirty specimens were meticulously prepared and subsequently categorized into six groups: one control group and five experimental groups, with five specimens for each group. A bacterial adherence test was done for all the six groups. According to the result of the pilot study, two percentages of CuO NPs additives (0.03 wt% and 0.04 wt%) were selected in addition to the control group.

A total of thirty specimens for the main study were prepared and categorized into three groups: one control group (A) and two experimental groups, group B (0.03 wt% CuO additive) and group C (0.04 wt% CuO additive). The shapes and dimensions of the test specimen molds were generated through the utilization of computer-aided software, specifically AutoCAD 2013 (Autodesk, USA). The template was subsequently constructed via a laser engraving cutting machine. (CNC) (JL-1612, Jinan Link Manufacture and Trading Co., Ltd., China). Three clear acrylic sheets (PT. Margacipta Wirasentosa, Indonesia) (the matrix, bottom, and cover) with 2±0.05 mm thickness were used. The matrix sheet was fabricated with circular perforations measuring 10 mm in diameter and was fixed to the bottom using chloroform (Weld-On, USA) as an adhesive to prevent movement during the pouring of silicone (Fig 1A). G-clamps, nuts, and screws were also utilized to fix the cover and provide further tightening (23).

According to the manufacturer's instructions, VST 50F silicone should be mixed at a 10:1 base to catalyst ratio. The CuO NPs powder was initially weighed in a mixing bowl using a 4-digit electronic scale (Denver Instrument, USA). Subsequently, a predetermined amount of silicone base was added to prevent the dispersion of the volatile NPs. The NPs were blended with the silicone base to produce the modified base, which was mixed for 10 minutes using a vacuum mixer (Dentaurum Airvac, Germany). To prevent the suction of NPs, the vacuum was switched off for the initial three-minute period. Subsequently,

the vacuum was activated for a duration of seven minutes, operating at a speed of 360 rpm and a vacuum pressure of (10-1) bar, with the intention of eliminating any air bubbles (24). The mixture was allowed to cool down for 2 minutes before adding catalyst. This delay was necessary due to the heat generated by the rotational motion of the mixer, which has the effect of reducing the material's available working time. The addition of the catalyst was performed based on weight and subsequently mixed with the modified silicone base for a duration of 5 minutes using a vacuum mixer. This process yielded a uniform and devoid-of-bubble mixture.25 The material was poured into the mold under standard conditions, which included a restricted temperature of 23±2°C and a humidity level of roughly 50±10% (25). The matrix cover was affixed over the poured material by positioning the margin on one side and gradually lowering it at the opposite end. The matrix was covered slowly and carefully to allow excess material to exit the mold. Then, a one-kilogram weight was placed on the mold cover's center. Nuts and G-clamps secured the mold until the specimens hardened (5). In accordance with the manufacturer's recommendations, the mixture was allowed to set for 2-4 hours, after which the specimens were retrieved, finished, and stored for 16 hours 20±25°C, 50 ±10% humidity according to ISO 23529 (26).

Bacterial adherence test Bacteria were collected from four male patients aged 50–55 years at Ghazi al-Hariri hospital in Baghdad's medical city using sterilized cotton swabs. To avert necrotic tissue, the swab was rotated across the infected area.27 The samples were inoculated into blood agar and mannitol salt agar (Oxoid, UK) that had been prepared following the manufacturer's instructions and then incubated aerobically at 37°C for 48 hours (Fig 1B) (28). S. epidermidis exhibits a rapid growth rate on blood agar, leading to the formation of non-hemolytic, cohesive colonies that are white in color and measure approximately 1-2 mm in diameter (29). While producing small pink, red, or colorless colonies with no color change to the medium after overnight incubation in mannitol salt agar (30). The positive catalase test indicated the presence of staphylococci, which was subsequently confirmed utilizing the VITEK 2 compact system (Biomerieux, France) pursuant to the guidelines provided by the manufacturer. A microscopical examination was also performed, and S. epidermidis appeared as cocci in grape-like clusters.

Brain heart infusion broth (Mast Group, UK) was used to prepare the bacterial suspension that would be utilized to test S. epidermidis' bacterial adhesion capacity on silicone specimens. It was prepared through the suspension of 34.5 g in 1000 ml of distilled water, followed by thorough mixing until complete dissolution was achieved. The sterilization process was carried out through autoclaving at a pressure of 15 pounds (lbs) and a temperature of 121°C for a duration of 15 minutes, according to the manufacturer's instructions. A suspension with a concentration of 107 colony forming units per milliliter (CFU/ml) was prepared at 0.5 McFarland standards using a McFarland densitometer (Biomerieux, France).

The silicone specimens underwent sterilization by autoclaving at 121°C for 20 minutes. Then the sterilized silicone specimens were deposited in sterilized plastic petri dishes containing the prepared bacterial suspension; the specimens were incubated for 1 hour at room temperature.22 Following the incubation period, the specimens were removed from the bacterial broth, rinsed with phosphate buffered saline for one minute with gentle rocking to remove all non-adhered bacterial cells, and dried with filter paper.22 The specimens then underwent staining with 1% crystal violet (HIMEDIA, India) for 15 minutes, and rinsed until no crystal violet residue remained in the washing solution, and dried with filter paper. Subsequently, the specimens were immersed in ethanol (96%) (Microm Microtech, France) for 20 minutes to solubilize the crystal violet [31-33].

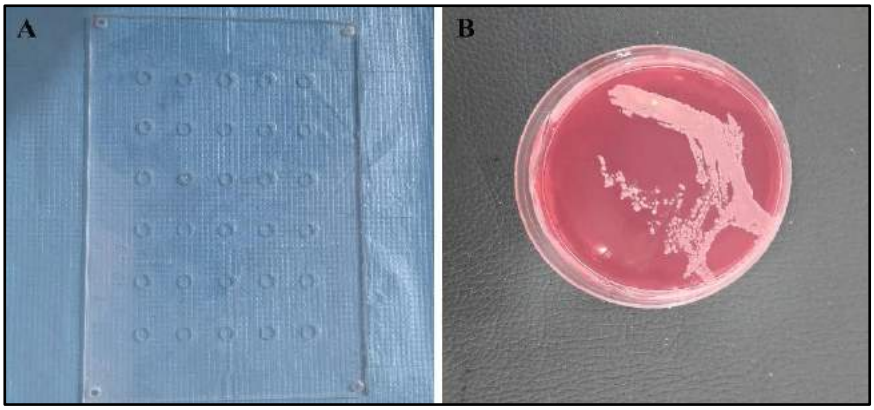

Figure 1. A: Specimens mold; B: Staphylococcus epidermidis on mannitol salt agar

RESULTS

Field emission scanning electron microscope (FE-SEM) A FE-SEM (Inspect F50, FEI, USA) was used to examine the topography and morphology of CuO NPs within the matrix of RTV silicone elastomer (VST 50F) specimens after the addition of CuO NPs. Silicone specimens were coated with metal or gold by a thin (1nm) metal sputter coated film by a sputter coated device, allowing electrons from FE-SEM to react with the specimens as directed by the company. In the FE-SEM pictures of the VST 50F silicone matrix, the CuO NPs were uniformly dispersed with some slight agglomeration, as seen in Fig 2 (A, B, and C).

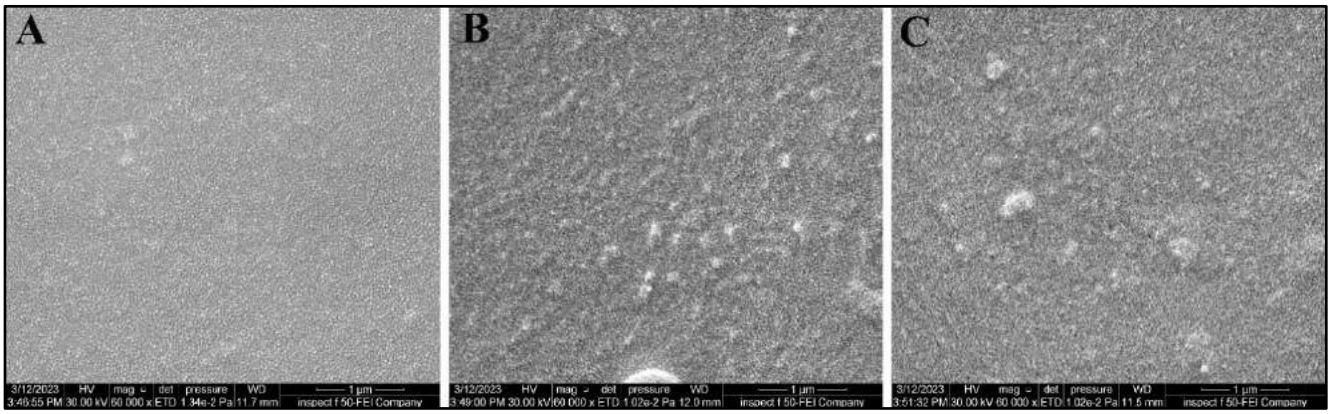

Figure 2. Images of field emission scanning electron mi croscope images at 60000 X magnifications (1 µm) showing an evenly distributed filler with a slight agglomerate: A: control specimen; B group B specimen C: group C specimen

Fourier transform infrared spectroscopy (FTIR)

FTIR (Spectrum Two N, PerkinElmer, USA) was used to investigate the potential chemical interaction between the silicone material and CuO NPs. Three specimens, one from each group (A, B, and C), were examined. The silicone specimens were fabricated as thin flushes with dimensions of 10x10x0.5 mm in length, width, and thickness, as per the manufacturer's instructions. FTIR results indicate that the addition of CuO NPs had no effect on the spectra of VST 50F silicone (no chemical interaction), as indicated in Fig 3 (A, B and C).

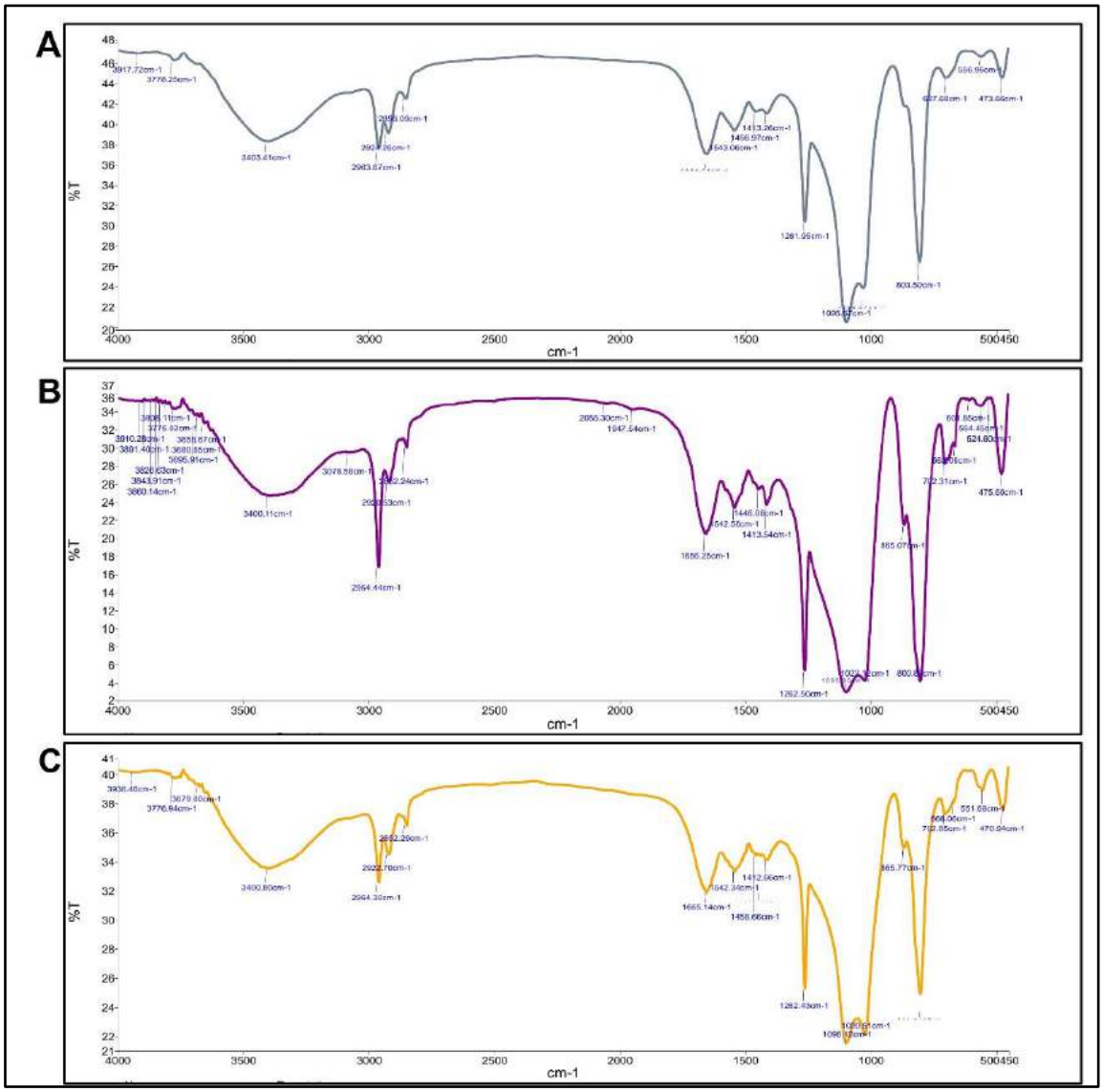

Figure 3. Fourier transforms infrared spectroscopy: A: control specimen (group A); B: group B specimen; C: group C specimen.

Statistical analysis

The statistical analysis was done by SPSS (statistical package for social science version 24) (IBM, USA) computer software. A probability "*P*" value of > 0.05 was deemed as non-significant statistically, ≤ 0.05 as significant.All variables in this this study were normally distributed among groups using Shapiro -Wilk test at $P > 0.05$ (Tab 1).

The results of the *S. epidermidis* adherence test showed that both experimental groups B and C had a mean value of OD less than that of control group A. It was observed that group C exhibited the lowest mean value among them and had a value of (0.012), followed by group B (0.029), and then group A with a mean value of (0.161), as shown in Fig. 4 and Tab 2.One-way ANOVA showed a significant difference among groups with a $P < 0.05$ (Tab 3).

Levene's test is used to assess variance homogeneity and, as a result, to determine the type of multiple comparisons post hoc test to use (Tab 4). Post-hoc Games-Howell test showed a significant difference between groups ($P < 0.05$) (Tab 5).

Table 1. Test of normality.

Groups	Statistic	df	*P*-value
Group A	0.944	10	0.593*
Group B	0.937	10	0.523*
Group C	0.968	10	0.872*

*= Non-significant at $P > 0.05$.

Table 2. Descriptive statistics of bacterial adherence test (optical density).

Groups	N	Mean	Std. Deviation	Std. Error	Minimum	Maximum
Group A	10	0.1612	0.020275	0.006411	0.133	0.190
Group B	10	0.0292	0.004566	0.001444	0.020	0.035
Group C	10	0.0127	0.002497	0.000790	0.009	0.017
Total	30	0.0677	0.068591	0.012523	0.009	0.190

Table 3. One-way ANOVA analysis of variance among groups.

	Sum of Squares	df	Mean Square	F	*P*-value
Between Groups	0.132	2	0.066	453.600	0.000**
Within Groups	0.004	27	0.000		
Total	0.136	29			

**= Significant at $P < 0.05$.

Table 4. Levene's test (homogeneity of variance).

	Levene Statistic	df1	df2	*P*-value
Based on Mean	18.749	2	27	0.000**
Based on Median	17.756	2	27	0.000**
Based on Median and with adjusted df	17.756	2	10.824	0.000**
Based on trimmed mean	18.742	2	27	0.000**

**= Significant at $P < 0.05$.

Table 5. Multiple comparisons of bacterial adherence test between groups using Games-Howell test.

(I) Groups	(J) Groups	Mean Difference (I-J)	Std. Error	*P*- value
Group A	Group B	0.132000	0.006572	0.000**
	Group C	0.148500	0.006460	0.000**
Group B	Group C	0.016500	0.001646	0.000**

**= Significant at $P < 0.05$.

CONCLUSION

Based on the findings of this study, the addition of CuO NPs to the maxillofacial silicone displayed antibacterial activity *in vitro* against *S. epidermidis*. CuO NPs in small percentages seem to significantly inhibit bacterial adhesion to the surface of VST 50F silicone elastomer and can serve as a basis for future long-term investigations regarding antimicrobial effectiveness.

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Evaluating the Serum CTLA-4 Levels in Patients with HBs Ag (-)/HBc IgG (+)/Hbs Ab (+): Across Sectional Study in the Najaf

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ABSTRACT

Background: *Hepatitis B virus is a virus that attacks the liver, leading to viral hepatitis, cirrhosis, and liver cancer in humans. The diagnostic markers for hepatitis B, anti-HBc IgG and anti-HBs Ab, are widely recognized. A cytotoxic T-lymphocyte antigen-4 called CTLA-4 is an immune checkpoint protein that stops the HBV infection from spreading. It accomplishes this by serving as a sort of inhibitory receptor, restricting the quantity of damage that an acute infection can cause to the hepatocyte and enhancing the infection's capacity to remain in the body throughout a chronic illness.* ***Aim of the study:*** *The study aims to evaluate serum CTLA-4 levels in individuals with HBsAg-negative, HBc IgG-positive, and HBs-positive Ab and explore the association between these findings and the existence and development of HBV infection.* ***Patients and methods:*** *A cross-sectional study was performed from July to October 2023. The serum was taken from 200 individuals, all of whom were tested by using an immunochromatographic assay for HBsAb, HBsAg, HBcAb, HBeAg, and HBeAb and also by using an ELISA technique for CTLA-4 and HBc IgG. The statistical analysis was conducted by using SPSS version 26.* ***Results:*** *Serum CTLA-4 level positively correlated with HBsAg-negative, HBc IgG-positive, and HBs-positive patient antibodies ($p = 0.000$), serum HBs Ab positivity ($P = 0.000$), and total HBc Ab positivity ($P<0.001$), all linked to the amount of CTLA-4. Serum HBe Ab negativity was not linked to CTLA-4 ($p = 0.181$).*

Conclusions: *Elevated serum CTLA-4 level in patients with HBs Ag-negative, HBc IgG-positive, and HBs-positive Ab.*

Keywords: Anti-HBs, Anti-HBc IgG, CTLA-4, ELISA, Hepatitis B virus.

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INTRUDUCTION

Hepatitis B virus continues to be a persistent global health concern despite the success of immunization strategies and the decline in HBsAg seroprevalence since 2000 due to the ongoing complications associated with chronic infection, which continue to contribute significantly to morbidity and mortality. The annual number of deaths from HBV-induced hepatocellular carcinoma and liver cirrhosis is approximately 820,000 (1). The hepatitis B virus releases numerous antigens, such as hepatitis B core, surface, and envelope antigens. Antigens exhibit the ability to induce an immune response as a result of their immunogenic characteristics (2). Approximately two billion people globally have been exposed to HBV, which is confirmed by the existence of antibodies to the hepatitis B core antigen (3). The markers can be successfully applied to aid in the selection of therapy options, predict illness severity in the

future, and facilitate the clinical care of CHB (4). The non-existence of HBsAg in the blood does not necessarily indicate the absence of HBV infection (5). Numerous mutations occur in the HBV genes due to a lack of a proofreading mechanism throughout replication (6).

The majority of investigations related to HBV reactivation have depended on the absence of detectable HBs Ag in individuals with occult hepatitis B infection and the existence of HBc Ab as a basis to identify occult hepatitis B infection (7). Individuals with seropositive occult hepatitis B infection (OBI) reveal antibodies in their blood circulation that are directed against the core antigen of the hepatitis B virus (anti-HBc) and/or antibodies that attack the surface antigen of the hepatitis B virus (anti-HBs). This particular kind of OBI represents roughly eighty percent of all OBI circumstances (8). The hepatitis B core antigen triggers the production of a significant amount of IgM, followed by IgG anti-HBc, due to its strong immunogenicity. Experts widely regard antiHBc as a highly sensitive and dependable marker for exposure to the hepatitis B virus. The levels of anti-HBc IgM rise fast after acute infection. Therefore, the presence of anti-HBc IgM is included in the initial diagnostic tests for HBsAg-positive patients who have elevated ALT levels, despite the existence or lack of a liver disorder (9). This diminished immune response makes the host's immune system incapable of eradicating HBV, resulting in a persistent infection (10). After a long-term HBV infection, the host's immune system frequently exhibits deficiencies or lacks the reactivity of T-cells specific to the virus. T-cell depletion is a state in which T cells exhibit impaired immune responses, like reduced production of cytokines, a decline in the ability to eliminate cells, and elevated levels of suppressive molecules like cytotoxic lymphocyte-associated antigen-4 (CTLA-4), programmed cell death-1 (PD-1), cytotoxic T, and lymphocyte activation gene-3 (11).

A co-inhibitory molecule that may be expressed on T cells is CTLA-4 (10). CTLA-4 (CD152) is an additional receptor that suppresses T cell function during prolonged viral infections (12). The CTLA-4 gene belongs to the immunoglobulin supergene family and resides on chromosome 2q33 (2). The upregulated inhibitory receptors on CD8 T lymphocytes in chronic hepatitis B individuals limit their immune defense process, leading to an exhausted phenotype (13). Elevated levels of CTLA-4 hinder the body's ability to resist certain kinds of infections. This is especially true for people who have HIV, where the virus temporarily weakens the immune system and makes the disease worse (14).

Immune checkpoint molecules have a significant impact on the progression of HBV infection. They help to minimize liver damage during acute infection and may contribute to the establishment of persistent infection in chronic HBV cases (15). There is a lack of studies that demonstrate an association between serum CTLA-4 levels and HBV infection in patients with HBs Ag (-)/ HBc IgG (+)/Hbs Ab (+). As far as our knowledge goes, this study is the first to evaluate the CTLA-4 immune checkpoint levels in relation to clinical parameters of the hepatitis B virus (HBV) infection in patients with HBs Ag (-)/HBc IgG (+)/Hbs Ab (+). The study aims to assess serum CTLA-4 levels in patients with HBsAg-negative, HBc IgG-positive, and HBs-positive Ab and investigate the correlation between these values and the presence and progression of HBV infection in the Najaf Government.

PATIENTS AND METHODS

The current study is a cross-sectional research project performed in Najaf- Iraq from July to October 2023. The study comprised a total of two hundred subjects and included exclusively Iraqi individuals who were recruited as patients from As-Sader Teaching Hospital in Najaf city. Among the overall sample, there

were 64 male and 136 female participants who varied from 18 to 80 years old. Every individual underwent screening for serum levels of CTLA-4, HBs Ag, HBs Ab, anti-HBc IgG, HBeAg, HBeAb, and HbcAb. The serum samples that confirmed negative for HBsAg but positive for HBc IgG and HBs Ab were chosen for inclusion whereas those that showed positive for HBsAg had not been included. The individual's data were acquired by giving a questionnaire and drawing a blood sample. Prior to being investigated, all participants were provided with an extensive description of the study's nature and aims and subsequently a permission was obtained for their participation.

The investigation comprised serum samples that screened negative for HBsAg, as well as individuals varying in age from 18 to 80 years old. Exclusion Criteria individuals who have the following were excluded:

1. a medical record of autoimmune illnesses, including systemic lupus erythematosus (SLE) and chronic inflammatory arthritis, and others.
2. a hepatic failure, persistent inflammatory disorders, or autoimmune illnesses.
3. a diagnosis of the hepatitis C virus (HCV), COVID-19, and CMV.
4. an HIV-related immunodeficiency and cancer.
5. a recent adoption of the HBV vaccine for immunization.

Five milliliters of venous blood were collected from every patient and transferred into a gel tube. After allowing the blood to clot, centrifugation separated the serum sample. Finally, the serum sample was placed in a plain tube to complete the necessary assays.

Presently, the techniques employed for clinical laboratory detection of HBV serological Hepatitis C virus rapid chromatographic immunoassay qualitative test, Human immunodeficiency virus rapid markers primarily consist of enzyme-linked immunosorbent assays (ELISA) and immunochromatographic assays. A fraction of the serum was used for a human hepatitis B virus screen test (immunochromaticographic assay): HBc Ab, HBs Ag, HBs Ab, HBe Ag, HBe Ab (Eugene Biotech/China), Human immunochromatographic test (Wondfo/China). The remaining portion was then divided into 1.5 ml Eppendorf tubes and stored in a refrigerator at -80°C for immunological analysis. After that, an ELISA method (Sun Long Biotech, China) was used to find a cytotoxic T lymphocyte antigen (CTLA)-4 and a qualitative HBc IgG. The procedures for all tests were conducted according to the instructions outlined in the kit's manual.

STATISTICAL ANALYSIS

The statistical analysis in this study was conducted with Version 26 of the Statistical Package for the Social Sciences (SPSS). The relationship between category and numerical data was illustrated by using the Mann-Whitney test. The findings are displayed in tables and figures, accompanied by a descriptive narrative, utilizing MS Word and Excel 2016.

The Ethics Council of the University of Kufa/Faculty of Medicine gave its approval before starting this research project. All participants gave their consent, and the As-Sader Teaching Hospital in Najaf also gave its approval.

RESULTS

The present study included a sample of 200 patients, who had undergone testing for HBV infection between July and October 2023, and were screened to detect the presence of HBc Ab, HBsAg, HBsAb, HBeAg, HBeAb, Hbc IgG, and CTLA-4. Fig 1 presents an overview of the general scheme followed in this investigation. Patients who were tested positive

for HBsAg, patients with hepatitis C infection patients with HIV, and patients with inadequate clinical data were excluded. This study has found that 10.5% of patients who tested negative for HBsAg, but positive for HBc IgG were also positive for HBs Ab. The study has also revealed that 75.5% of patients had HBc IgG, 77% had HBc Ab, 10.5% had HBs Ab, and 16.5% had Hbe Ab.

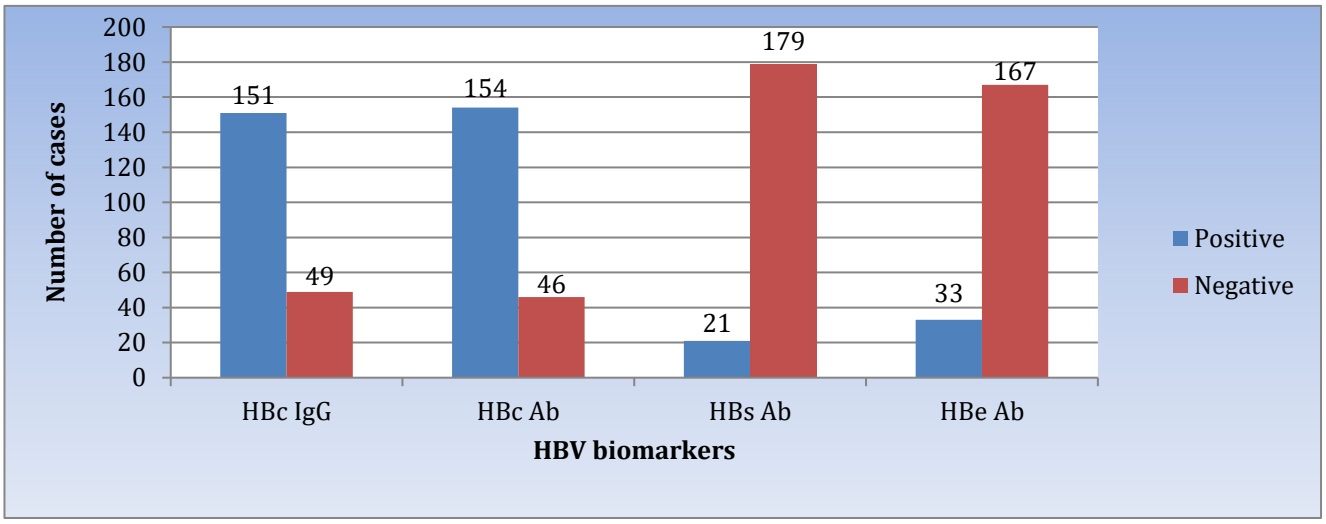

Figure (1): Count of HBV indicators in the study group.

A statistically significant association was observed between CTLA-4 levels and HBc IgG antibodies (p value<0.05, Z = -8.770). Specifically, individuals with positive HBc IgG (N = 151, M = 14.8131pg/ml) were more likely to have higher CTLA-4 levels than those without HBc IgG antibodies (N = 49, M = 4.5637pg/ml). As seen in Table 1., There was actually a statistically significant link between being positive for HBc Ab and CTLA-4. The p-value was less than 0.05, and Z was -8.292. Specifically, people who were tested positive for HBc Ab (N = 154, M = 14.5960 pg/ml) had a higher likelihood of producing higher levels of CTLA-4 than those who were tested negative for HBc Ab (N = 46, M = 4.6222 pg/ml). As seen in Table 1., A statistically significant association was found between CTLA-4 and the presence of HBs Ab (p value<0.05, Z = -5.076). Patients who were tested positive for HBs Ab (N = 21, M = 39.1348 pg/ml) had a higher likelihood of producing higher levels of CTLA-4 compared to patients who were tested negative for HBs Ab (N = 179, M = 9.1540 pg/ml). As illustrated in Table 1. However, there was no statistically significant link observed between the presence of HBe Ab and the production of CTLA-4 (p > 0.05). A strong association was found between individuals who have been evaluated for a positive result for HBsAg (-)/HBc IgG(+)/HBs Ab (+) and the production of CTLA-4 (p value<0.05, Z=-5.076-). Specifically, individuals with positive results (N = 21, M = 39.1348 pg/ml) had a significantly higher likelihood of producing higher levels of CTLA-4 compared to individuals with negative results (N = 179, M = 9.1540 pg/ml). As indicated in table 2.

Table (1): The relation between CTLA-4 and viral markers by Mann-Whitney test.

Test Result type	HBc IgG (+)	HBc Ab (+)	HBs Ab (+)	Hbe Ab (+)
Mann-Whitney U	612.500	686.000	606.000	2349.000
Z	-8.770-	-8.292-	-5.076-	-1.338-
P value	0.000	0.000	0.000	0.181
p value=probability value (level of significance at <0.05)				

Table (2): The relation between CTLA-4 level and HBsAg (-)/HBc IgG/HBs Ab (+) group by Mann-Whitney test

Test Result type	HBs Ab (+)/HBc IgG/HBsAg (-)
Mann-Whitney U	606.000
Z	-5.076-
P value	0.000
p value= probability value (level of significance at <0.05)	

DISCUSSION

The current study has demonstrated that anti-HBc (+) had the highest detection rate at 77% (154/200), followed by anti-HBc IgG (+) at 75%, and anti-HBs (+)/anti-HBc (+) at 10.5% (21/200). These results were in line with the findings of a study conducted by Cai *et al*. (2022), which found that the pattern of HBV serologic markers was found in anti-HBc(+) (3.73%, 10/268) and anti-HBs(+)/anti-HBc(+) (1.87%, 13/695) (16). The generation of anti-HBc is intimately associated with the synthesis of cccDNA and HBcAg (17). The variations in antibody frequencies can originate from variations in various geographical regions with various sample sizes, and the serologic window in the incubation period after infection, can be responsible for the differences in HBV infection rates worldwide.

Regarding the HBsAg-negative/anti-HBc-positive state as an OBI stage in the HBV infection's natural course. Over 90% of people who are anti-HBc positive could have OBI. The "alternative" antiHBc test is considered the most acceptable and practicable marker for occult hepatitis B infection diagnosis (18). The overall frequency of occult hepatitis B infection (OBI) was 6.2% among blood donors who tested negative for HBsAg but positive for anti-Hbc (19). They found that the HBsAg-negative/anti-HBc-positive state is a stage of occult hepatitis B virus (HBV) infection that happens naturally as the disease gets worse (20). HBsAg clearance often indicates a previous HBV infection solely through the presence of anti-HBc. People who do not have HBsAg during the last stage of either resolved or occult HBV infections, on the other hand, usually have 1000 times lower anti-HBc levels than people who do have HBsAg (21).

Furthermore, in the present study, 16.5% of HbeAb seroprevalence is less than the 33.4% HbeAb-positive Chinese population (22). Ndako *et al*. (2021) found a 45.8% positive rate among participants with HbeAb (23), differing

from the results of the current study. HBV infection naturally triggers HBeAg seroconversion, a crucial event in the disease progression. This is when you lose HbeAg and make anti-HBeAg antibodies. This usually happens years after the replicative phase and marks a shift to a low/non-replicative phase where the liver's necro-inflammation can decrease and the infection may be resolved (24). However, the correlation between the proteins of HBV and the production of CTLA-4 after CHB infection is still unclear (22). The current investigation revealed a notable association between increased levels of CTLA-4 and markers of HBV in patients with HBs Ag (-)/HBc IgG (+)/Hbs Ab (+). Dharma (2023) found a strong association between the amount of CTLA-4 in the blood serum and the progression rate of chronic hepatitis B ($P<0.001$), which aligns with the current study's findings. Patients with chronic hepatitis B, hepatocellular cancer, and liver cirrhosis exhibited elevated serum levels of CTLA-4. The researchers found a positive correlation between the serum level of CTLA-4 and the progression of chronic hepatitis B (25).

The present study discovered a significant association between increased levels of CTLA-4 and positive markers of HBV. Tang *et al.* (2016) discovered that CD4+ T cells from people with chronic hepatitis B had more CD28 family receptors, like PD-1 and CTLA-4, than CD4+ T cells from healthy people (26). These results are very similar to what this study has found. A new study by Peng *et al.* (2011) showed that looking at the presence of CTLA-4 on CD8+ T cells in people who had a CHB infection gave different outcomes. However, the overall trend suggests an increase in CTLA-4 levels on CD8+ T cells. People who have HBeAg-positive chronic hepatitis B experience this effect, and having HBeAg around makes CD8+ T cells make more CTLA-4. While these discoveries hold the possibility of major progress, the underlying mechanism of these events remains unknown (27). Although Wang *et al.* (2018) found something similar, the new results do not support earlier research that said HBV infection lowers the expression of CTLA-4 on T cells (28). This reduction may affect their capacity to regulate immunological responses.

The importance of CTLA-4 levels in patients with HBV has significant clinical consequences. High levels of CTLA-4 can serve as an indicator of more severe liver inflammation and worse outcomes. Additionally, using immunotherapy to target CTLA-4 appears to be a possible way to manage HBV infection. One study found that blocking CTLA-4 increased T cells that are specific to HBV and decreased inflammation in the livers of people who had HBV (29).

CONCLUSIONS

1. Patients who tested negative for HBs Ag, positive for HBc IgG, and positive for Hbs Ab exhibited elevated levels of CTLA-4 in the serum. CTLA-4 levels may serve as indications of the immune response to HBV infection or the severity of a disease.
2. The study found a strong correlation between serum CTLA-4 levels and virus-specific proteins such as HBs Ab, HBc Ab, and HBc IgG, but did not find a significant correlation between anti-HBe and CTLA-4 levels.
3. Researchers need to conduct additional multicenter investigations to determine the role of CTLA-4 in the intensity and progression of HBV infection.
4. Elevated levels of CTLA-4 in individuals exposed to HBV infection may provide a chance to explore novel therapeutic approaches. These strategies could involve targeting the regulation of CTLA-4 levels to minimize the adverse development of HBV and enhance patient response to therapy.

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Meningioma as a Rare Presentation

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ABSTRACT

Background: *The most common extra axial primary central nervous system (CNS) tumor is meningioma, accounting for 36% of all CNS tumors. On neuroradiologic and gross assessment, the typical meningioma is lobulated. Cystic variants, although uncommon, are well recognized, and possibly be confused with metastatic or glial tumors.* ***Case presentation:*** *A 40-year-old female patient complaining of headache, Magnetic resonance imaging (MRI) revealed frontoparietal brain cystic lesion with intracystic nodule, given the differential diagnosis of low-grade gliomas, hemangioblastoma, pleomorphic xanthoastrocytoma, pilocytic astrocytoma and rarely meningioma. The histopathological slides showed the diagnosis of meningothelial meningioma (WHO/Mayo Clinic_ Grade I). Immunohistochemistry (IHC) was performed for epithelial membrane antigen (EMA) showing positive membranous stain but IHC staining for glial fibrillary acetic protein (GFAP) was negative for the cytoplasm.*

Conclusions: *Patient with cystic meningioma was reported. There is no absolute test for preoperatively distinguishing it from the most prevalent other gliomas. So avoiding any extra cost and time loss, angiographic assessment and cooperation with a histopathologist are of clinical importance for the identification of these possibly curable neoplasms.*

Keywords: Diabetes Mellitus, Pure Tone Audiometer, Sensorineural Hearing Loss.

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INTRUDUCTION

Meningioma is the most prevalent extra axial primary central nervous system tumor; it accounts for 36% of all CNS tumors. It arises from arachnoid cells associated with dura mater or choroid plexus, grows along external surface of brain, spinal cord or occasionally, within the ventricular system [(1)].Three grades were exist based on WHO criteria [(2)], female affected more than male [(3)], hormone replacement therapy or oral contraceptives may be a risk factor [(4)]. On neuroradiologic and gross assessment, the typical meningioma is lobulated, solid, or globular mass that is anchored broadly to the dura mater. Cystic variants, although uncommon, are well identified, meningiomas with intramural cysts, may easily be mistaken with other glial or metastatic tumors [(5)].

CASE PRESENTATION

A 40-year-old female patient complaining of headache, MRI revealed frontoparietal extra-axial brain cystic lesion with mural nodule (Figure 1 A, B), given the differential diagnosis

of glioma, pleomorphic xanthoastrocytoma, pilocytic astrocytoma and rarely meningioma. In April 2023, about 10 ml of yellow colored fluid aspirated during surgical removal of the brain cystic lesion, and the histopathological slides showed the diagnosis of meningothelial meningioma (WHO/Mayo Clinic_ Grade I) (Figure 2 A, a). IHC was performed for EMA showing positive membranous stain (Figure 2B, b), and IHC staining for GFAP was negative for the cytoplasm to exclude the other diagnoses (Figure 2 C,

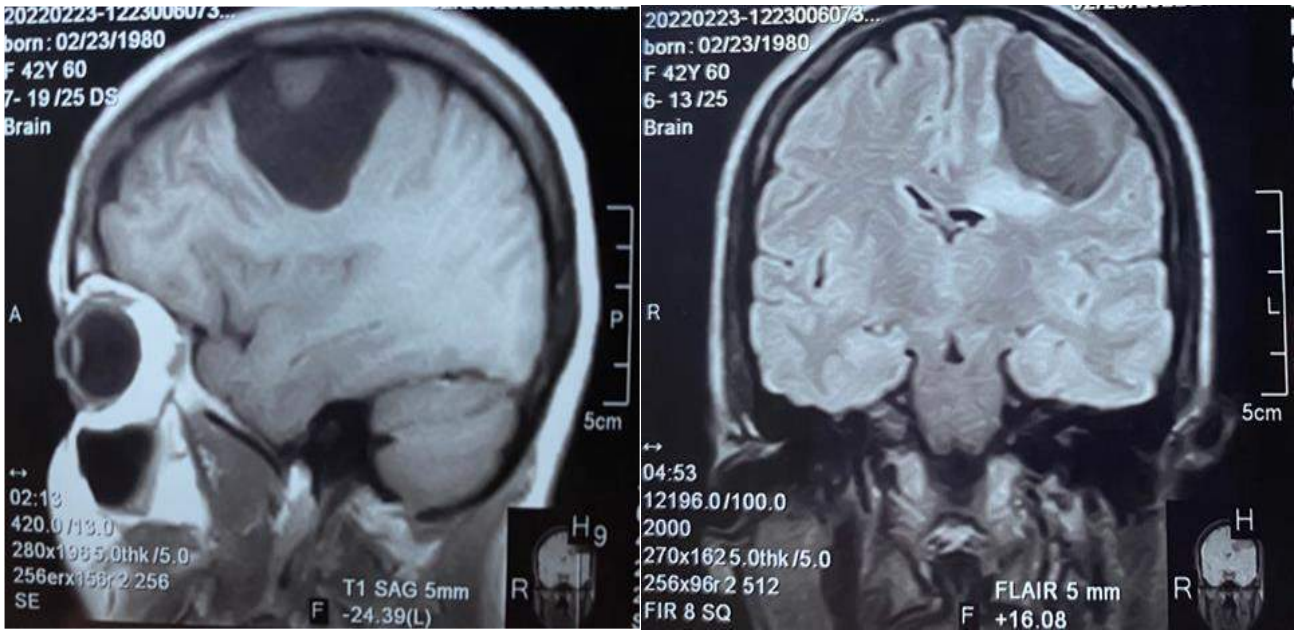

Figure (1): MRI showing left parasagittal frontoparietal cystic brain lesion, hypointense on T1 with enhanced mural nodule, mild edema and compression of left lateral ventricle. (A; sagittal view) (B; coronal view).

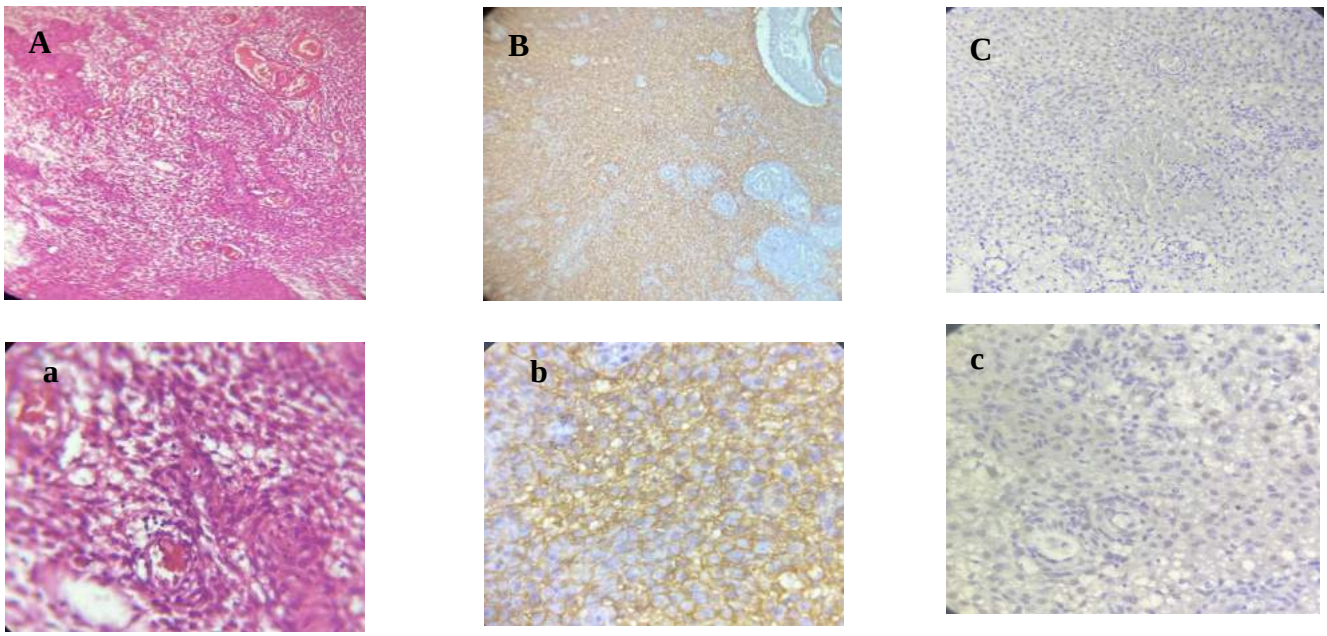

Figure (2): Morphological structures and IHC findings of the specimen slides. Hematoxylin and eosin staining (HE) showed the lobular microarchitecture with many meningothelial whorls (A, 10x; a, 40x). IHC showed that the neoplastic cells were positive for EMA, (B, 10x; b, 40x) and negative for GFAP (C, 10x; c, 40x).

DISCUSSION

Cyst with a mural nodule tumor (CMNT) is a pattern of the radiological findings for central nervous system (CNS) lesions seen in fluid-secreting neoplasms, including; commonly, hemangioblastoma[6], pilocytic astrocytoma[7] ,ganglioglioma[8], and pleomorphic xanthoastrocytoma[9].

Meningioma rarely manifested as cystic lesions with mural nodule and may be confused as one of the intraparenchymal cystic brain tumors. We here present a 40-year-old woman with a frontoparietal brain lobe cystic tumor, demonstrating MRI enhanced mural nodule. At first, differential diagnoses made including low-grade gliomas, hemangioblastoma, and cystic meningioma. Total removal of the lesion was done by surgical approach. Intraoperative evaluation showed that the tumor was extra axial, the cyst lined by a thin membrane and the content was yellow color fluid. The final diagnosis after the histopathological assessment was meningothelial meningioma, WHO grade I. Although hemangioblastoma, ganglioglioma, pilocytic astrocytoma, and pleomorphic xanthoastrocytoma, commonly exhibit such MRI finding, meningioma had to be added to such differential diagnosis.

CONCLUSIONS

Patient with cystic meningioma was reported. The computed tomographic scans or magnetic resonance images of the tumor similar to those of glial or metastatic neoplasms with necrotic or cystic changes. Meningioma should be added to differential diagnosis of CMNT. Preoperatively, there is no absolute test for distinguishing cystic meningiomas from the most common other gliomas. So avoiding any extra cost and time loss by other investigations, angiographic assessment and contacting the neuropathological results are of importance for the identification of those possibly treatable neoplasms.

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Terms Used to Describe Abnormalities of Joint Kinematics: An Overview

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ABSTRACT

Background: *In the joints, the movements, active or passive, occur normally in certain physiological planes: axes or directions, and within certain limits. In certain pathologies, the joint kinematics may be affected in one of the following ways: limitation of the normal movements, movement beyond the normal limits, or movement in the non-physiological planes. Different terms were used to describe the abnormalities of the joint kinematics. Nowadays, terms like joint hypermobility, joint laxity, joint instability, and ligamentous laxity are, synonymously or not, still used in some literature in their correct place. The current article aimed to review the abnormalities of joint kinematics and the new concepts of optimum application of the terms used to describe them. It has reached to some conclusions like: limitation of joint movement is the term used to describe any incomplete joint movement within a physiological plane and standard limits; Joint hypermobility is the term used to describe the ability to do a joint movement in a physiological plane but beyond the usual limits. Joint instability is the term used to describe the liability of a joint to move in non-physiological planes.*

Keywords: Abnormalities, Joint hypermobility, Joint instability Kinematics, Joint movement, Stiffness.

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INTRODUCTION

A joint is a site where the bones are connected together [(1)]. Synovial joints usually have a range of movement (ROM) more than that of cartilaginous ones. Kinematics is the branch of mechanics concerned with the motion of objects without reference to the forces that cause the motion [(2)]; in the human being, it deals with the possible motions of a whole body or its parts [(3)]. In the joints, the movements, whether active or passive, normally occur in certain physiological planes: axes, or directions, and within certain limits. Every joint has its specific movement(s) that occur(s) within a specific plane(s) and range(s). The ROM of each movement has its limits usually determined by certain anatomical structures or otherwise by the tightness of the restraining ligaments. Age, gender, and ethnic differences tend to make different capabilities of joint mobility [(4,5)].

In certain pathologies, the joint kinematics may be affected in one of the following ways: limitation of the normal movements, movement beyond the normal limits, or movement in the non-physiological planes. The abnormalities of joint kinematics had been described in the literature separately. Different terms are used to describe abnormalities of movements. Previously these terms had been used with some ambiguity. Recently, each term gained its special meaning with a specific definition for the medical field. Nowadays, terms like joint

hypermobility, joint laxity, joint instability, and ligamentous laxity still are used in some literature as synonymous or not in their correct place. Thus, the current article aimed to review the abnormalities of joint kinematics, the terms used to describe them, and the current concepts of optimum use of the terms used to describe the different types of joint kinematic abnormalities.

METHODS

The study design and registration protocol

The study was done according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses extension for Scoping Reviews (PRISMA-ScR) checklist [6,7]. A review protocol was prepared and registered with the Open Science Framework website on December 18. 2023. Available from: https://osf.io/28rey

Information sources

Electronic databases such as PubMed, Europe PMC, and Google Scholar were used to search for relevant articles. The keywords or phrases in the search process were: joint kinematics, limitation of movement, joint stiffness, joint hypermobility, and joint instability. Specific regions were excluded from the search process; In addition to the electronic database search, six books were also involved in the search for the relevant data.

Eligibility criteria

The inclusion criteria here were journal articles discussing joint movement abnormalities and those related to the human species. The search process was further restricted to those articles published in English only. The exclusion criteria, however, were literature discussing non-musculoskeletal, locomotor, joint abnormalities, and those concerning the treatment of joint movement abnormalities.

Search period and strategy

The search process was done to find the relevant articles during the period December 18, 2023, till December 22, 2023. It was done by the author and the peer review of the electronic search strategies (PRESS); a checklist was taken into consideration during the search process [8].

Data extraction

The extracted data from the articles were collected and then sorted by using a Microsoft Office Word software sheet (Microsoft Corporation, Microsoft Office Word, 2019. Redmond, Washington, USA). According to the scoping review methodology, an assessment of the quality, risk of bias, of the included studies was not performed [6,7].

RESULTS

A total of 560 articles were found at the onset of the search process but finally, only 34 were included in this review. The detail of the search process was summarized in the figure below. The included articles were 30 from journals and four books. Twenty-eight of the journal articles were original and two were systematic reviews. Three books specialized in orthopedic disorders and the fourth was on the physiology.

The retrieved data were divided into three parts: limitation of the normal movements, movement beyond the normal limits, and movement in the non-physiological planes:

Limitation of the normal movements

Limitation of joint movement is a general term used to describe any incomplete joint movement within a physiological plane and standard limits for that specific joint. It is the inability to complete the usual movement within the usual plane and the usual limits. It may be

due to pain from any cause of inflammation, trauma, infection, …, or loss of the ability to actively do a movement by any of the neuro-muscular-tendinous disorders. There are other terms used to describe the limitation of joint movement but in specific conditions. Paralysis is the term used to describe the inability to perform the movement(actively) from a neurological cause [9]. Stiffness is the term used to describe the inability to complete a particle joint movement both actively and passively. It may be due to congenital problems such as arthrogryposis and synostosis, or acquired from a post-traumatic and post-operative sequel, chronic inflammatory disorders, degenerative disorders, or tumors. Locking is the term applied to the sudden inability to complete a particular movement from a mechanical block [9].

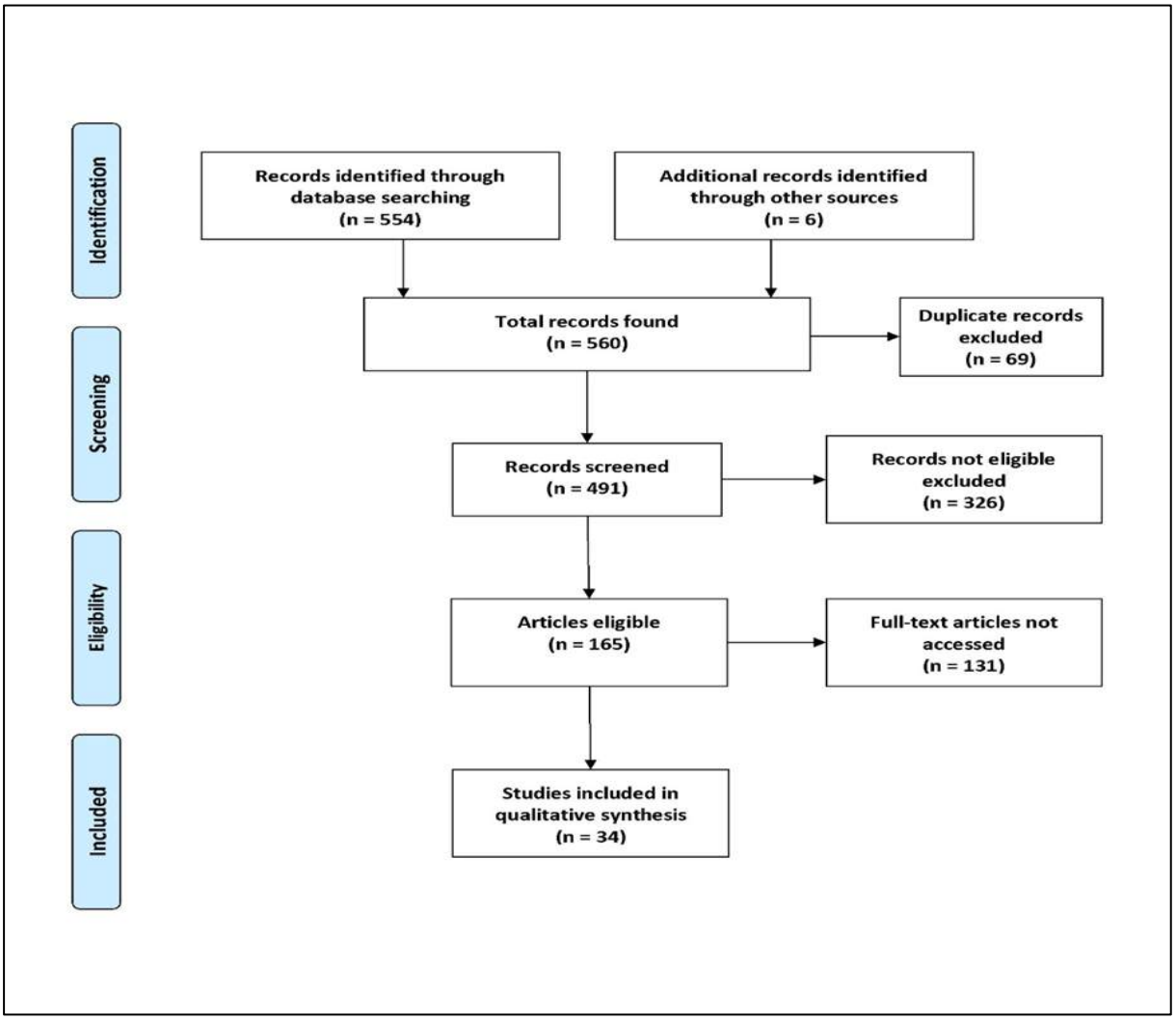

Figure (1): The flow diagram for the search process.

The ROM is recorded in degrees of a circle, with the joint as its center. It starts from the neutral or the extended anatomical position of the joint, a starting position, which is designated to be the zero degree, rather than the 180 degrees. Then the degrees are added in the direction in which the joint moves and finishes where movement stops due to anatomical limitation [10]. For accuracy, it is ideal to measure the ROM objectively with a goniometer. For example, 'knee flexion 0–140 degrees mean that the range of flexion is from zero, the straight knee, through an arc of 140 degrees, the leg making an acute angle with the thigh [10].

Movements beyond the normal limits

Joint hypermobility (JH) is a term commonly used to describe the ability to move a joint, passively and/or actively, in a physiological plane but beyond the usual limits of the age, gender, and race. Hence, it is a descriptive term for the increased ROM rather than a diagnostic term for a disease or a syndrome. It may exist as a separate diagnostic result but often a feature of a larger syndromic diagnosis [11,12]. Synonyms of JH include joint hyperlaxity, joint hyperextensibility, loose joints, and double-jointedness [11]. It occurs due to excessive laxity of the constraining soft tissues especially the capsule-ligamental structures [5,13,14]. This excessive laxity occurs due to either a congenital disorder, or genetic defect affecting connective tissue matrix proteins which may occur as an isolated defect or part of an inherited disorder like Ehler-Danlos' syndrome, Marfan's syndrome, Down's syndrome, osteogenesis imperfecta, skeletal dysplasia as achondroplasia, … [11]. It, however, could occur as an acquired one from training with stretching physical exercises [5,15], widespread inflammatory or degenerative diseases of the joints [11,16], neuromuscular disorders as Ullrich congenital muscular dystrophy, congenital muscular dystrophy with joint hyperlaxity [17,18], or endocrinal as hypothyroidism [11].

Joint hypermobility may be localized, observed in less than five joints [11], generalized, in five or more joints, usually symmetrical in four limbs and axial skeleton, peripheral, observed in hands and/or feet of infants and children, or historical in adults who lost their JH [19,20].

Movement in the non-physiological planes

Joint instability is a term used to describe the liability of a joint to move in an unusual plane, subluxation or dislocation, during usual life activities. It is mainly used as a patient's complaint or symptom when feeling the joint to be insecure, such as the give way of the knee joint. The term joint laxity is used during the clinical examination (sign) when a joint is found to be liable for a movement passively in an unusual plane by performing special tests [11]. It occurs due to a deficiency in one of the supporting structures of the joint, like bones, ligaments, and muscles. It may result from congenital, developmental, and hereditary disorders (a primary bone defect like glenoid dysplasia in shoulder instability, trochlear dysplasia in patellofemoral instability, and generalized JH from excessive ligamentous laxity) [21-24], or acquired sequel of trauma (limb bone malalignment, torn ligament, tendon insufficiency, …), repeated movement or overuse over time, and sequel of neuromuscular disorders [25,26]. It is localized in most of the cases but may be generalized (only in generalized JH cases).

DISCUSSION

Unawareness of the different abnormalities that occur in joint kinematics and the terms used to describe them may lead to misunderstanding among the medical personnel. The current study aims to increase awareness about these terms, which are used ambiguously nowadays, and the current agreement on their applications.

Limitation of joint movements

Patients often have difficulty in distinguishing the type and cause of limitation of movement. They may use the term stiffness for all kinds of limitations of joint movements. Stiffness should never be assumed until verified by examination. Using the goniometer is essential for the ideal estimate of the ROM angle of a joint. However, with practice, it may be possible to estimate the angles, to an acceptable extent, by eyeballing [9]. Furthermore, the goniometer may not be available nearby all the time. Describing the range of movement accurately may be difficult. Although it is ideal to cite or span the ROM, since the beginning till

the end, in degrees [9] , it may be difficult to interpret the degree of limitation by degrees for all persons. For example, if there was a limitation of elbow flexion and the ROM was 115 degrees, it may be difficult to imagine the magnitude of the present limitation by everyone, even if it was supplemented by the normal range reference. Terms such as 'full', 'good', 'limited', and 'poor' may be misleading. Hence, there is a need for a grading system for the limitation of joint movement. The author of the present study suggests a subjective grading based on objective measurements depending on how much the normal ROM is deficient: mild (less than 25%), moderate (25-75%), severe (more than 75%) permitting for little movement, and complete (100%), no movement occurs.

Since most of the human body joints are working together in a synchronized manner, the functional sequel of a single joint stiffness may be buffered by the others. Therefore, a single joint stiffness, even if it is severe or ankylosed or fused, might not have a serious impact on daily life activities provided the nearby surrounding joints are normal or have a considerable ROM. If multiple joints are affected, the additive effect, even of a mild degree, subsequently may cause a significant impact on the function of an extremity or a body region.

Joint hypermobility

The abnormality of joint kinematics can be diagnosed when the obtained ROM is compared with the normal parameters for that joint, sex, age, and race. In general, the joints have higher angular values in females than males, young especially children, and adolescents more than in old adults [27]. Negros and Asian backgrounds are generally more hypermobile than Caucasians [12,28]. Some synonymous terms were used for JH like joint hyperlaxity and joint hyperextensibility. The first one cannot be used for pure clinical description because it gives a clue about the nature of pathology which is the excessive ligamentous laxity, and the second one gives a clinical description for one direct of JH which is hyperextension which may not be true for all the joints. The terms like loose joints and double jointedness seem to be non-academic.

Joint hypermobility and joint hypermobility syndrome

The term joint hypermobility syndrome (JHS) is used to describe connective tissue disorder involving mainly the joint capsule-ligamental structure and characterized by chronic musculoskeletal clinical features, symptoms and signs, due to JH [29]. It may be in a localized, generalized, peripheral, or historical form. It is sometimes referred to by different other terms like hypermobility spectrum disorder (HSD), benign joint hypermobility syndrome (BJHS), and benign hypermobility syndrome (BHS) [5,13,19,20,28,30]. The Beighton Scoring System measures joint hypermobility on a 9-point scale, while the Brighton diagnostic criteria, major and minor, is used to diagnose JH syndrome [31-35]. The term syndromic JH is used when at least a second tissue/structure other than the joint capsule-ligamental structure is involved like connective tissue disorders, such as Ehlers-Danlos syndrome, Marfan's Syndrome, osteogenesis imperfecta, skeletal dysplasia, such as Larsen syndrome, spondyloepimetaphyseal dysplasia, hereditary myopathies, such as Bethlem myopathy, chromosomal and genomic disorders, (such as Down's syndrome [5,11,35].

Joint instability, joint hypermobility, and joint laxity

Joint instability and JH have been used in the past as synonyms. However, by inference, they prelude to different forms of joint kinematics abnormalities. Hypermobile joints may also be unstable and lax, but not all unstable/lax joints are hypermobile [11,12]. Clinical symptoms and signs are the first clues to differentiate between

them. Joint instability usually presents with certain characteristic symptoms like recurrent attacks of a particular joint dislocation, such as shoulder and patella-femoral joint, recurrent attacks of pain around the affected joint with certain activities [35], abnormal sounds as clunks or crepitations may be heard or felt [25,35,37], and decrease joint, and limb, function also known as activity-limiting symptoms as in shoulder , dead arm syndrome [35,38]. Abnormal signs may be found during the clinical examination of a joint by performing drawer or stress tests, such as of elbow and knee. The term joint laxity is used to describe these abnormal clinical findings [11]. Investigations like imaging by doing radiological stress views and MRI are usually valuable and diagnostic. Arthroscopy may also be valuable in some cases of joint instability [39,40]. Joint instability usually disturbs the usual life and ends with early degenerative changes and osteoarthrosis due to biomechanical disturbance in the joint [11,41-43].

CONCLUSION

Limitation of joint movement is the term used to describe any incomplete joint movement within a physiological plane and standard limits. Joint hypermobility is the term used to describe the ability to do a movement joint in a physiological plane but beyond the usual limits. Joint instability is the term used to describe the liability of a joint to move in non-physiological planes.

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Usefulness of Phase Sensitive Inversion Recovery MRI Sequence in the Detection of Cortical Lesions in Multiple Sclerosis

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Abstract

Background: The presence of cerebral cortical lesions in multiple sclerosis has an important clinical impact on the prognosis of the disease and associated disability. However, the accurate detection of cortical lesions using conventional magnetic resonance imaging sequences remains challenging. The study aims to assess the value of phase-sensitive inversion recovery sequence in the detection of cortical lesions in multiple sclerosis patients and to evaluate their relation with clinical subtypes, duration, and clinical disability of the disease. **Patients and Methods:** Seventy cases, 51 females and 19 males, of multiple sclerosis, confirmed by McDonald criteria, were enrolled in this cross-sectional study and phase-sensitive inversion recovery images, axial and coronal sections, were obtained for each patient in every MRI session. Cortical lesions were subclassified into intracortical, leukocortical, and juxtacortical. Clinical disability was assessed using the extended disability status scale. The number of detected cortical lesions on phase-sensitive inversion recovery images was calculated and compared with that detected on conventional T2-weighted and fluid-attenuated inversion recovery images. **Results:** The number of cortical lesions detected on phase-sensitive inversion recovery was lesser compared to the T2-weighted sequence, a total of 1151 versus 1258 lesions respectively. The T2-weighted sequence was significantly better in the detection of leukocortical and juxtacortical. On the other hand, phase-sensitive inversion recovery was better than fluid-attenuated inversion recovery in detecting intracortical, while fluid-attenuated inversion recovery was better in detecting juxtacortical, and both sequences detected the same number of leukocortical lesions. The overall number of detected cortical lesions showed a statistically significant correlation only with the extended disability status scale and not with the clinical subtype or duration of multiple sclerosis.

Conclusion: phase-sensitive inversion recovery detected more intracortical lesions and fewer juxtacortical lesions than fluid-attenuated inversion recovery, cortical lesions were significantly correlated with the degree of clinical disability of multiple sclerosis.

Keywords: Brain, Cortical, Inversion Recovery, Magnetic Resonance Imaging, Multiple Sclerosis.

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INTRUDUCTION

Multiple sclerosis (MS) is a global problem, with rising incidence both worldwide (1) as well as in Iraq (2). The symptoms of MS are tremendously diverse and depend on the severity and location of lesions within the CNS (3,4). MS is a clinical diagnosis but the McDonald criteria incorporating magnetic resonance imaging (MRI) have been established (5) while diagnostic criteria combining clinical, imaging, and laboratory evidence have evolved (6). MRI is of utmost importance in the

diagnosis of MS and is particularly helpful in excluding other pathologies or showing features suggesting alternative diagnoses (7,8). The T2-weighted (T2W) and fluid-attenuated inversion recovery (FLAIR) as well as contrast-enhanced T1-weighted (T1W) are most commonly and routinely utilized sequences in the imaging evaluation of MS (9, 10). The white matter (WM) lesions characteristic of MS do not represent the whole pathology of MS (11). There was radiological and pathological evidence of the presence of the so-called cortical lesions (CL) that were found to be closely associated with cognitive impairment independent of WM lesions (12,13). However, unlike WM lesions, not all these CLs can be depicted using conventional MRI sequences (12, 14).

Therefore, there was a need for a feasible imaging technique to detect CLs with reasonable accuracy. A double inversion recovery (DIR) sequence has been utilized for this mission (15) by suppressing both WM and cerebrospinal fluid signals, hence improving the conspicuity of demyelinating plaques (16, 17). However, the DIR sequence is generally susceptible to image artifacts and both false-negative and false-positive results with a low signal-to-noise ratio (18). Another T1-W MRI sequence, the phase sensitive inversion recovery (PSIR), has also been used for better detection of CLs (19), utilizing its advantages of the wider range of signal intensity resulting from the combination of both negative and positive longitudinal magnetization, with consequent higher contrast resolution with lesser acquisition time (1:30 minutes for whole brain) than DIR, FLAIR and routine T1-W FSE sequences (19, 20, 21). However, most of the conducted studies utilized high-strength MRI machines (3-7 Tesla), which are generally scarce in our region and not feasible like the conventional 1.5 Tesla devices.

Therefore, the current study was conducted to evaluate the accuracy of the PSIR sequence in detecting cerebral CLs in MS patients using a 1.5 T machine commonly utilized and readily available MRI machine for daily clinical practice in our neuroscience center and regional hospitals. The study also aimed to assess the relation of CLs with the subtypes, duration, and clinical disability of the disease.

PATIENTS AND METHODS

This cross-sectional diagnostic study was conducted on 70 MS patients at Middle Euphrates Neuroscience Center, AL-Sadir Medical City in Najaf Province – Iraq, during a period of one year from January 2021 to January 2022 .According to the inclusion and exclusion criteria, patients with a confirmed diagnosis of MS using McDonald criteria (2017) (6) of both genders and their age above 10 years were included. However, patients with the presence of any of the following were excluded: MRI contraindications, past medical history of neurological, major medical or neuropsychiatric illnesses; failure to complete the MRI exam, or

missing of one or several sequences; significant artifact and refusal to participate in the study.

The study was approved by the Institutional Review Board and informed oral agreement was taken from all patients for acceptance to participate in the study.

Clinical data including age, gender, medical history, type and duration of MS, drugs used by the patients, and expanded disability status scale (EDSS) were obtained from the registry and history.

Due to the imaging protocol and analysis, all examinations were conducted using a 1.5 T MRI machine (Achieve Philips, Netherlands, 2011). The PSIR sequence was performed for each patient as part of routine MRI sequences. The routine examination also included conventional T1-W, T2W-TSE, and FLAIR. A board-certified specialist radiologist, with more than 13 years of experience in neuroradiology, interpreted the T2-W-TSE, FLAIR, and PSIR images. CLs were subclassified into three locations: intracortical (IC), when they are confined to the GM; leukocortical (LC), when lesions involve both grey and white matters of the cortex; and juxtacortical (JC) when lesions involve the subcortical U-fibers (figure 1) (21). The total number of CLs in each cerebral region was assessed separately on the T2-W-TSE, FLAIR, and PSIR sequences respectively.

STATISTICAL ANALYSIS

The statistical analysis of the data was carried out by using SPSS-27 (IBM Inc., Chicago, IL, USA for Windows ®); the data were presented in frequency, percentage, mean, and standard deviation. The significance of difference was tested by using the students-t-test for two independent means, Paired-t-test for paired observations, two dependent means, or ANOVA test for more than two independent means. P-values less than (0.05) were considered statistically significant.

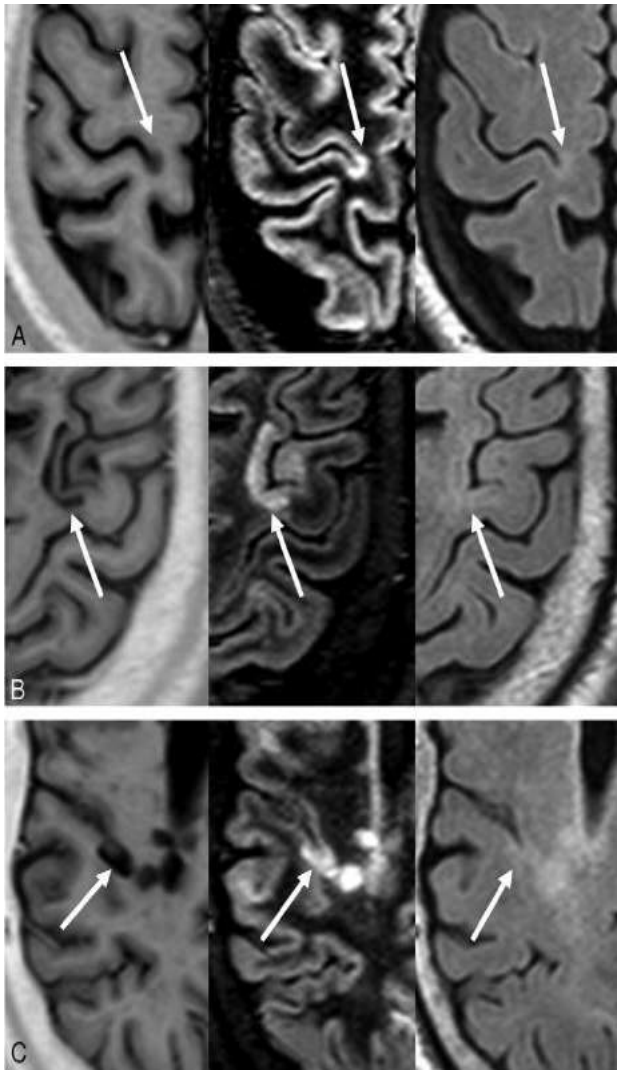

Figure 1. Classification of cortical lesions according to the location into intracortical (A), leukocortical (mixed) (B), and juxtacortical (C) (Adapted from Nelson F et al (19) with permission from American Society of Neuroradiology) .

RESULTS

This study included a total of 70 MS patients, with a mean age was (31.1± 10.1) years, females (72.9%) more than males (27.1%), with a mean duration of MS of (4.1 ± 4) years. The most common treatment used was Betaferon, (29 41.4 %). Remitting relapsing (RR) type was the most common type of MS (71.4 %), followed by clinically isolated syndrome (CIS) (13 patients, 18.6 %), and the least was primary relapsing (PR), only one patient, 1.4%. Most of the patients (57, 81.5%) had a mild disease with EDSS of less than three (Table 1).

Table 1. General characteristics and MS variables of the study sample.

Variable		Number	%
Gender	Male	19	27.1
	Female	51	72.9
Age (years)	≤ 20	12	17.1
	21-30	24	34.3
	31-40	19	27.2
	> 40	15	21.4
Treatment	Betaferon	29	41.4
	Tysabri	17	24.3
	Rebif	14	20
	Gelenia	7	10
	Avonex	2	2.9
	Rituximab	1	1.4
Type of multiple sclerosis	Remitting relapsing	50	71.4
	clinically isolated syndrome	13	18.6
	Primary progressive	4	5.7
	Secondary progressive	2	2.9
	primary relapsing	1	1.4
Duration (years)	1_5	54	77.1
	6_10	10	14.3
	> 10	6	8.6
EDSS	< 3	57	81.5
	≥ 3	13	18.5

EDSS-Expanded disability status scale.

The total number of IC lesions, detected on T2-W sequence was more than on PSIR sequence, 123 versus 119 respectively, but without statistical difference (P=0.49). Regarding LC lesions, a total number of plaques were detected more on T2-W than PSIR sequences (292 versus 228 respectively) with statistically significant difference (p value 0.0001). Regarding JC lesions, more lesions were detected using the T2-W sequence than PSIR, 870 versus 804 respectively, with statistically significant difference, p value of 0.004. (Table 2).

Compared to FLAIR, a higher number of IC plaques were detected by using PSIR images, 50 versus 20 respectively, with a statistically significant difference (p-value 0.001), more JC lesions were seen on FLAIR than PSIR, 624 versus 580 respectively, with a statistically significant difference (p-value o0.003) while both FLAIR and PSIR sequences found a similar number of LC lesions, 147 for each. (Table 3).

Table 2: Number of cortical lesions detected on T2W and PSIR sequences.

Location	IC		LC		JC	
	T2-W	PSIR	T2-W	PSIR	T2-W	PSIR
Frontal lobe	63	55	119	95	399	378
Temporal lobe	6	5	37	27	58	38
Parietal lobe	49	53	93	78	248	245
Occipital lobe	5	6	43	28	165	143
Mean	1.76	1.70	4.17	3.26	12.43	11.49
Std. Deviation	3.241	2.634	3.538	3.855	8.050	9.002
Total	123	119	292	228	870	804
P Value	0.49		0.0001		0.004	

IC=intracortical; LC=leukocortical; JC=juxtacortical; T2-W=T2-weighted; PSIR=phase sensitive inversion recovery.

Table 3: Number of plaques detected on FLAIR and PSIR sequences.

Location	IC		LC		JC	
	FLAIR	PSIR	FLAIR	PSIR	FLAIR	PSIR
Frontal lobe	9	26	61	58	269	246
Temporal lobe	0	5	32	28	55	51
Parietal lobe	9	16	36	48	181	170
Occipital lobe	2	3	18	13	119	113
Mean	0.10	0.25	0.74	0.74	3.12	2.90
Std. Deviation	0.481	0.671	1.044	1.354	2.806	2.855
Total	20	50	147	147	624	580
P Value	0.001		0.277		0.003	

IC=intracortical; LC=leukocortical; JC=juxtacortical; FLAIR=fluid attenuated inversion recovery; PSIR=phase sensitive inversion recovery.

The total number of the detected CLs was statistically correlated with EDDS for all locations: IC, LC, and the JC lesions. However, it was most significant for IC lesions (p values of <0.001, 0.004 and 0.02 respectively) while no statistically significant correlation (p values > 0.05) was found with age, gender, duration of the disease, type of the treatment and type of MS (Table 3).

Table 3: Correlation between cortical lesions and study variables.

	Intracortical lesions	Leukocortical lesions	Juxtacortical lesions
	P value	P value	P value
Age	0.515	0.974	0.473
Gender	0.683	0.671	0.927
Duration	0.887	0.694	0.673
Treatment	0.171	0.269	0.141
Type of multiple sclerosis	0.634	0.633	0.810
*EDSS	<0.001	0.004	0.020

EDSS-Expanded disability status scale.

DISCUSSION

Cortical lesions in MS have an impact on neurological and cognitive functions; their diagnosis plays an important role in predicting the disease prognosis (22). However, CLs are difficult to visualize by conventional MRI sequences (23) and clinical implications and value for follow-up is not yet established (24, 25). PSIR provides a better grey matter/white matter contrast and differentiation than the T1-W sequence (18, 19, 22) and was applied in the evaluation of neoplastic space-occupying lesions whether extra-axial or intra-axial and in the detection of what is called "black hole" lesions (26, 27). The present study was conducted to compare the accuracy of PSIR sequence with conventional MRI sequences (T2-W and FLAIR) in the detection of CLs in MS patients using a 1.5 Tesla MRI scanner. PSIR images were more capable in the detection of IC lesions compared to the FLAIR images, which is consistent Hashemi et al's study which found a significantly higher number of IC and LC plaques by using the PSIR sequence compared to FLAIR sequence(18) Using 3 Tesla scanner MRI, studies have confirmed the same results with a higher number of CLs detected by PSIR sequence compared to FLAIR (16, 19, 20), with the advantage of improved classification of CLs subtypes(21)

The current study has shown no significant difference between PSIR sequence and T2-W in detecting IC lesions. However, other types of lesions like LC and JC were detected more on FLAIR and T-W images than PSIR sequences. This discrepancy between this study's results and other studies can be attributed to several factors: firstly; many lesions in the JC region are WM lesions rather than genuine cortical lesions which are easier to detect on FLAIR and T2W-TSE sequences similar to what was found by Wattjes et al study (28). Secondly, this discrepancy is related to the efficiency of MRI device used as significantly

higher numbers of CLs were detected using 3T MRI devices (29) and except for Hashemi et al (18), all previous studies were done by using the higher strength (3-7 Tesla) MRI devices. Despite the higher detectability of the CLs in T2-W sequence, this could be attributed to the fact that lesions detected on PSIR would be more reflective of the actual clinical situation because the hypointensity on T1-W images is thought to be more important than the hyperintensity seen on T2-W images during the follow-up of MS disease progression. Those T1-W hypointense lesions are potentially independent parameter of MS pathological process **(Error! Reference source not found.)** and correlated with the degree of pathological severity [30]. Moreover, the hyperintense lesions detected on T2-W images in MS may be overestimated due to the associated Wallerian degeneration [31, 32] in addition to the non-significant high T2-W signals incidentally found in healthy subjects [33] and various migraine and vascular headaches [34].

Studies have found an association between the presence of CLs and clinical disability [26, 35]. In the current study, the overall number of plaques seen was statistically correlated to the severity of the disease reflected by EDSS for all locations: IC, LC, and JC lesions, with the most significant correlation being with IC lesions. This supports the finding that IC plaques usually occur in the advanced stages of MS disease and their presence reflects the extent of clinical disability [36]. Furthermore, Rovaris et al found that the cognitive function of MS patients, reflected by EDSS is markedly influenced by the severity and extent of cortical and subcortical MS lesions. [37]

This study has some limitations. Most of the included MS patients in this study were of RRMS type, in which the probability of CL is thought to be lower compared to PPMS and SPMS [38]. The true maturity of JC lesions that were better detected on T2-W and FLAIR could be related to the WM and genuine cortical lesions. All images were evaluated by a single radiologist, hence the interobserver variation could not be assessed. The long-term relationship between the clinical course of the disease and the CL burden could not be evaluated.

CONCLUSIONS

The study has concluded that LC and JC lesions were detected more on T2-W than PSIR. There was no difference between T2-W and PSIR in the detecting IC lesions. LC was detected more on FLAIR than PSIR. There was no difference between FLAR and PSIR in the detection of LC lesions. IC lesions were detected more on PSIR than FLAIR. All subtypes of CL were statistically correlated with EDDS, most significantly for IC subtype. PSIR is as an adjuvant with T2-W and FLAIR sequences when a specific evaluation of IC location is required in regions where only the 1.5 Tesla strength MRI device is available. Future studies to investigate the value of PSIR in the progression of disease as well as in monitoring clinical response were recommended.

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Investigation the Influence of Stress on Salivary Features, Oral Hygiene and Gingival Health Condition among a Group of Adolescents Male Students

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Abstract

Background: *Stress is a condition that causes pressure and is caused by several factors in teens, such as unplanned events.* ***Subjects and Method:*** *This observational comparative study included 260 male students divided into three groups: low stress, moderate stress, and high stress who were provided according to the Adolescent Stress Questionnaire Scale (ASQ-S). Oral hygiene examination, including plaque index, was done according to Silness and Loe,1964 index, and calculus examination was done according to Ramfjord,1959 index, while gingival health examination was performed according to Loe and Silness,1967 index. Salivary pH, flow rate, salivary Vitamin B_{12}, and Folic acid were measured in unstimulated saliva samples from the low and high-stress groups.* ***Results:*** *It has been shown that the higher means of plaque and gingival indices among high-stress level students was statistically non-significant ($p>0.05$). The high-stress group has a reduced salivary flow rate, with significant differences ($P < 0.05$). Vitamin B_{12} and Folic acid levels were more critical in the high-stress group ($P > 0.05$). Among both low and high-stress groups, there was a negative, non-significant correlation between pH and flow rate with plaque and gingival indices. In high-stress students, the correlation of Vitamin B_{12} and Folic acid with plaque and gingival index was damaging.* ***Conclusion:*** *Augmentation of stress is linked with disturbance of psychological well-being, which tends to influence oral hygiene and gingival health by lowering the rate of saliva flow and impacting the levels of Vitamin B_{12} and Folic acid in saliva.*

Keywords: Folic acid, Gingival health, psychological well-being, Stress, Vitamin B_{12}.

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INTRUDUCTION

Stress can refer to a physical, mental, or emotional issue that generates tension in the body or mind. Adolescence is a crucial time of life because of the rapid physical, social, cognitive, and emotional development that occurs then (1). These changes substantially affect one's health and well-being in later years (2). Stress leads to an increase in glucocorticoids and catecholamines, as well as an alteration in the immune response, which in turn influences health-related behaviors. It may lead to infections and periodontal tissue loss. The activation of these hormones decreases the activity of natural killer cells and the number of lymphocytes, among other immunological processes.(3)

Saliva is an ideal and non-intrusive biological specimen for the quantitative and qualitative evaluation of chemical and physiological

mediators linked to many conditions, including stress-related disorders[4]. The sympathetic and parasympathetic nervous systems control the volume and composition of saliva during stress, which can reduce the rate at which saliva is secreted from the salivary glands, a condition frequently accompanied by dry mouth [5]. A nutritional deficiency might result from a lack of vitamin intake, which substantially impacts dental and general health. Vitamins have a considerable impact on oral health[6]. Micronutrients such as vitamins C, B9, and E, calcium, zinc, copper, iron, and anti-inflammatory and antioxidant properties play a role in development. A lack of certain micronutrients contributes significantly to developing periodontitis and caries [7]. Vitamin B12 (cobalamin) and B9 (folic acid) are B complex vitamins required for cell metabolism [8]. Vitamin B_{12} is a vital micronutrient for brain development and function. It is essential for a child's early development because it affects memory, reasoning, attention, metabolism, the formation of red blood cells, and oral health [9,10].

This study was conducted because the Iraqi school system currently lacks a mental health philosophy and the Iraqi environment is undergoing several stressful life events. To avoid parental control, the presence of complete permanent dentition was ensured to minimize the effect of gender variation. Therefore, this study aims to determine the impact of stress on salivary variables, oral hygiene, and gingival health in a sample of 15-year-old male students. The null hypothesis proposes no correlation between salivary state and oral hygiene and gingival health about stress.

PATIENTS AND METHODS

Study Design

This descriptive cross-sectional study was done in Al Najaf City Center/Iraq, from 16th February 2023 until the end of May 2023. The 15-year male students were randomly selected from a third-grade intermediate of ten Iraqi schools. The researcher initially explained the purpose of the study to the students. A total of 260 students accepted to participate and those who fulfilled the study's conditions and agreed to participate were asked to sign a consent form. Any student with systemic disease that might interfere with oral health conditions like diabetes or disorders of salivary glands, a student on vitamin B12 and folic acid supplements therapy, taking medications, on orthodontic treatment, a smoker, and a student who refuses to participate in the research were excluded.

The adolescents' stress level was estimated using the Adolescent Stress Questionnaire Scale (ASQ-S) [11]. An oral examination included measuring the plaque index according to Silness and Loe,1964 index criteria [12] as shown in (Fig 1,2,3) calculus index according to Ramfjord 1959[13] for oral hygiene assessment as shown in (Fig 4), and gingival index according to Loe and Silness,1967 index criteria [14] for gingival health assessment, the level of gingival inflammation shows in (Fig.4,2).

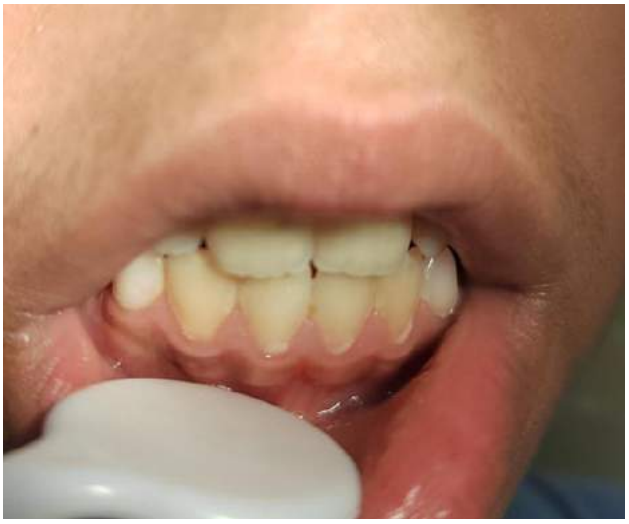

Figure 1: Moderate accumulation of plaque within the gingival pocket, or the tooth and gingival margin which can be seen with the naked eye.

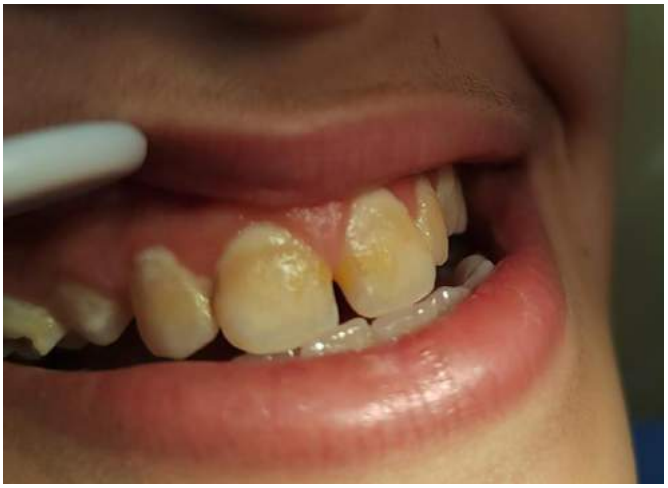

Figure 2: Abundance of soft matter within the gingival pocket and/or on the tooth and gingival margin, with mild inflammation, slight redness, edema, no bleeding on probing.

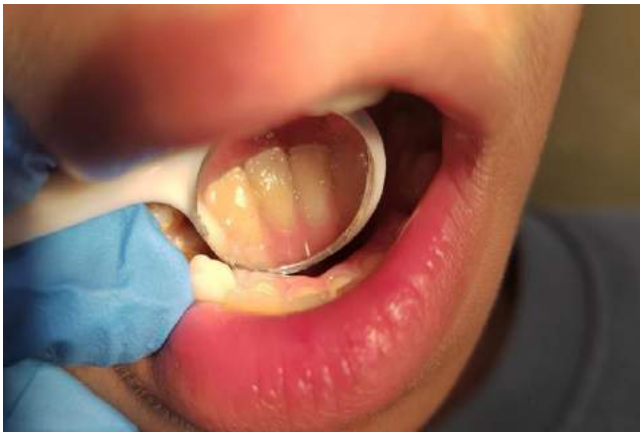

Figure 3: A film of plaque adhering to the free gingival margin and adjacent area of the tooth. The plaque may be seen only after the application of the disclosing solution or by using the probe on the tooth surface. No calculus presented. No inflammation of gingiva

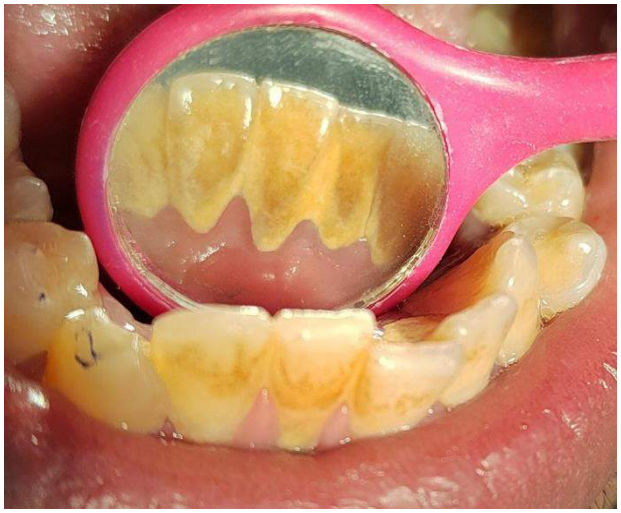

Figure 4: Moderate lingual supra gingival calculus with gingivitis

Data Collection Method

The students' stress levels were measured by using the ASQ-S, a self-recording questionnaire taken from a previous study [11]. There were fifty-four items in the initial version of the questionnaire; after introducing the scale to experts, eighteen items had been dropped out; the adjusted questionnaire consisted of 36 items related to stress resulting from daily lives; adjustments were made considering the Iraqi culture. The stress of becoming an adult, dealing with peer pressure, home life, taking on adult responsibilities, attending school regularly, balancing work and play, and performing well in school, and the stress of financial pressure are among the 36 self-rated items included in the ASQ-S. The answer for each item was scored as: Not stressful to me= 1, moderately stressful to me=2, severely stressful to me=3, very severely stressful to me=4; and I had not passed=0 .

The validity of the ASQ-S was checked to establish the correlation between each item's score and the scale's total scores (Table 1).

Pearson correlation was employed to determine the correlation between each item's score and the sample's total score, which consisted of 260 questionnaires; the correlation coefficient ranged from (0.85-0.36), and results demonstrated that each relationship was statistically significant when compared to the essential Pearson correlation value (0.12) at the P-value (0.05). The Cronbach α method was employed to assess a scale's reliability using this approach, giving the results of the reliability coefficient, which was 0.90.

Table 1. Items validity of ASQ scale using the method of correlation of item score and total score of the scale

No	Pearson correlation	No	Pearson correlation	No	Pearson correlation
1	0.40	13	0.53	25	0.48
2	0.40	14	0.42	26	0.43
3	0.51	15	0.42	27	0.42
4	0.54	16	0.54	28	0.43
5	0.39	17	0.47	29	0.53
6	0.44	18	0.46	30	0.36
7	0.40	19	0.57	31	0.40
8	0.36	20	0.41	32	0.58
9	0.37	21	0.53	33	0.49
10	0.39	22	0.47	34	0.49
11	0.47	23	0.43	35	0.51
12	0.45	24	0.55	36	0.57

Saliva Collection Method

The collection of unstimulated salivary samples was conducted within a specially designated area at the school during the morning hours from 9 to 11 AM. The samples were obtained by the saliva collection procedures established by the University of Southern California School of Dentistry [15]. The students were instructed to take their seats and rinse their mouths with distilled water, allowing themselves to rest for a minimum of five minutes. They were requested to minimize their physical activity and to position their forehead above and the test cup below. Subsequently, they were directed to keep their lips open to facilitate the collection of saliva into the cup for five minutes. Upon the conclusion of the designated collecting period, the students were instructed to expel any residual saliva from their oral cavity expeditiously by expectorating it into the provided cup. Every student's cup was assigned a distinct code, denoted by a serial number on their case sheet. The measurement of salivary flow rate was conducted using the equation SFR = volume ÷ time (ml/min) [16]. The pH measurement was conducted by using a pH meter manufactured by Hanna Instruments, a company based in the United States. The saliva samples were centrifugated at 3000 rpm for 15 minutes in a researcher's laboratory at the Medical College, University of Kufa. Following centrifugation, the resulting clear supernatant was carefully separated using a micropipette. The supernatant was then divided into two sections, each placed in separate Eppendorf tubes, for subsequent analysis of Vitamin B_{12} and Folic acid.

Immunological Assay and Statistical Analysis

Both main B_{12} and Folic acid were analyzed by kit using enzyme-linked immune sorbent assay (ELISA) based on the Biotin double antibody sandwich technology to assay the Human Vitamin B_{12} and Folic acid (YLbiont, Human). The data description, analysis, and presentation were performed by using Statistical Package for

Social Science (SPSS version -22, Chicago, Illinois, USA), for frequency and percentage for qualitative variables and mean and standard error (SE) for the quantitative variables. Pearson correlation is a parametric test for the linear correlation between two quantitative variables while Analysis of Variance (ANOVA) is used for testing the difference between independent groups using a Games-Howell post hoc test. The level of significance is P≤0.05.

RESULTS

The total number of students is classified into three groups with different stress levels. The sample distribution was done according to the stress level scale, as shown in Table 2. About two-thirds (65.38%) of students had moderate stress levels, whereas 16.92% and 17.69% showed low and high stress levels respectively. The mean values of plaque, calculus, and gingival indices (1.590, 0.100,1.306), respectively, were higher in the high-stress level group with a statistically non-significant difference (P>0.05) (Table 3)

Table 2. The distribution of the sample according to stress level.

Stress levels	N	%
Low"<=40"	44	16.92
Mod."40-60"	170	65.38
High ">=60"	46	17.69

Table 3. Plaque and gingival index according to stress levels.

Vars.	Stress levels								Total	
	Low"<=40"		Mod."40-60"		High ">=60"					
	Mean	±SE	Mean	±SE	Mean	±SE	F	P value	Mean	±SE
PLI	1.449	0.081	1.440	0.038	1.590	0.075	1.620	0.200	1.468	0.032
CalI	0.071	0.022	0.083	0.016	0.100	0.067	0.140	0.869	0.084	0.016
GI	1.047	0.138	1.000	0.060	1.306	0.100	2.712	0.068	1.062	0.050

In Table 4, the mean pH value was lower in the group experiencing low-stress levels. However, the observed difference did not reach a statistical significance (P>0.05). In contrast, the group subjected to high levels of stress had a lower mean flow rate value, and this difference was shown to be statistically significant (P<0.05); the means of both vitamin B_{12} and Folic acid were high in the high-stress level student group with a statistically non-significant difference (P>0.05)

Table 4. Salivary physical and chemical constituents among students with low and high stress levels with statistical difference

Vars.	Stress				Statistical tests	
	Low"<=40"		High ">=60"			
	Mean	±SE	Mean	±SE	T	P value
PH	6.923	0.162	7.113	0.036	1.169	0.246
SFR	0.980	0.043	0.735	0.032	4.595	**<0.001**
B12	117.656	1.381	118.111	1.576	0.216	0.829
Folic acid	1.705	0.052	1.759	0.089	0.511	0.610

The pH and flow rate of the low and high-stress groups had negative relationships with plaque, calculus, and gingival indices among the low and high-stress students, except the calculus index, had a positive connection (P>0.05) for both low and high-stress levels. This is in Table 5. Table 6 shows that vitamin B12 correlated positively with plaque index and negatively with calculus and gingival indices in low-stress groups. Folic acid negatively correlated with plaque, calculus, and gingival index. B12 and Folic acid correlated negatively with plaque and gingival indices and positively with calculus index in high-stress individuals. None of these associations were significant.

Table5. Correlations of salivary physical and chemical constituents with oral hygiene and gingival health condition according to stress level.

Stress level		pH		SFR	
		r	p	r	P
Low"<=40"	PLI	-0.077	0.620	-0.115	0.456
	CalI	-0.006	0.969	0.067	0.666
	GI	-0.043	0.780	-0.046	0.766
High ">=60"	PLI	-0.100	0.507	-0.238	0.111
	CalI	0.150	0.319	0.165	0.272
	GI	-0.049	0.745	-0.010	0.948

Table 6. Correlations of salivary chemical constituents with oral cleanliness and gingival health condition according to stress level.

Stress level		B12		Folic acid	
		r	P	r	P
Low"<=40"	PLI	0.105	0.499	-0.166	0.280
	CalI	-0.189	0.218	-0.242	0.114
	GI	-0.187	0.223	-0.153	0.321
High ">=60"	PLI	-0.055	0.715	-0.261	0.080
	CalI	0.057	0.708	0.059	0.697
	GI	-0.048	0.750	-0.262	0.079

DISCUSSION

Psychology-related factors may influence the levels of plaque and the state of the gingiva in adolescent students. The current study has shown that the percentage of students with moderate and high-stress categories was high; this agreed with studies[17‘18‘19‘20‘29]. The results of the study showed that means of plaque, calculus, and gingival indices were higher in high-stress levels with statistically no significance; this coincided with [19‘20‘22] and may be connected to the fact that stressful conditions activate the neuroendocrine system, which in turn causes the continuous release of pro-inflammatory mediators and an alteration in the normal microbiota, which ultimately leads to periodontal inflammatory disorders [22]. Another study revealed a significant stress-related gingival index mean difference; results may vary due to age range, periodontal variable studied, study design, and stress measures employed[18]. According to this study's results, saliva's flow rate was significantly ($P<0.05$) lower in the high-stress group, as seen in previous studies[20‘22]; this phenomenon can be explained by the fact that the autonomic nervous system mainly controls the secretion of saliva. Therefore, when individuals are exposed to stressful conditions, they cause a gradual accumulation of stress on various systems involved in maintaining stability in the body, such as the central nervous system, the autonomic nervous system (ANS), and the hypothalamus-pituitary-adrenal axis (HPA). Consequently, the ANS and HPA axes become dysregulated over an extended period .[23]

Salivary pH was not significantly lower in the low-stress group compared to the high-stress group; this finding agrees with [25] but contrasts the results of [20], which indicated that both flow rate and pH decreased as stress increased. Although the sympathetic and parasympathetic nervous systems influence salivary pH regulation, the current study does not demonstrate a significant decrease in salivary pH levels under high stress. This discrepancy may be attributed to variations in saliva sampling methods, the study population, and the parameters utilized[33]. By [18‘20‘26], salivary flow rate and pH negatively correlated with gingival and plaque indices in both high and low stress levels. This finding opposes the conclusion drawn by [19]; the plaque accumulation in the high-stress group of this study may have been caused by a reduction in flow rate. Furthermore, low pH conditions promoted the proliferation of various pathogenic bacteria responsible for the induction and advancement of periodontal disease.[18]

The results of the current study show a positive correlation of pH and SFR with calculus formation, which agrees with[34]. The results of this research revealed that the values of B_{12} and Folic acid were statistically non-significantly higher in high-stress students, although there was disagreement with [28].

However, a cross-sectional study by [29] found no connection between psychiatric symptoms and vitamin B_{12} and Folic acid. It is important to note that the subjects' poor intake of these nutrients, as well as variations in eating patterns, lifestyle, nutritional evaluation techniques, classifications of psychiatric symptoms, and other confounding variables, may have influenced the findings. The correlation between vitamin B_{12} and Folic acid was negative and non-significant, with plaque and gingival indices among the high-stress level group. This agrees with [30‘31‘32]. This may be related to the existence of high levels of vitamins, which are crucial for the maturation process of the oral mucosal epithelium and the enhancement of hematopoietic factors that contribute to the prevention and treatment of infection in oral mucosa.[32]

The limitation of the study was that some students were shy, which led to difficulty in oral examination and salivary samples. The researcher had to take samples from students in the intermediated third grade early in the

morning, which conflicted with the school and teachers' classes.

CONCLUSIONS

Depending on the findings, an increased stress condition has considerably reduced salivary flow, which negatively influences oral hygiene and gingival health. Vitamins B_{12} and Folic acid, two members of the B complex vitamins, have been linked to better periodontal health.

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The Correlation between Serum Levels of Progranulin and Inflammatory Markers in Patients with Chronic Obstructive Pulmonary Disease

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Abstract

Back ground: *Chronic obstructive pulmonary disease, or COPD, is an inflammatory chronic illness of the parenchyma and/or airways that is typically accompanied by increasing, irreversible dyspnea, coughing, sputum production, and/or exacerbations of respiratory symptoms. The pathological process of COPD includes bronchitis and/or emphysema; inflammation in the lungs has systemic effects as well. The most frequent causes of COPD are indoor and occupational pollution. Progranulin, or PGRN, is a precursor of pleiotropic glycosylated protein that plays a major role in wound healing, angiogenesis, neoplasia, cell development, the cell cycle, inflammation, and the modification of the autoimmune process. It is highly prevalent in a variety of cell types, including respiratory epithelial cells.* ***Objective:*** *The aim is to study relationship between serum level of PGRN and inflammatory markers in COPD patients.* ***Material and method:*** *A case control study composed of 40 patients with COPD and 45 controls, demographic characteristic, blood investigation include CRP-titer, WBC and plasma PGRN were measured and made comparison of data in cases with controls, data collected were statically analysis by SPSS .*

Result: *The study showed that, there was no significant correlation between serum progranulin level, WBC and C-Reactive Protein titer in COPD patients (P value >0.05).* ***Conclusions:*** *It was concluded that PGRN level in COPD patients don't correlate with CRP titer level or with WBC.*

Keywords: Chronic Obstructive Pulmonary Disease, Progranulin, Spirometry, C-Reactive Protein.

Article Information

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INTRUDUCTION

In 2019, chronic obstructive pulmonary disease (COPD) was third globally in terms of causes of mortality, with 3.23 million of those fatalities taking place in middle- and low-income nations. Males, individuals over 40, smokers, and former smokers had higher rates of COPD prevalence. According to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) criteria, the prevalence of COPD worldwide in 2019 was 10.3%.(1,5,18,24) .

Multiple types of inflammatory cells and mediators are involved in the progression of COPD, which is a complex sickness that affects the lungs and remains an inflammatory disease. Over an individual's lifetime, complex, dynamic, and cumulative interactions between genes and environment can alter normal lung development pathways and cause harm. This is the cause of it(3,8) .

Tobacco Smoking considered the most common cause of COPD (10), even though smoking is thought to be the main cause, epidemiological research revealed that 20% to 40% of COPD patients had never smoked (6), also cannabis, pipe, water pipe, cigar all consider as risk factors for COPD.(12) Genetic reasons include alpha-1 antitrypsin deficiency (AATD) (14) as an example. Pollution resulting from exposure to gas particles in the environment, whether indoors or outdoors; prior infections, such as recurrent respiratory infections primarily in the lower respiratory tract during early childhood (risk increased by two to three times); prior tuberculosis increases the risk of developing COPD (9) and increased susceptibility to pollution and reduced nutrition in low-income environments.(1)

Patients with COPD experience an altered version of their typical inflammatory response to long-term irritants such as smoke ingested from cigarettes and other particles. Although the primary origin of this modified form of inflammation remains unknown, certain research indicates that genetics might be involved (8). In inflammatory disorders including COPD and asthma, PGRN—also referred to as acrogranin or PC cell-derived growth factor (PCDGF)—plays a critical role. Its anti-inflammatory properties stem from its interaction with TNF R1/2 and inhibition of the TNF-α-TNFR1/2 signal, which prevents neutrophil degranulation. Research has indicated that elevated levels of PGRN are present in the blood of patients with COPD, possibly as a result of reduced or inhibited neutrophilic inflammation. The GRN gene encodes PGRN, which is made up of 593 amino acids and has a molecular weight of 68.5 kDa (23). Different proteinases can break down PGRN, and certain substances, including secretory leukocyte protease inhibitor (SLPI), can prevent it from having an anti-inflammatory effect. The pro-inflammatory granulins produced by PGRN degradation can counteract the anti-inflammatory effects of the protein .(21,23)

The inflammatory process, systemic inflammation, smoking status, and ischemic heart disease may all contribute to the elevated CRP levels observed in COPD patients. Elevation of CRP has been associated with myocardial infarction, unstable angina, stroke, and sudden coronary mortality.(20,25,28)

The white blood cell (WBC) is regarded as a key element of immunity. It plays a role in defense against infections and shielding the body from diseases. The five types of WBC are neutrophils, basophils, eosinophils, lymphocytes, and monocytes. Abnormalities in the count of WBC can indicate a number of diseases, including inflammation, allergies, bacterial, fungal, and viral infections, leukemia, and lymphoma.

PATIENTS AND METHODS

40 COPD patients (26 men and 14 women) who met inclusion criteria and were not excluded from exclusion criteria were gathered from Al-Sader Teaching Hospital in Al-Najaf city between January and August of 2023.

Forty-five healthy individuals, sixteen men and twenty-nine women, were included in the study. They were selected at random from the general community, free of COPD, and possessed normal pulmonary function tests as determined by spirometry. After being informed about the purpose of the study, the methods of the research, the spirometry test, and the blood draw, participants gave their verbal agreement. Those with COPD who were over 35, stable at diagnosis (13), and had not previously received treatment with long-acting beta-agonis, anti-inflammatory medications, or oral corticosteroids were included in the study (11,15). Exclusion criteria include renal or hepatic failure, inflammatory bowel disease, connective tissue disorders, cancer within the previous five years, and chronic illnesses that cause systemic inflammation .(17)

Methods

Each participant gave a five-milliliter venous blood sample, which was placed in a gel tube. Serum progranulin was measured using the Human PGRN ELISA Kit (Elabscience/USA), and CRP was measured for each participant using the Human CRP ELISA Kit (Elabscience/USA), all in accordance with the precise manufacturing source's protocol and WBC blood investigation.

STATISTICAL ANALYSIS

The data for the study were analyzed using the Statistical Package for the Social Sciences (SPSS 23.0 for Windows), and the results showed descriptive statistics as mean±SD, frequency, and percentage. For categorical variables, chi-square tests were used to compare groups, and for nonparametric data, the Mann-Whitney U-test was employed. For normally distributed data, Pearson's correlation coefficient was employed, with a significance threshold of $p < 0.05$.

RESULT

The current study included a total of 85 participants (40 COPD patients and 45 as a control). Two thirds of COPD group were male against one third of the control group. The analysis of data showed that there was significant statistical difference in the mean Plasma progranulin levels between the COPD and the control group (table-2 and figure1). The results concluded that there was no significant correlation between the mean progranulin level and the mean levels of the inflammatory markers of the COPD group and also of the control group ($p>0.05$) as shown in table3 below.

Table NO.1: Demographic characteristics of the COPD and control group.

Characteristics		Patients n=40	Control n=45	P value
Age (years)	Below 45	13 (32.5) %	27 (60) %	0.06
	45-54	13 (32.5)	8 (17.7)	
	55-64	7 (17.5)	7 (15.6)	
	65 and above	7 (17.5)	3 (6.7)	
Gender	Male	26 (65)	16 (35.6)	**0.007**
	Female	14 (35)	29 (64.4)	

Table NO.2: Comparison of progranulin level between patient and control.

Variable		mean±SD	P value
Plasma progranulin level (ng/ml)	Patients	230.52±189.01	**0.006**
	Control	114.19±68.41	

Figure NO.1: Plasma progranulin levels in COPD and control group.

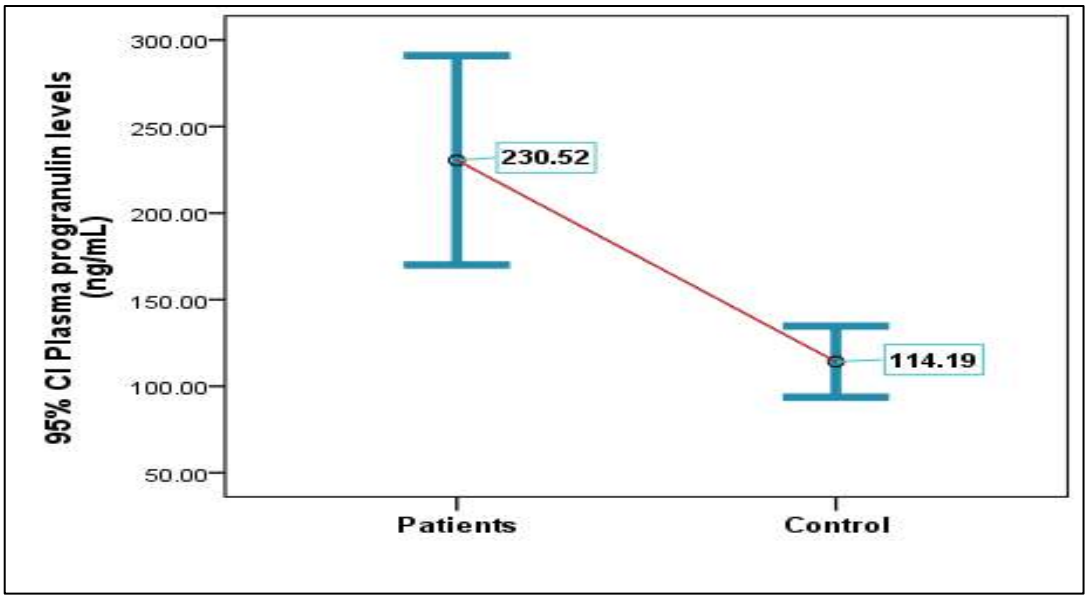

Table NO.3: Correlations between progranulin level and inflammatory markers.

Variables	Patients		Control	
	Pearson Correlation (r)	P value	Pearson Correlation (r)	P value
WBC	0.099	0.542	-0.047	0.758
CRP	0.023	0.889	-0.090	0.557

DISCUSSION

This study involved 40 COPD patient (patient group) and 45 non-COPD subjects. There is no difference in age between patients and controls P value (0.06) ,the age consider as risk factor for developing of COPD due to either the lung function decline with increase age or due to increase time of exposures to the other causes such as tobacco smoking ,air pollution and occupational pollution during the life (1),this consistent with other studies(12) ,65% of the patients in this study were males ,this consistent with study of (18). Systemic review composed of more than 150 previous study ,that found prevalence of COPD was larger in males than females but the difference in prevalence between males and females in developed countries and high income countries was not significant statistically, this appear due to environmental factors and behavioral factors , genetic factors and prevalence of smoking high in men all these causes lead to difference in prevalence of COPD between genders, the diagnosis of COPD in developing countries still higher in males than females, but because of increase in smoking among females and working in males occupations that have risk of develop COPD due to pollution(3),but in other research (4). that found no difference in prevalence of COPD between men and women. (18), explain in high income countries prevalence of COPD higher in females than males, due increase exposure to tobacco smoking and found females have more susceptibility to harmful effect of tobacco smoking due to smaller size of lung in female so become more susceptible to oxidative stress .(22)

This study showed a significant difference in progranulin level between cases of COPD and controls (230.52±189.01 vs 114.19±68.41 , P value 0.006) ,which is similar to finding of other study which found that progranulin level higher in stable COPD than controls(10)..Also shows that there is no significant association between progranulin level and inflammatory markers in patients and controls, which differs from the finding by a study of (10) found that progranulin correlated positively to CRP and ratio of neutrophil/lymphocyte . (26) found that there is no significant association between progranulin level and white blood cells count. This study shows that there is no significant association between progranulin level and inflammatory markers in patients and controls may be due to small sample size and there is similarity between cases and controls in age,smoking habit,past medical history and the cases we take them in stable stage for at least one month prior to examination and they are 99% of them in GOLD 2 stage.

CONCLUSIONS

We concluded for this study that elevated levels of PGRN are linked to COPD and are no significant association with inflammatory markers.

RECOMMENDATION

we recommended further studies with larger sample size for clarify the effect of PGRN on the severity of COPD and inflammatory markers.

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The Effect of Age on Right Ventricular Systolic Function Using Traditional Echocardiographic Measures

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ABSTRACT

Background: *In a wide range of clinical settings, comprehensive evaluation of the right ventricle has become increasingly essential. Measures of right ventricle systolic function, including fractional area change, tissue Doppler, S velocity, tricuspid annular plane systolic excursion, show a significant variation in previous studies and different data known regarding how these measurements change with age.* ***Aim of the study:*** *The aim of this study is to evaluate the effects of age on right ventricle systolic function by using conventional 2D echocardiographic assessment.*

Patients and methods: *Comprehensive transthoracic echocardiography examinations were performed on 103 healthy adult volunteers, mean age 37.1±11.9 years (range: 20-66), to determine age related changes in right ventricle dimensions and function.* ***Results:*** *2DE assessment of right ventricle function demonstrates slightly significant differences in tricuspid annular plane systolic excursion (r= -.197, p = .047), and FAC (r= -.241, p = .015), among age groups, with a non-significant very small negative relationship between age and tissue Dopplers (r= -.0925, p = .355).*

Conclusions: *This study has demonstrated that age-adjusted measures are required for the evaluation of right ventricle function. Further, the conventional techniques may ignore mild or slight changes leading to underestimated assessment which regarded as a fundamental challenge for their use; so, the use of advanced techniques which allow early identification of right ventricle dysfunction are recommended since they can detect subclinical dysfunction before anomalies revealed by traditional echocardiography occur.*

Keywords: Age, Right Ventricle, Systolic Function, Traditional Echocardiography.

Abbreviation: (RV) Right Ventricle, (TAPSE): Tricuspid Annular Plane Systolic Excursion, (Tds): Tissue Doppler Derived Tricuspid Lateral Annular Systolic Velocity, (FAC): Fractional Area Change, (TDI): Tissue Doppler Imaging.

Article Information

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INTRUDUCTION

The right ventricle (RV) has been traditionally considered an irrelevant cardiac chamber, with little attention given to its specific physiologic and pathophysiologic characteristics. However, recent research has highlighted the need for a deeper understanding of RV characteristics. Accurate assessment of RV performance can significantly impact prognosis, especially in patients with right-sided heart failure and congenital heart disorders[1-7].

Echocardiography is the primary method for RV assessment due to its simplicity and diagnostic data. However, challenges like complex cavity, myocardial fiber architecture, and nonconcentric contraction mechanism make RV function assessment one of the most technically challenging tasks in echocardiography[1, 8-12]. The RV has a unique myocardial fiber arrangement, with most fibers oriented longitudinally. This structure explains RV contraction primarily based on longitudinal shortening. To accurately assess

RV systolic function, a multi-parametric method with global and regional measurements are recommended. Global measurements include fractional area change (FAC), while regional measurements focus on specific RV function aspects like tricuspid annular plane systolic excursion (TAPSE) and tricuspid annular longitudinal velocity. Conventional 2DE parameters assess both radial and longitudinal RV contraction elements, but there is a lack of information on how these new modalities differ depending on age and gender [9, 13-15][16-20]. The tricuspid annular peak systolic velocity (S') is a reliable technique for evaluating the systolic function of the RV. It correlates positively with RVEF determined by CMR and has better prognostic value than other parameters like FAC and TAPSE. TAPSE is a reliable index for primary RV function evaluation and monitoring, but has limitations such as being angle-dependent and not fully representative of RV global function. FAC measures the change in RV area from end-diastole to end-systole, but may overlook outflow tract contraction, which is crucial for congenital heart disease cases. Studies show that RV FAC is a significant predictor of cardiovascular and overall mortality [18, 21-23].

The aim of this study is to evaluate the effects of age on RV systolic function by using conventional 2D echocardiographic assessment, which are important for distinguishing between pathological conditions and normal physiological changes. It improves a diagnostic precision by identifying initial RV impairment signs and understanding mechanisms of age-related modifications in the RV.

PATIENTS AND METHODS

This cross-sectional study investigated 102 healthy male participants from November 2022 to October 2023 who were referred to the echocardiography unit at al-Foratul-Ausat Teaching Hospital in Najaf-Iraq. They were screened for cardiovascular disease, including medical history, medication use, risk factors, and lifestyle choices. A physical examination was performed to rule out cardiovascular and metabolic co-morbid disorders. The participants were divided into four groups based on age: Group A (aged 20-29), Group B (aged 30-39), Group C (aged 40-49), and Group D (aged 50-60). The study excluded participants with a history of coronary artery disease, diabetes mellitus, systemic arterial hypertension, smoking, bicuspid aortic valve, congenital heart disease, heart failure, cardiomyopathy, sinus rhythm disturbances, or respiratory disorders, treatments affecting the heart, poor quality echocardiogram images, and athletes.

The study involved a single examiner conducting echocardiography examinations using Vivid E9 equipment. Participants were informed about the study's purpose and given the option to accept or reject participation. Methods included recording height and weight, determining BMI, and computing body surface area (Tab. 1). The study assessed RV function through 2DE echocardiography exam, with a 3.5-MHz phased array transducer, while participants in the left lateral decubitus posture

Table 1: Anthropometric data of the study

Data (M±SD)	Group A (20-29 yrs) No. 33	Group B (30-39 yrs) No. 25	Group C (40-49 yrs) No. 25	Group D (50-60 yrs) No. 20	Total No. 103
Age	24.9±2.8	32.7±2.99	43.7±3.0	55.7±5.4	37.1±11.9
Weight (kg)	73.5±15	78.3±12.0	81.4±11	81.1±14	78±13.6
Height (cm)	171.7±6.5	172.2±4.5	172±4.9	171.7±6.1	171.9±5.5
BMI (kg/m^{2})	24.6±4.4	26.2±3.2	27.3±2.90	27.2±3.9	26.1±3.8
BSA (m2)	1.8±0.1	1.8±1.9	1.8±0.1	1.8±0.1	1.8±0.1

By passing an M-mode cursor through the tricuspid annulus in the typical apical four-chamber window and calculating the difference between the end-diastolic and end-systolic measured longitudinal movement of the annulus, in mm, taken at the onset of an electrocardiogram QRS complex, TAPSE was calculated as an indicator of the RV longitudinal systolic function (24, 25).

RV FAC (%) = 100 × (EDA − ESA)/EDA is the formula employed to calculate fractional area change, after the end-diastolic and end-systolic RV areas derived from planimetry of the endocardial boundary in the apical four-chamber view. It represents the radial and longitudinal elements of RV contraction (18).

By pulsed-wave DTI, peak systolic tricuspid annular motion velocity (S) in conjunction with simultaneous electrocardiography were measured at the lateral corner of the tricuspid annulus from the apical four-chamber view (18, 26-28).

STATISTICAL ANALYSIS

The data were analyzed per age categories [group A (20-29 year), group B (30-39 year), group C (40-49 year) and group D (50-60 year or more)]. The mean and standard deviations were calculated. To compare the study groups, SPSS version 28 was used along with analysis of variance (ANOVA) and correlation regression testing. An analysis of the relationship between the different variables and age was conducted by using the correlation coefficient (r). Probability value P less than 0.05 was considered statistically significant (α=0.05).

RESULTS

The mean values by age for the RV systolic indices (TAPSE, FAC, TDs) derived by 2DE and TDI:there was a statistically significant decrease in FAC with age. In contrast, TAPSE and the tissue Doppler S wave doesn't show a significant variation with age. (Tab. 2).

FAC also was statistically different among group A-C and A-D. TAPSE and S wave were not significantly different in these four groups. The Correlation of RV systolic indices (TAPSE, FAC, TDs) derived by 2DE and TDI with age: According to the person correlation results, there was a significant small negative relationship between age and TAPSE (r= -.197, p = .047), as well as age and FAC (r= -.241, p = .015). There was a non-significant very small negative relationship between age and TDs (r= -.0925, p = .355). (fig: 1, 2, 3).

Table 2: The values of TAPSE, TDs, FAC according to age groups.

Data (M±SD)	Group A (20_29yrs) No. 33	Group B (30_39yrs) No. 25	Group C (40_49yrs) No. 25	Group D (50>60yr) No. 20	Total No. 103	P value
TAPSE (mm)	22.8±2.893	23.3±2.408	21.6±3.202	22.1±2.685	22.5±2.85	Non-Sig.
TDs (m/s)	0.13±0.021	0.14±0.029	0.12±0.028	0.13±0.031	0.13±0.02	Non-Sig.
FAC (%)	51.1±7.639	47.2±7.059	43.3±10.94	45.9±8.676	47.2±9.01	Sig.
	P value< 0.05: significant. P value< 0.01: highly significant.					

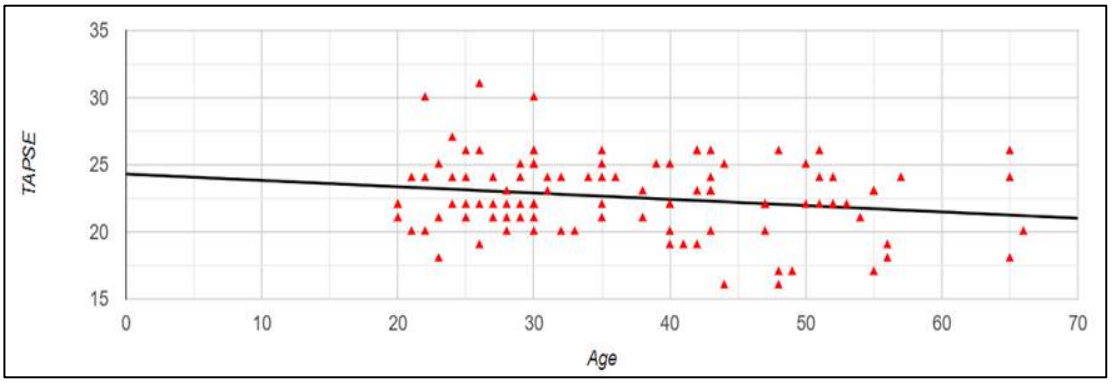

Figure 1: linear regression plot demonstrating the relationships between TAPSE and age in years.

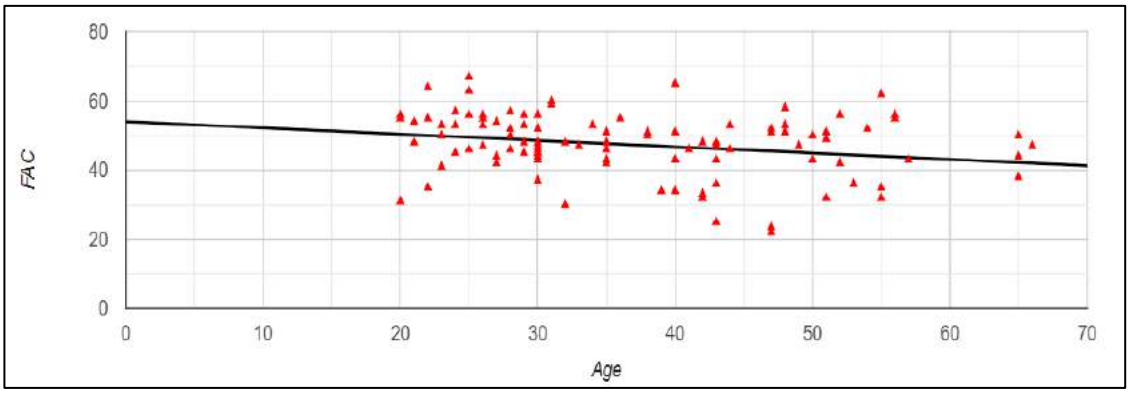

Figure 2: linear regression plot demonstrating the relationships between FAC and age in years.

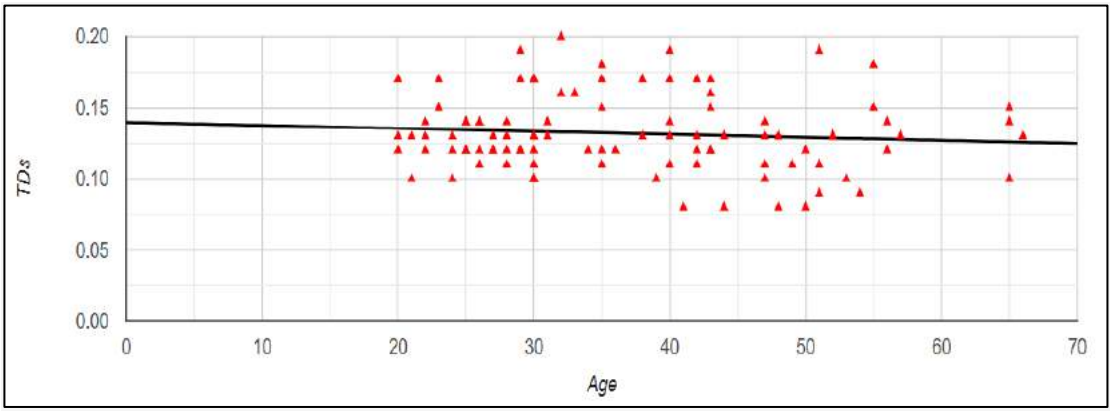

Figure 3: linear regression plot demonstrating the relationships between TDs and age in years.

DISCUSSION

This study has demonstrated that age has a significant effect on certain clinical parameters of RV systolic function. Age-related reductions were observed in longitudinal systolic function, as expressed by TAPSE, and RV contractility, estimated by FAC. There was a slight but non-significant negative correlation between age and the tissue Doppler S wave. The finding highlights the importance of routine cardiac assessment in elderly people to identify early aging-related changes. These results have significant implications for both present and future clinical and research settings. Specifically, they indicate that age normalized values have to be considered when interpreting RV parameters in clinical scenarios, to establish normality or the degree of abnormalities. This is particularly noteworthy as the RV functional capability serves as an important indicator of outcome in a variety of disorders.

RV contraction is mostly caused by longitudinal shortening. The architecture of the cardiac muscle fibers in the LV and RV varies depending on the myocardial level. While circumferential fibers predominant in the lateral wall of LV, longitudinally arranged fibers predominate in the RV free wall. Most previously proposed assessments of RV function(11) have relied on the imaging of peak systolic motion and TAPSE which provides easy indication of RV longitudinal shortening and FAC which is a primary indicator of RV contractility.

Like the study conducted by (29), the current study has observed a small but significant negative correlation between TAPSE and age. However, the slight negative relationship found here between age and S velocity was not statistically significant. It means that these parameters are possibly less vulnerable to the subtle changes in RV performance brought on by aging. Other research (11, 30-32), with the exception of the study of McGhie et al., which reported a non-significant correlation between TAPSE and age, found a decline in basal RV longitudinal function exhibited by TAPSE and s velocity. However, no significant change in RV systolic velocities with age was observed in other studies employing pulsed wave tissue Doppler (33-38). Perhaps as a result of the somewhat younger participants in current study.

The current study observed reduction in FAC which indicates an aging-related decline in right ventricular contractility and pumping capacity. The structural and functional alterations in the myocardium may be responsible for this age-related decrease in FAC. Lower RVFAC may result from impaired RV contraction caused by myocardial stiffness and fibrotic remodeling from collagen fiber deposition with age.

According to three earlier investigations (31, 37, 39), there was no significant change in FAC with aging. Further research is required to offer a more comprehensive knowledge of the correlation between RVFAC and age, as the current body of research is very restricted.

CONCLUSIONS

2DE derived FAC and TAPSE were shown to decrease significantly with age. RV longitudinal systolic function as measured by tissue Doppler s wave showed a small negative correlation **WITH** age, which demonstrate that conventional techniques may ignore mild or slight changes leading to underestimated assessment regarded as a fundamental challenge for their use. So, the use of advanced techniques which allow early identification of RV dysfunction are recommended since they can detect subclinical dysfunction before anomalies revealed by traditional echocardiography occur.

RECOMMENDATIONS

1. A long-term follow-up of the participants would support the findings of this cross-sectional analysis.
2. It is recommended that a greater number of participants including female gender be examined.

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The Sensitivity and Specificity of Measuring the Thickness of Myometrium to Predict the Time of Spontaneous Labour in Preterm Prelabor Rupture of Membranes and Oligohydramnios

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ABSTRACT

Background: Preterm prelabour rupture of membranes (PPROM) accounts for one- third of cases of preterm labour which is the leading cause of perinatal morbidity and mortality. The time from the rupture of membranes until labour, i.e. the latent period (LP), is an important factor in neonatal outcome. **Aim of the study:** The present study aims to determine the sensitivity and specificity of measuring the thickness of myometrium for the prediction of LP. **Patients and methods:** This cross-sectional observational study was performed in Az-Zahraa Teaching Hospital in Najaf-Iraq during the period from the first of August 2016 to the first of October 2017. A total of 89 patients who admitted with the diagnosis of PPROM at the 26th to the 34th weeks of gestation with oligohydramnios were included in the study. The thickness of myometrium was measured via transabdominal ultrasound in the lower uterine segment (LSMT), anterior (AMT), posterior (PMT) and fundal (FMT) parts of uterus within 24 hours of membranes rupture. **Results:** The mean measurements of LSMT, AMT, PMT and FMT in patients with PPROM and oligohydramnios were 7.25±2.31, 7.87±3.45, 8.71±3.88 and 8.65±3.72 respectively. The sensitivity and specificity of measuring AMT for prediction of LP > 7 days were 80% and 53%; those for PFT were 80% and 34.1% respectively at a cut-off point of 6.5 mm while the FMT was found to be 60% sensitive and 34.1% specific. LSMT was 80% sensitive and 43.9% specific for recognition of LP > 7 days at a cut-off point of 7.5mm. **Conclusions:** measurement of the thickness of the myometrium may be a sensitive but non-specific tool for the prediction of the latent period.

Keywords: Latent Period, PPROM, Sensitivity, Specificity, Thickness of Myometrium.

Article Information

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INTRUDUCTION

Preterm prelabour rupture of membranes PPROM is a syndrome characterized by spontaneous rupture of the fetal membranes before 37 completed weeks in the absence of uterine contractions, i.e. before the onset of labour(1). It occurs in up to 3% of all pregnancies and accounts for approximately one-third of all deliveries before 37 weeks gestation (2). As preterm delivery is the most common consequence of PPROM, prediction of the time of spontaneous labour cannot be overemphasized. Approximately 50% of patients with PPROM deliver within 7 days, 75% within 14 days and 85% within 30 days (3). Currently, there is a limited capability to predict latent period (LP), i.e. time from rupture of membranes until labour; this results in difficulties in counseling the patients that suffer from PPROM. Several factors are postulated to affect the LP. An inverse relationship exists between gestational age (GA) at the time of PPROM and LP (4,5).

The relationship between the oligohydramnios and LP is controversial. Some studies showed that oligohydramnios is associated with shortening of LP (6) while another study by Test et al (7) showed that the LP was prolonged in women who had oligohydramnios. A more recent study could not reveal any association between oligohydramnios and LP (8). A twin pregnancy complicated with PPROM has shorter LP than a singleton pregnancy (9). Pregnancy complications such as chorioamnionitis, placental abruption or non-reassuring fetal testing result in early delivery and shorter LP. It has been suggested that shorter length of cervix might be associated with shorter LP. The digital cervical examination has been investigated as a predictor for LP and has been shown to be of limited value. Furthermore, a digital cervical examination may increase the risk of ascending infection. A study done by Fischer et al (10) that measured the length of the cervix by trans-labial ultrasound revealed that cervical length had no association with the duration of the LP in patients with PPROM.

Measurement of the thickness of the myometrium by transabdominal ultrasound in patients with PPORM as a predictor for the time of spontaneous labour has been studied and it has been suggested that the thicker the myometrium the longer the LP. Uterine contractions during labour are associated with diffuse thinning of the myometrium (11). This is consistent with the well-known fact in physics that the applied force per unit of cross-sectional area, i.e. wall stress, is directly proportional to the intra-cavitary pressure and radius of the curvature but inversely proportional to the thickness of the myometrium. Thus, the thinner the myometrium during contraction, the greater will be the generated uterine wall stress(12). These findings suggest that the direction of the force paths, fundal dominance, is not determined by asymmetrical myometrial hypertrophy but, rather may be a function of increased myometrial mass that results from the increased surface area at the fundus (13). A positive correlation between the thickness of myometrium and LP has been revealed by several studies (11, 14). However; the data regarding this association is contradictory and the sensitivity and specificity of measuring the thickness of myometrium in the prediction of LP are not yet verified (14).

The aim of the current study is to determine the sensitivity and specificity of measuring the thickness of myometrium for the prediction of LP in patients with PPROM and oligohydramnios.

PATIENTS AND METHODS

This cross-sectional study was conducted upon 89 pregnant women at 26th to 34th weeks of GA who were admitted to Az-Zahra`a Teaching Hospital with the diagnosis of PPROM and oligohydramnios, amniotic fluid index (AFI) < 5 cm) during the period starting from first of August 2016 to the first of October 2017. The protocol of the study was approved by the Scientific and Ethical Committees at College of Medicine at the University of Kufa. Written informed consents were taken from all patients who participated in the study. A comprehensive history, general, abdominal and sterile speculum examinations were performed for all patients and those who are suitable for expectant management are included in the study. All patients underwent sterile Cusco's speculum examinations to detect cervical dilatation and amniotic fluid pooling, aided if necessary by mild pressure on the abdomen or Valsalva manoeuvre. A Confirmation of the leakage of amniotic fluid by a positive ferning test, i.e. observation of a crystallization pattern on a dried sample of fluid under the microscope and a positive nitrazine test, i.e. alkaline pH determination of fluid, were achieved for all patients in the study.

The exclusion criteria included:

- Multiple pregnancy.
- Intra-uterine growth restriction.

- Uterine or fetal anomalies.
- Uterine scar or fibroid.
- Placental abruption or placenta previa.
- Other maternal or fetal conditions that necessitated termination of pregnancy such as chorioamnionitis or fetal distress.

During the follow-up, five patients were excluded from the study as they required emergency termination of pregnancy by cesarean section: three of them due to development of chorioamnionitis, one of them due to non-reassuring fetal heart rate pattern and one of them due to placental abruption. Thus, the final analysis of data included only 84 patients.

Trans-abdominal ultrasound scan was performed for all patients initially within 24 hours of membrane rupture by the same experienced sonographer for determination of GA, placental localization, fetal well-being, congenital anomalies, amount of liquor by AFI and thickness of the myometrium at the lower uterine segment (LSMT), fundus (FMT), anterior wall (AMT) and posterior (PMT) wall of the uterus. All scans were performed abdominally by the use of GE (voluson E6) ultrasound via a transabdominal curvilinear probe with a 3.5 MHZ frequency.

Data from the study subjects were dedicated on the age, gravidity, parity, GA based on menstrual history and/or first-trimester ultrasound, LP, thickness of myometrium, i.e. LSMT, AMT, PMT and FMT. The thickness of the myometrium was measured between the serosa and decidua. For determination of LSMT, the ultrasound probe was positioned about 2 cm above the urinary bladder reflection anteriorly; for AMT the probe was positioned 1cm above the maternal umbilicus; for FMT the probe was positioned so that the total fundal curvature could be recognizable. The PMT was determined where the maternal pulse of the aorta was detected. During follow-up, all patients received prophylactic antibiotics and steroids for enhancement of lung maturity. Maternal monitoring was performed clinically and by laboratory investigations, including complete blood counts and C-reactive protein. Fetal monitoring was accomplished regularly including daily non-stress test, twice-weekly biophysical profile, and fetal heart rate monitoring four times a day. Digital vaginal examination and tocolytics were avoided. However, if the patient complained of pain and contractions, she could be fully assessed to identify the start of labour. LP was defined from the time of membrane rupture until the start of the spontaneous labour.

STATISTICAL ANALYSIS

Statistical analysis was done by using SPSS version 20. Independent sample T-test, Pearson's correlation coefficient and receiver operator curve (ROC) were used as needed. A P value of <0.05 was set as significant.

RESULTS

As the final analysis of data involved 84 patients with PPROM and oligohydramnios, Tab. (1) shows the demographic characteristics of those patients. The descriptive data of the mean, minimum, maximum thickness of myometrium at the lower uterine segment, fundus, and the anterior wall and posterior wall of the uterus were shown in Tab. (2). Pearson’s correlation between the LP and the thickness of myometrium at different parts of the uterus was estimated as seen in Tab. (3). There were significant positive correlations between the LP and LMST, AMT, PMT and FMT, as P= 0.01, 0.001, 0.036 and 0.041 respectively. The sensitivity and specificity of measurement of the thickness myometrium at different parts of the uterus for the prediction of the LP >7 days were

determined by the ROC curve analysis as seen in Fig. (1) and Tab. (4). Area under the curve (AUC) for anterior (AMT), posterior (PMT), fundal (FMT) parts and the lower segment (LSMT) of the uterus were 0.700, 0.602, 0.566 and 0.629 respectively.

Table (1): Demographic characteristics of the patients enrolled in the study.

Characteristic	Mean	Minimum	Maximum	SD
Age/years	28.66	20	35	±6.34
Gravidity	3.53	1	10	±2.98
Parity	1.81	0	8	±1.53
GA*/weeks	31.28	26	34	±3.88
AFI*	2.36	1	4	±1.7
LP */days	8.34	1	19	±1.1

* GA= gestational age, AFI= amniotic fluid index, LP= latent period.

Table (2): The measurements of the thickness of myometrium at different parts of uterus.

Thickness of myometrium /mm	Mean	Minimum	Maximum	SD
LSMT*	7.25	5.2	11.3	±2.31
AMT*	7.87	5.4	12.5	±3.45
PMT*	8.71	5.6	14.8	±3.88
FMT*	8.65	5.9	13.7	±3.72

* LSMT= lower segment myometrial-thickness, AMT= anterior myometrial-thickness, PMT=posterior myometrial-thickness and FMT= fundal myometrial-thickness.

Table (3): The correlation between the thicknesses of myometrium at different parts of the uterus with the LP.

Thickness of myometrium /mm	r	P value
LSMT*	0.64	0.01
AMT*	0.71	0.001
PMT*	0.38	0.036
FMT*	0.56	0.041

* LSMT= lower segment myometrial-thickness, AMT= anterior myometrial-thickness, PMT= posterior myometrial-thickness and FMT= fundal myometrial-thickness, r=Pearson's correlation coefficient.

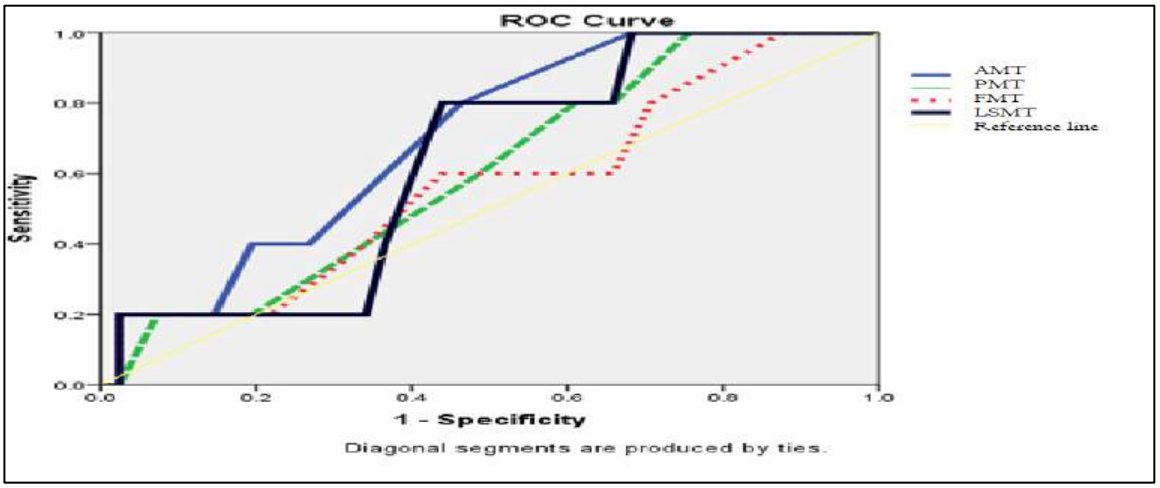

Figure (1): ROC curve represents the sensitivity and specificity of measurement of the thickness of myometrium and area under the curve (AUC) at different parts of uterus for patients whose LP was >7 days.

Table (4): Sensitivity and specificity for detection of LP >7 days.

	AMT	PMT	FMT	LSMT
Sensitivity	80%	80%	60%	80%
Specificity	53.7%	34.1%	34.1%	43.9%
Thickness of myometrium/mm	6.5	6.5	7.5	7.5
LP/ days	>7	>7	>7	>7

DISCUSSION

Several predictors have been investigated for their role in the assessment of spontaneous labour time in PPROM. None of these studied factors was found to be of reasonable predictive power. The focus of current study is to find practical, easy and available ultrasound-based measures that might be helpful in predicting the LP. One of these measures is to estimate the thickness of myometrium and to find out its relationship with the duration of LP. Although several studies have revealed a positive correlation between the thickness of myometrium and the length of LP, there is a paucity of data regarding sensitivity and specificity of this measure to predict the LP [11, 14, 15].

In this study, the mean thickness of myometrium at lower segment (LSMT), anterior (AMT), posterior (PMT) and fundal (FMT) parts of the uterus were found to be 7.25±2.31, 7.87±3.45, 8.71±3.88 and 8.65±3.72 mm respectively. These findings were comparable to those found by Hamdi et al [16] who found out AMT, PMT and FMT to be 8.23 ±2.59, 8.9 ±2.86, and 9.1±3.54 mm respectively. Atarjavadan et al [17] and Gupta et al [18] also found similar ranges. However, the mean AMT, PMT and FMT were found to be higher in a previous study done by Buhimschi's et al [11] which were10. 6 ±0.6, 9.6 ±0.6 and10.7 ±0.7 mm respectively while they were found to be lower in Kalantari's et al [15] study, i.e. 6.5 ±1.5, 6.5 ±1.7, 7.9±2.4 mm

respectively. A small sample size, as in Kalantari's et al [15] study which included 24 PPROM patients only, and the use of 5.5-7.7 MHz probe frequency, as in Buhimschi's et al [11] study, might explain these differences.

Comparing the demographic data of the current study with that of previous studies revealed that the mean maternal age here was 28.66 ± 6.34 years, as in Tab. (1), while it was 29.2 ± 1.2 in Buhimschi et al's [11] study, 28.9 ±5.1 years in kalantari et al's [15], 27.2 ± 4.9 in Hamdi et al's [16], 27.9 ± 4.7 in Atarjavdan et al [17], 26.2± 4.9 mm in Gupta et al's [18] and 29.44±6.1 in Elshikh's [19]. Nevertheless, no association between the maternal age and the thickness of myometrium was found in the previous studies [14-17].

The mean gravidity and parity in the current study were 3.35, ranging from 1-10, and 1.81, ranging from 0-8, respectively. Previous studies had involved patients with different ranges of gravidity and parity [14-17]. However, no relationship was found on review of the literature between gravidity or parity and the thickness of myometrium or LP [17].

The mean gestation age in this study was 31.28±3.88 weeks, as seen in Tab.(1), while the mean gestation age was 29.5 weeks in Buhimschi's [11] study, 29.1 weeks in Kalantari's [15], 30.6 weeks in Hamdi's [16], 28.6 in Atarjavdan et al's [17] and 32 weeks in Gupta et al's [18]. These differences might explain difference in the results.

A significant positive correlation between the thickness of myometrium at the lower segment, anterior, posterior and fundal parts of the uterus and LP was evident in this study as seen in Tab. (3). These findings were consistent with the findings of Buhimschi et al's [11], and Elshikh's [19]. In contrary, the findings of Kalantari et al [15], Hamdi et al [16] and Naleini et al [20] had revealed no association between the thickness of myometrium and LP. Atarjavdan et al [17] found a significant positive correlation between the thickness of the myometrium at the anterior wall, fundus and lower uterine segment in patients with GA below 30 weeks while no such association was revealed in those with GA of 30 weeks and more. Finally, Gupta et al [18] found a significant positive correlation between the thickness of myometrium at the anterior wall and lower uterine segment whereas no significant correlation was found in the posterior and fundal myometrium.

The current data has revealed that the sensitivity and specificity of measuring AMT for prediction of LP > 7 days were 80% and 53% and those for PFT were 80% and 34.1% respectively at a cut-off point of 6.5 mm while the FMT is found to be 60% sensitive and 34.1% specific and LSMT was 80% sensitive and 43.9% specific respectively for recognition of LP > 7 days at a cut-off point of 7.5mm.

There were two previous studies that estimated the sensitivity and specificity of measuring the thickness of myometrium for the prediction of LP. In a study performed by Atarjavadan [17], FMT was 79% sensitive and 39% specific for prediction of LP >8 days at a cut-off point of 6.9 mm, AMT was found to be 89% specific and 42% specific at a cut-off point of 5.5 mm and LSMT was 89% sensitive and 85% specific for prediction of LP >8 days at a cut-off point of 7.35 mm respectively.

Buhimschi et al [11] found that the thickness of fundal myometrium (FMT) was 89%sensitive and 62.5% specific for prediction of LP > 7 days at a cut-off point of 12.6 mm.

CONCLUSIONS

The thickness of the myometrium at the lower segment, fundus, anterior and posterior parts of the uterus might be sensitive but not specific for prediction of LP > 7 days.

RECOMMENDATIONS

These data might be helpful in counseling and to optimize the management of patients with PPROM. Further studies are needed to confirm these results and to find better predictors of LP in patients with PPROM.

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Long Term Effect of Post-Covid-19 Syndrome on Hematological Parameters in Iraqi People

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ABSTRACT

Background: The COVID-19 epidemic, which is still ongoing, has a significant effect on health care both in Iraq and worldwide. Further strain on health systems was caused by successive waves of altered viruses, further enhancing virus dissemination. Persistent symptoms may last for several months following the initial COVID-19 infection. The illness is called post-acute sequelae of SARS-CoV-2 infection, or Long COVID (PASC). Long-term COVID-19 infection can affect various organs and systems, including the hematological system, and cause many symptoms. Hematological problems have been observed in individuals previously infected with COVID-19, in several investigations. Poor outcomes and an increased risk of severe disease are linked to the majority of these changes. **Aim of the study:** This aims to find the post-COVID-19 effects on the hematological parameters. **Patients and methods:** This study was a case-control investigation at the As-Sadr Teaching Hospital in Maysan City- Iraq between October 13, 2023, and November 13, 2023. There were two groups: fifty-eight-patient case or experimental group along with a fifty-eight- individual control group. In this study, the randomly selected healthy individuals in the control group were matched to patients with COVID-19 according to symptoms. Except for not having COVID-19 disease, the controls had to fulfill the same requirements as the cases. Clinical profiles and hematological markers were examined and contrasted between the two groups. **Results:** The data show no significant differences ($P > 0.05$) in age, BMI, and sex between case and control groups. The results confirm alterations in hematological parameters. Blood sample analysis in the case group show a significantly higher mean of RBC and a decline in MCHC, and MCV compared to the control group. However, no significant differences in hemoglobin and hematocrit parameters exist between them. The mean HCT% and HGB are significantly lower for the age group 18-29 years than for 30-39 and 40-49 years and the alterations were more noticeable in male COVID-19 patients.

Conclusions: The results have shown altered hematological parameters after 3 years of COVID-19 infection. The changes include reduced Hg, MCH, and MCHC and higher RBC values that might affect oxygen transport through the body. The alterations were more noticeable in male COVID-19 patients, indicating that males are more affected by the disease than females. In light of the increasing number of individuals with Long-COVID, more research is required to determine the precise underlying causes of the extended altered RBC and hemoglobin value among different age groups.

Keywords: Hematological parameters, Hemoglobin, Hypoxia, Long Covid-19, SARS-CoV-2.

Article Information

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INTRUDUCTION

In December 2019, Wuhan, China, announced the first case of the extremely contagious new coronavirus, known as COVID-19 and SARS-COV-2 (1). This has led to widespread illness and death across the globe (2). On February 24, 2020, in Najaf City, the first COVID-19 case in Iraq was reported. As of April 9, 2020, confirmed cases have significantly increased to 1232 (3). The respiratory system is the main organ affected by the condition. Nevertheless, COVID-19 can also affect the hematological system, among other organs and systems. Hematological abnormalities in COVID-19 patients have been identified in several investigations. Some changes include an increase in the number of white blood cells, a decrease in red blood cell and hemoglobin levels, and an increase in total bilirubin and ferritin levels, which may occur in severe COVID-19 infection (4, 5). Additionally, there have been reports of large changes in spleen size in deceased COVID-19 patients, which is a normal reaction to anemia (6).

The symptoms of COVID-19 can vary in severity and include fever, coughing, sore throat, exhaustion, shortness of breath, body aches, and loss of taste or smell. It is crucial to emphasize that hematological abnormalities are not a common symptom of COVID-19 and that patients might differ greatly in the severity of these abnormalities(5). Certain symptoms may last for several months following the initial COVID-19 infection. In this instance, the illness is called post-acute sequelae of SARS-CoV-2 infection, or Long COVID (PASC). Even those with moderate or asymptomatic illnesses may experience these symptoms.

The consequences of Long COVID are still being studied, however, there is a wide range of symptoms. Fatigue, breathlessness, joint or chest pain, headaches, foggy thinking, trouble focusing, loss of taste or smell (7). On the other hand, people may sustain organ damage, including heart, lung, or kidney problems (8). There is evidence that the development and severity of Long COVID can be influenced by age, sex, and race (9, 10). Both men and women can contract long COVID, while some research suggests that women are more susceptible (9, 10). Furthermore, lengthy healing times and chronic symptoms are often more common among the elderly. Different racial and ethnic groups exhibit different cognitive symptomology related to long-term COVID-19 infection (11).

A recent meta-analysis revealed that smoking, high body mass index, age, and female sex were linked to a higher risk of Long COVID-19 (12). Additionally, a study showed that COVID-19 altered the size and shape of red blood cells, which may have an impact on oxygen transport (13). Blood biomarkers may be able to predict Long's COVID status and help with medical intervention and treatment. Hematological parameter changes continue in Long Covid (14).

PATIENTS AND METHODS

A case-control study was conducted from October 13, 2023, to November 13, 2023, at the As-Sadr Teaching Hospital in Maysan City-Iraq. Fifty-eight patients and fifty-eight controls are included in the total. The study paired patients exhibiting COVID-19 symptoms with randomly chosen controls. The controls had the same requirements as the cases, except not having COVID-19 disease. The two groups were compared and their clinical profiles and hematological indicators were analyzed. The ages of the studied groups are randomly selected, all ages. An informed consent was achieved from every patient and control included in this study. Every participant underwent a clinical evaluation and a thorough medical history was obtained from both patients and controls, covering details such as age, name, gender, phone number, place of employment, history of COVID-19 infection, and symptoms like fever, cough, headache, loss of taste and smell, vomiting, diarrhea, runny nose, shortness of breath, chest pain, and others, in addition to the length of the sickness and the kind of medication or vaccine that is applied.

Hematological parameters

Blood collection

Five milliliters of venous blood were taken from each person. The blood samples were taken and placed in two separate tubes. One ml was deposited in Ethylenediaminetetraacetate (EDTA) anticoagulated blood tubes for hematological parameter measurements by Auto Hematology Analyzer, including: The number of circulating blood cells, Hemoglobin (Hb), Hematocrit (the proportion of whole blood volume occupied by red cells), Red blood corpuscles Count-RBCs count (cells/mm3). Level of hemoglobin in blood (g/dl), Packed cell volume (PCV%), Mean corpuscle volume-(MCV) (femtoliter, fl), Mean corpuscular hemoglobin (MCH, Picogram) and Mean corpuscle hemoglobin concentration (MCHC)(g/dl).

STATISTICAL ANALYSIS

Statistical analyses were done by using the SPSS program (version 26). Categorical variables were presented as percentages and frequencies while continuous variables were presented as means ± standard deviations. The chi-square test was used to assess the qualitative data between categorical variables (age groups, gender). An independent t-test was used to compare the means between cases and controls. Error bars of means and standard deviations were used to compare the hematological parameters according to age groups and gender. The P values less than 0.05 will be considered statistically significant results.

RESULTS

Characteristics of the Study Patients

Table (1) displays the baseline demographic information between healthy controls and cases who became infected with COVID-19. The groups that had recovered from COVID-19 infection and the healthy controls had mean ages of 34.1±9.8 and 35.9±11.7 years, respectively, and BMIs of 29.4±8.01 and 28.6±6.1 (Kg/m2). It indicates that there is no significant difference (P > 0.05) in age, BMI, or sex between the groups.

Table (1): Demographic data of study population

Variable	Subgroup	Group		Total	P
		Cases (n=66) No.(%)	Controls (n=67) No.(%)		
Age group (years)	18-29	29 (43.9%)	24(35.8%)	53(39.8%)	0.2
	30-39	17(25.8%)	25(37.3%)	42(31.6%)	
	40-49	13(19.7%)	7(10.4%)	20(15.0%)	
	50-65	7(10.6%)	11(16.4%)	13(13.5%)	
Mean age(years) ±SD		34.1±9.8	35.9±11.7		0.3
Sex	Male	38(57.6%)	42(62.7%)	80(60.2%)	0.5
	Female	28(42.4%)	25(37.3%)	53(39.8%)	
BMI	Normal	15(22.7%)	17(25.4%)	32(24.1%)	0.8
	Overweight	26(39.4%)	28(41.8%)	54(40.6%)	
	Obese	25(37.9%)	22(32.8%)	47(35.3%)	
Mean BMI (Kg/m^2)±SD		29.4±8.01	28.6±6.1		0.6

P value< 0.05: significant. P value< 0.01: highly significant.

Hematological parameters differences between cases and control

Table (2) indicates a statistically significant (P <0.05) variation in the blood parameter values (RBC, MCHC, MCV, and MCH) between the case and control groups. The cases have a higher RBC mean of 5.2±0.7 compared to the control mean of 4.7±0.6. Comparing the mean of MCHC in cases (30.4±1.1) to the mean in controls (32.4±1.5), there is a reduction in cases mean. In addition, the cases' MCV (82.8±7.5) and MCH (24.04±2.6) mean values are lower than those of the control group (85.7±3.8 and 27.1±1.7, respectively). Nonetheless, the table shows that the parameters of hemoglobin and hematocrit are not significantly different between the patients and control groups (P > 0.05).

Table (2): Hematological parameters differences in cases and control.

Hematological assessment	Cases (n=66) mean±SD	Controls(n=67) mean±SD	P value
RBC(10^12/L)	5.2±0.7	4.7±0.6	0.0001
MCHC(g/dL)	30.4±1.1	32.4±1.5	0.0001
MCV(fL)	82.8±7.5	85.7±3.8	0.006
MCH(pg)	24.04±2.6	27.1±1.7	0.0001
HCT%	39.8±3.9	40.02±3.9	0.8
HGB(g/dl)	12.2±1.4	12.6±1.3	0.05

Hemoglobin (Hb), Hematocrit HCT%, Red blood corpuscles Count (RBCs), Packed cell volume (PCV%), Mean corpuscle volume (MCV), Mean corpuscular hemoglobin (MCH), Mean corpuscle hemoglobin concentration (MCHC). Statistically significant (P <0.05).

Comparison of the hematological parameters between cases and control across different age groups

Table (3) revealed that the age group (18-29, 30-39, and 40-49 years) had a significant increase in RBC and a decrease in MCHC, MCH MCV, and HCT% in cases than in control, while the 50-65 years old have a nonsignificant increase in RBC value. However, the hemoglobin, MCV, and HCT% decrease insignificantly in all age groups except (18-29) year which decrease significantly between cases and control. The results show no significant difference in RBC, MCHC, MCH, and MCV in cases among age groups. The mean values of the hemoglobin and hematocrit 38.5±3.9, 11.6±1.3 significantly lower in cases of the age group (18-29 years) compared to the 30-39 and 40-49 age group 41.02±3.3, 12.7±1.1and 41.1±4.1, 12.6±1.4 respectively.

Table (3): Comparison of the hematological parameters between cases and control across different age groups (years).

Age group(years)	Hematological assessment	Cases (n=66) mean±SD	Controls(n=67) mean±SD	P value
18-29	RBC(10^12/L)	5.2±0.7	4.8±0.6	0.01
	MCHC(g/dL)	30.2±0.9	32.9±1.4	0.0001
	MCV(fL)	82.2±7.5	85.6±3.8	0.01
	MCH(pg)	23.8±2.6	27.5±1.8	0.0001
	HCT%	38.5±3.9	41.7±3.4	0.002
	HGB(g/dl)	11.6±1.3	13.1±1.3	0.0001
30-39	RBC(10^12/L)	5.1±0.6	4.6±0.6	0.007
	MCHC(g/dL)	30.6±1.5	32.3±1.7	0.001
	MCV(fL)	81.9±8.4	84.3±3.6	0.2
	MCH(pg)	24.1±2.9	26.9±1.8	0.0001
	HCT%	41.02±3.3	39.5±3.6	0.2
	HGB(g/dl)	12.7±1.1	12.4±1.2	0.4
40-49	RBC(10^12/L)	5.2±0.7	4.4±0.5	0.03
	MCHC(g/dL)	30.4±0.8	31.6±1.6	0.03
	MCV(fL)	85.4±7.3	87.2±4.3	0.6
	MCH(pg)	24.6±2.3	26.6±2.1	0.08
	HCT%	41.1±4.1	38.8±3.7	0.2
	HGB(g/dl)	12.6±1.4	12.6±1.6	0.9
50-65	RBC(10^12/L)	5.2±0.7	4.6±0.4	0.07
	MCHC(g/dL)	30.6±0.8	32.1±1.5	0.02
	MCV(fL)	82.5±6.2	85.9±3.7	0.2
	MCH(pg)	23.8±2.1	26.9±1.2	0.001
	HCT%	40.3±4.1	38.3±5.2	0.4
	HGB(g/dl)	12.5±1.4	12.2±1.3	0.6

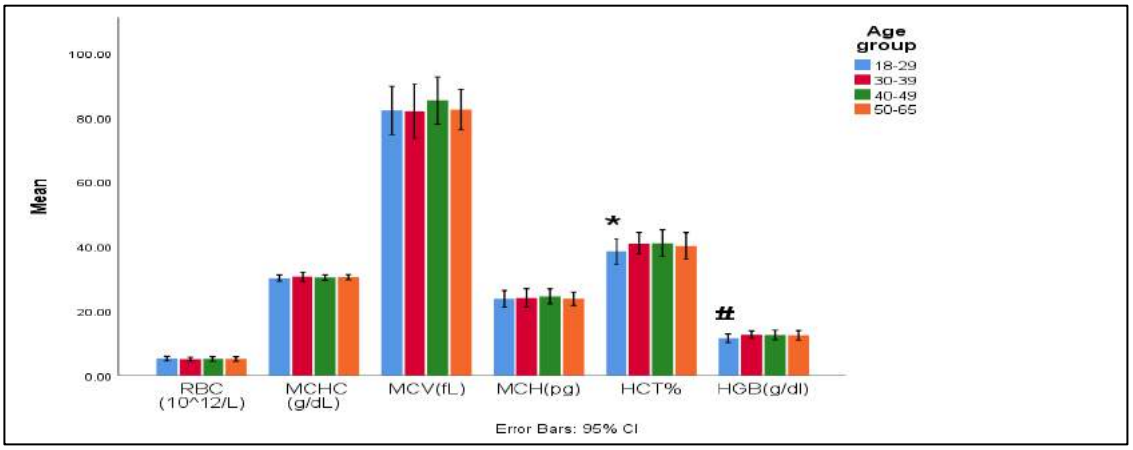

Figure (1): Comparison of the hematological parameters between cases and control across different age groups (years).

* Mean HCT% is significantly lower for the age group 18-29 years than for 30-39 and 40-49 years
#Mean HGB is significantly lower for the age group 18-29 years than 40-49 years

Hematological parameters change between males and females in cases and control

Table (4) demonstrates that males and females have a significant difference in RBC, MCHC, and MCH values in both groups (P =0.0001). The level of hemoglobin, hematocrit, and RBC increased significantly in males 12.9±1.1, 41.6±3.2, 5.3±0.6 than in females 11.2±1.1, 37.4±3.6, 5±0.7, respectively. Male hemoglobin and HCT% indicated no significant difference P= 0.9 between patients and controls, however, female cases' hemoglobin significantly decreased P= 0.007.

Table (4): Hematological parameters for male and female in cases and control

Sex	Hematological assessment	Cases (n=66) mean±SD	Controls(n=67) mean±SD	P value
Males	RBC(10^12/L)	5.3±0.6	4.8±0.5	0.0001
	MCHC(g/dL)	30.6±1.2	32.6±1.4	0.0001
	MCV(fL)	82.6±7.8	86.6±4.1	0.006
	MCH(pg)	24.1±2.6	27.2±1.6	0.0001
	HCT%	41.6±3.2	40.8±4	0.3
	HGB(g/dl)	12.9±1.1	12.9±1.2	0.9
Female	RBC(10^12/L)	5±0.7	4.3±0.5	0.0001
	MCHC(g/dL)	30.1±0.9	32.4±1.8	0.0001
	MCV(fL)	83.04±7.2	84.1±2.7	0.5
	MCH(pg)	23.9±2.5	26.9±1.9	0.0001
	HCT%	37.4±3.6	38.6±3.6	0.2
	HGB(g/dl)	11.2±1.1	12.2±1.4	0.007

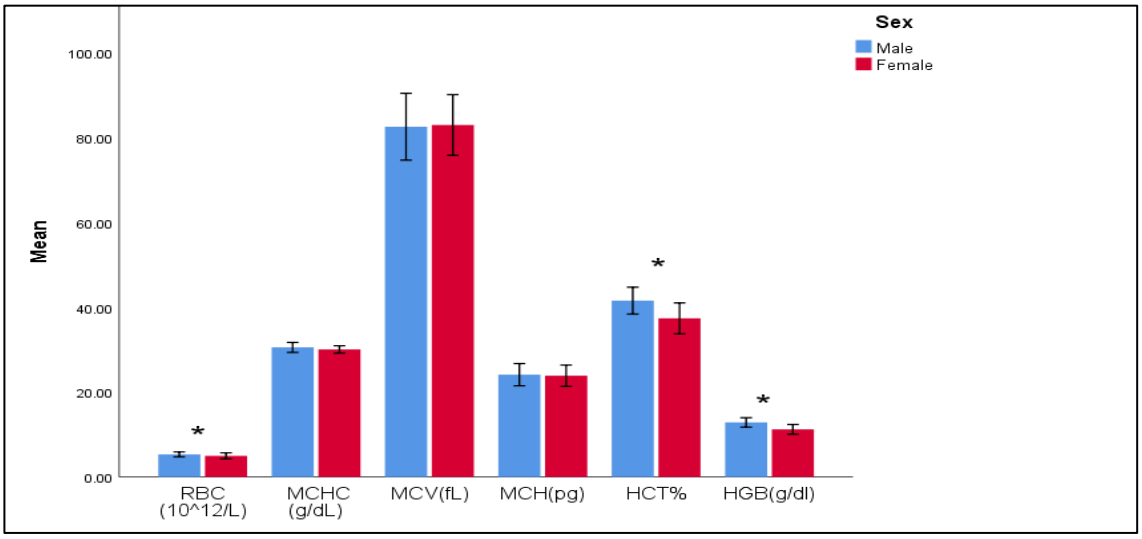

Figure (2): Hematological parameters alteration between male and female cases.

*Mean RBC, HCT%, and HGB increase significantly in male cases than in female.

DISCUSSION

The frequency of COVID-19 cases has increased worldwide. SARS-CoV-2 infection is associated with a change in RBC shape and compromised cell function (13). Looking at the COVID-19 outbreak, it is critical to evaluate clinical, demographic, and hematological characteristics. In Iraq, there is a limited number of research have examined hematological markers linked to COVID-19. However, further research is needed to fully comprehend this phenomenon, as it is becoming increasingly clear that COVID-19-related infections can last for a long time and result in long-term COVID-19. Therefore, hematological parameters such as RBCs, MCHC, MCH, MCV, HCT%, and HGB and their relation to long-term COVID-19 were examined in the current study.

This research offers a good deal of information on hematological parameters and demographic data, which indicated no significant difference in gender between cases and control; this could be explained by the impact of behavioral decisions or gender immunological variations (15). The data were gender-separated since the outcomes support previous research that found clear variations in the overall hematological parameters of males and females (13). This was linked to either periodic menstrual blood losses in women or increased testosterone levels in men (16). The present results demonstrate that the mean of RBC, HCT%, and HGB increase significantly in male cases than in females. This data is consistent with previous research (17); thus the other study also detected higher hemoglobin levels in males compared to females (18).

According to the comparison of the outcome of RBC count and its indices, MCHC, MCV, and MCH, there was a highly significant decrease observed in all mean values of indices and an increase in RBC count as compared with the control. For instance, the mean value of hemoglobin concentration of the control group was 12.6±1.3 whereas its concentration has decreased non-significantly in cases (mean 12.2±1.4) (P value=0.05). These findings were crucial in confirming that the effects of the COVID-19 virus on MCH (pg/cell), MCV (µm3), and MCHC (g/dl) concentrations were greater than those on other RBC indices like hemoglobin and HCT% because the differences were negligible and not statistically significant. The explanation of the current results which found an

increase in RBC count and normal or decreased hemoglobin level in cases compared with controls, is that the majority of COVID-19 patients experienced dyspnea and decreased physical fitness, which may be associated with lung injury and changed red blood cell (RBC) consumption of oxygen, resulting in hypoxemia. Even so, no changes were seen in hematocrit, mean corpuscular hemoglobin concentration, or RBC count (19). Based on these studies, the usual red blood cell count is acceptable; however, the red blood cells' role in oxygen transfer may have changed. According to the aforementioned research, COVID-19 has short-term impacts on red blood cells (RBCS); however, it may also have long-term effects on RBCs (20). There is sufficient data to suggest that the numbers of red blood cells (RBC) and hematocrit (HCT) rise dramatically in hypoxic environments, which is comparable to the alterations that occur by living in high altitudes (21). In response to hypoxic conditions, the kidneys produce more erythropoietin, which in turn stimulates the bone marrow to produce more red blood cells to improve oxygen transport to the body's tissues. This process leads to an increase in the number of reticulocytes, which are newly formed red blood cells that differ from mature ones (22). In that, they are typically less flexible and more spherical, and their cell membrane contains endoplasmic reticulum remnants (23). However, the research group's levels of hemoglobin were noticeably lower than those of the controls, which matched the current study results (24). COVID-19 infections cause a drop in hemoglobin levels through a variety of intricate mechanisms, by retaining iron in macrophages and decreasing its absorption from the intestinal tract leading to alterations in iron homeostasis (25). As a result, hemoglobin synthesis and circulation iron levels both decline. Additionally, compared to cases aged 30-39, 40-49, and 50-59 years, the COVID-19 patients in the age group 20-29 years old had a noticeably lower mean of hemoglobin and hematocrit. The mean age per years ±SD of COVID-19 patients in this study was 34.1±9.8, which was found younger than in other studies. This is also supported by a previous study in Saudi Arabia which suggested that this condition affects a younger age group (26). Studies have shown that patients with COVID-19 and older populations have worse results (27). This may be due to a combination of factors, including the physiological aging process and, more significantly, the higher incidence of frailty and comorbidities in older adult patients, which lower functional reserve and impair intrinsic capacity and resilience as well as the body's ability to fight infections (28).

This study had various limitations. It is possible to determine the course of the hematological consequences of prolonged COVID by evaluating the patient's hematological profile during the COVID-19 acute phase, which was not done in this study. Further, the results might be restricted by the small number of participants of older age in this study. To the best of the researcher’s knowledge, however, this is the first study to link abnormalities in hematological parameters with clinical outcomes and offer insights into these parameters for patients with long COVID up to two years. To eliminate variations that occur naturally, nonetheless, earlier research has demonstrated that hematological values remain consistent over an extended length of time.

CONCLUSIONS

The results of this study indicate a statistically significant (P <0.05) variation in the blood parameter values (RBC, MCHC, MCV, and MCH) between the case and control groups. The cases have a higher RBC mean, lower hemoglobin, MCHC, MCH, and MCV. This current study shows that the patient's age and gender have a substantial impact on individual hematological parameters in post-COVID-19 syndrome and the results demonstrate that the mean of RBC, HCT%, and HGB increase significantly in young male cases than in young

females. The current study's findings supported earlier research's observations and other findings on variations in hematological parameters and a few other variables in long-term COVID-19-infected patients. Thus, more hematological research is required to validate these findings.

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Shear-Wave Elastographic Evaluation of Splenic Stiffness in Patients with Chronic Liver Diseases as A Predictor of The Oesophageal Varices Grade

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ABSTRACT

Background: *Chronic Liver disease (CLD) is a global public health problem and one of the leading causes of mortality worldwide with many complications like portal hypertension (PH) and esophageal varices (EV). Researchers have investigated the role of shear-wave elastography (SWE) as a non-invasive method to assess patients with cirrhosis and PH. However, studies evaluating the role of splenic stiffness (SS) have shown variable results.* ***Aim of the study:*** *This study aims to assess the SS in a patient with CLD compared to controlled cases and to evaluate its role in the prediction of EV grade.*

Patients and methods: *The study included 60 participants; 30 patients diagnosed with CLD by the clinical, laboratory, and radiological investigation, who underwent upper gastrointestinal endoscopy; and 30 control health individuals. The mean splenic size, SS, and platelet counts were measured for all participants. Mean SS was compared between two groups, and then its validity in differentiated between low and high-risk groups of EV was assessed.*

Results: *Patients with CLD and controls were significantly different in SS values with cut-off value of 12.49 Kpa. Among CLD patients, the low and high-risk EV subgroups were significant different in SS values with cut-off value of 15.125 KPa. Compared with splenic size and platelet count, SS had the highest accuracy (93.3%) in predicting high-grade EV.*

Conclusions: *Patients with CLD showed higher SS values than control with stepwise increase in SS with increasing grade of EV. SS can accurately predict high-risk groups of EV hence may help decrease patients' burden by avoiding unnecessary endoscopy.*

Keywords: Chronic liver disease, esophageal varices, splenic stiffness, Ultrasound, shear wave Elastography.

Article Information

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INTRUDUCTION

Chronic Liver disease (CLD) is a global public health problem [1] and one of the leading causes of mortality worldwide [2]. In patients with CLD, complications are mainly related to portal hypertension (PHT) and esophageal varices (EV) [3,4]. PHT carries mortality and recurrence rates and evaluation is important for early treatment and improving outcomes [5,6].

Because EV usually don not cause signs and symptoms unless they bleed, patients are advised to undergo esophagogastroduodenoscopy (OGD) upon diagnosis of cirrhosis [7] being the gold-standard

tool for EV detection [8,9]. Liver biopsy is an accurate test for the diagnosis of CLD, but is invasive and generally less reliable in the assessment of complications [10],[11].

Hepatic vein portal gradient (HVPG) is an excellent predictor of clinical decompensation [12], but is an invasive and technically difficult procedure [13]. Therefore, non-invasive methods have been recently developed, but were proved inaccurate for the early prediction of clinical decompensation in cirrhotic patients[14,15]. Two-dimensional shear wave elastography (2D-SWE) has emerged as the most frequently used diagnostic ultrasound tool for hepatic fibrosis quantification by measuring liver stiffness (LS) [16,17] and was found to correlate well with HVPG and can detect clinically significant PHT[18], but the measurements can be compromised by several practical considerations [19] [20].

Splenic elastography has been also used in patients with CLD [21,22,23] and splenic stiffness (SS) values were found to correlate well with the stage of liver fibrosis [24] and PHT [25]. However previous studies have either concluded variable cut-off values of SS or included only patients with certain etiology of CLD and there was no emphasis on the EV grading. Therefore, the current study was conducted to assess the SS values in patient with CLD of different aetiologies and to evaluate the value of SS in the prediction of EV grade.

PATIENTS AND METHODS

This case-control study was conducted on 60 participants (30 CLD patients with PHT and 30 healthy control) at the Radiology department of Al-Sadir Medical City / Annajaf Health Directorate, from February 2022 to February 2023.

The Study Design

It is a case-control study.

Subjects

The following cases were excluded: 1) patients with a history of intervention for PHT (splenic embolization, trans jugular intrahepatic shunt, splenectomy, etc.) or splenic and hepatic surgery; 2) unproved diagnosis of EV because of an unavailable EG report; 3) presence of technical difficulty due to cooperation, inability to hold breath, ongoing gastrointestinal hemorrhage, hemodynamic instability, severe ascites, severely decompensated liver disease, and an unusual small-sized spleen; and 4) patient with splenic SOL or diffuse enlargement due to underlying infectious, hematological or infiltrative disease. The study was approved by the Intuitional Review Committee and informed consents to participate in the study were taken directly from all participants.

Exclusion criteria

Thirty patients, regardless of age and gender, were recruited from the gastrointestinal center in Al-Sader Medical City with a proven diagnosis of CLD and PHT with EV documented and graded by OGD were selected. They were age-and gender matched with 30 healthy individuals, who have no clinical or laboratory evidence of CLD as control. CLD and PHT were diagnosed and documented by the clinical, laboratory and radiological. OGD

was the gold standard for evaluating PHT and EV diagnosis and grading using Baveno classification [26].

Data collection:

The clinical history and data laboratory investigations and OGD reports were obtained from all the patients directly and /or from their records.

Instruments

All ultrasound and SWE examinations were done by a single radiologist, using LOGIC E9 XDClear ultrasound system (GE Healthcare, 2019, USA) and C1-6 convex probe. All participants were examined after fasting for at least 4 hours. SWE measurements were performed in the supine position, during normal breathing. A rectangular box was placed in the splenic parenchyma, so it did not contain vessels. The SS was calculated by placing a region of interest (ROI) measuring 1.5 × 0.5 cm drawn in the largest possible diameter into the rectangular box (Figure 1). ROI box was placed 1.0 cm below the capsule of the spleen and then adjusted to be in a region free of the blood vessels and rib shadowing. Then the stiffness value was obtained in kilopascals (KPa) and the measurement was validated using Grgurevic method [27]. Three to six valid measurements were taken from the upper, mid, and lower portions of the spleen, and their average was accepted as the mean SS value.

STATISTICAL ANALYSIS

Statistical analysis was carried out using SPSS (version 27). Student t-test was used to compare means between the two groups while ANOVA test was used to compare means among three groups or more. Pearson Chi-Square test was used to find the association between categorical variables. A p-value of ≤ 0.05 was considered significant.

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RESULTS

A total number of 60 participants (30 patients and 30 control) were included and showed no statistically significant difference regarding mean age and gender distribution (table 1).The mean splenic size was (16.17 ± 1.80) cm, the mean SS was (26.84 ± 14.49) KPa and the mean platelet count was (194.77 ± 137.83) (cell x10^3/microliter) as shown in table 2. The mean differences of splenic size, SS, and platelet count were not statistically significant different according to etiology of liver disease Table 4. There was a stepwise increase in SS values and a relative decrease of mean platelet count with an higher grades of EV, with statistically significant difference while no statistically significant difference between mean splenic size and grade of EV was found (table 5). The ROC curve for SS in predicting differentiate cases of liver diseases revealed optimal cut-off value was ≥ 12.49 KPa with overall accuracy of 85.0% (figure 2, A).

For practical purposes and clinical implication, we considered grade I varices as a low-risk group while grad II and III are a high-risk group as these groups have different treatment strategies directed by gastroenterology specialists. The optimal SS cut-off value to predict high-risk groups of EV was ≥ 15.125 KPa , with highest level accuracy in predicting high-risk EV compared to that of platelet count and splenic size as shown in figure 2 (B, C and D) and table 6.

Table 1: shows the association between gender and study group (N=60).

Study variable	Study group		P-value
	Patients	Control group	
Gender			
Male	17 (56.7)	17 (56.7)	1.000
Female	13 (43.3)	13 (43.3)	
Total	30 (100.0)	30 (100.0)	
Age (Mean ± SD years)	51.83 ± 14.97	51.10 ± 17.17	0.861

Table 2. Splenic size, splenic stiffness and platelet count of patients with liver diseases. (N=30)

Study parameters	(Mean ± SD)	Range
Splenic size (cm)	(16.17 ± 1.80)	(13.0-21.0)
Splenic stiffness KPa	(26.84 ± 14.49)	(9.82-55.98)
Platelet count (cell x10^3/microliter)	(194.77 ± 137.83)	(56.0-686.0)

Table 3: Mean splenic size and splenic stiffness values of the study groups

Study variable	Study group	Mean ± SD	P-value
Splenic size (cm)	Patients	16.17 ± 1.80	**<0.001***
	Control group	11.17 ± 1.37	
Splenic stiffness (KPa)	Patients	26.84 ± 14.49	**<0.001***
	Control group	9.33 ± 3.52	

Table 4: The mean differences of study variables according to the type of liver disease (N=30).

Study variables	Type of liver disease	N	Mean ± SD	P-value
Splenic size (cm)	Alcoholic liver disease	9	15.56 ± 1.74	0.316
	Chronic HCV	7	15.86 ± 1.86	
	Cryptogenic CLD	5	17.80 ± 2.17	

	NASH	5	15.80 ± 1.64	
	Primary biliary cirrhosis	2	17.00 ± 0.00	
	Wilson disease	2	16.00 ± 0.00	
Splenic stiffness (KPa)	Alcoholic liver disease	9	27.09 ± 15.05	0.954
	Chronic HCV	7	25.87± 14.43	
	Cryptogenic CLD	5	28.24 ± 19.52	
	NASH	5	25.87 ± 15.45	
	Primary biliary cirrhosis	2	35.05 ± 16.30	
	Wilson disease	2	19.76 ± 5.32	
Splenic stiffness (m/s)	Alcoholic liver disease	9	2.90 ± 0.83	0.961
	Chronic HCV	7	2.81 ± 0.86	
	Cryptogenic CLD	5	2.92 ± 1.05	
	NASH	5	2.83 ± 0.90	
	Primary biliary cirrhosis	2	3.37 ± 0.81	
	Wilson disease	2	2.56 ± 0.35	
Platelet count (cell x 10^3/microliter)	Alcoholic liver disease	9	219.67 ± 108.84	0.888
	Chronic HCV	7	191.29 ± 123.29	
	Cryptogenic CLD	5	220.80 ± 263.40	
	NASH	5	194.00 ± 104.22	
	Primary biliary cirrhosis	2	107.50 ± 41.71	
	Wilson disease	2	119.00 ± 80.61	

Table 5: The mean differences of study variables according to grades of esophageal varices (N=30).

Study variables	Grades of esophageal varices	Number	Mean ± SD	P-value
Splenic size (cm)	Grade I	10	16.70 ± 2.11	0.304
	Grade II	8	15.38 ± 1.77	
	Grade III	12	16.25 ± 1.48	
Splenic stiffness (KPa)	Grade I	10	12.63 ± 1.87	**<0.001**
	Grade II	8	25.94± 9.29	
	Grade III	12	39.27 ± 11.97	
	Grade II	8	2.90 ± 0.55	

	Grade III	12	3.57 ± 0.60	
Platelet count (Cell x10^3/microliter)	Grade I	10	318.30 ± 171.78	**0.001**
	Grade II	8	153.25 ± 47.25	
	Grade III	12	119.50 ± 56.70	

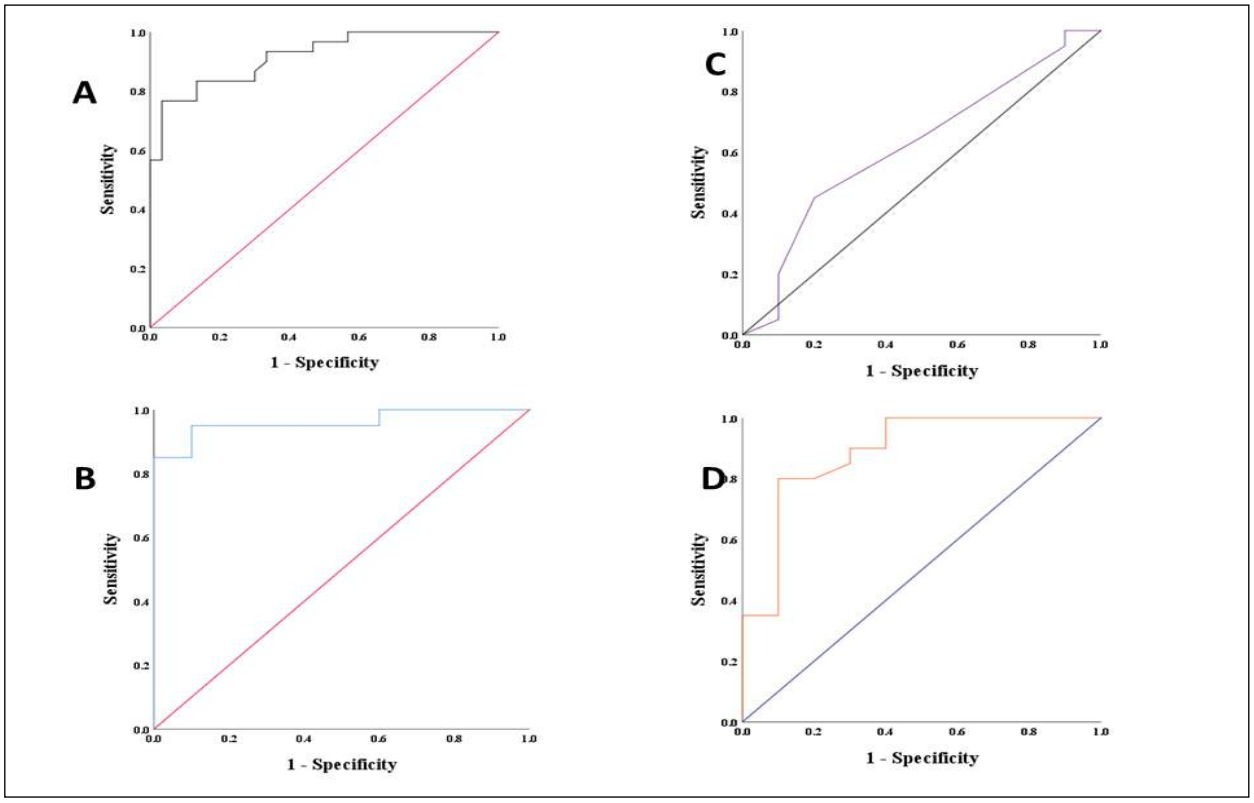

Figure 2: ROC curve of splenic stiffness in predicting liver disease (A). ROC curves of splenic stiffness (B), splenic size (C) and Platelet count to predict high-risk groups of oesophageal varices.

Table 6: The sensitivity, specificity, PPV, NPV, and overall accuracy of study variables to predict high-risk groups of oesophageal varices.

Study variable	Cut-off point	Sensitivity	Specificity	PPV*	NPV**	Overall accuracy
Splenic size (cm)	≤ 16.50	65.0%	50.0%	72.2%	41.7%	60.0%
Splenic stiffness (KPa)	≥ 15.125	95.0%	90.0%	95.0%	90.0%	93.3%
Platelet count (cell x 10^3/microliter)	≤ 173.50	80.0%	90.0%	94.1%	69.2%	83.3%

**PPV= Positive predictive value; **NPV= negative predictive value.*

DISCUSSION

The prediction of the risk of EV can be done using simple parameters like splenic size and platelet counts or more invasively by OGD and HVPG. With the feasibility and recent advances in elastography, researchers have investigated the role of the LS and SS in assessing cirrhosis and PHT because anatomically, the portal vein arises by the confluence of the superior mesenteric and splenic veins; therefore, disorders in portal blood flow may lead to spleen congestion, increasing its stiffness [28] . However, these studies have shown variable results[29]. In this study, the mean elasticity values for the spleen did not exhibit a significant gender or age difference which was in agreement with [30,31] .

The mean SS cut-off value that discriminated between healthy and CLD patients in the current study was 12.49 Kpa while 15.125 Kpa was as a cut-off value that predicted high-risk groups of EV, both values were slightly lower than [32] who found mean SS of 15 KPa in normal subjects and 20.2KPa in patients with CLD complicated by EV.

Considering validity in predicting the presence of EV in patients with CLD, the current study revealed that SS was more accurate in than splenic size and platelet count, a finding that was consistent with [33] with higher overall accuracy in our study. According to findings of current study, we belief that SS can serve as a surrogate for the dynamic component of PHT, with resultant improved discrimination between clinically significant PHT and subsequent EV. Moreover, the SS values were significantly increased in patients with high-grade EV, confirming that portal flow is directly affecting SWE values.

Ford et al [34] measured SS in patients with chronic hepatitis C virus–related cirrhosis and concluded that they were more accurate than the other parameters. However, patients with different aetiologies of CLD included in our study have showed no significant difference in mean SS, reflecting that SWE technique may be clinically applicable regardless of the etiology of CLD.

The sensitivity and specificity of SS in detecting high-risk groups of EV in this study were higher than previously found in a systematic review (95% vs 78% and 90% vs 76% respectively) [35]. Therefore, considering the excellent accuracy of SS using SWE in detecting EV with good sensitivity and specificity, we think that our results could have important clinical implication by differentiating between low and high-risk groups of EV in addition to the ability to rule out EV (high NPV) so that avoiding unnecessary endoscopy and subsequently decreasing the burden on patients, because HVPG and OGD are invasive and not accepted easily by patients, with continuous need for non-invasive tests to assess EV[36].

This study is not without limitations. First is the relatively small sample size, however, the results are preliminary and future studies with a larger sample will be more helpful. Results of the current study cannot be generalized on all patients because those with comorbidities, were excluded, hence, conducting similar research on such patients in future is suggested. Interobserver variation was not assessed in this study, although it was not the main aim, dedicated studies to address both interobserver and intra-observe variation may be required.

Limitations

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such patients in future is suggested. Interobserver variation was not assessed in this study, although it was not the main aim, dedicated studies to address both interobserver and intra-observe variation may be required.

CONCLUSIONS

The SS is significantly higher in a patient with CLD compared to healthy volunteers. SS values were not affected by age, gender nor aetiology of CLD. Among patients with CLD, SS values increase with increasing grade of EV. The optimal cut-off value of SS to differentiate normal from diseased liver was 12.5 KPa while the cut-off value to differentiate low-risk from high-risk groups of EV was 15.49 KPa. Compared with splenic size and platelet counts, SS had the highest accuracy in predicting high-risk groups of EV in patients with CLD, so that SWE can be a reliable and noninvasive option for monitoring EV in CLD patients and may consequently reduce the rate of unnecessary endoscopies. A future study addressing SS as an adjuvant with liver stiffness in predicting the presence of EV in CLD seems clinically useful.

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Investigating the Panton-Valentine Leukocidin Gene in Methicillin-Resistant Staphylococcus aureus from Diabetic Foot Infections

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ABSTRACT

Background: Methicillin-resistant *Staphylococcus aureus* is a significant pathogen associated with multidrug resistance among diabetic foot infections. The *Panton-Valentine Leukocidin (pvl)* gene is an MRSA marker frequently present in community-acquired infections; it causes deep skin and soft tissue infections and osteomyelitis. **Aim of the study:** This study aimed to detect the prevalence of the *PVL* gene in MRSA isolates among diabetic foot infections (DFIs). **Patients and methods:** A cross-sectional study conducted from August 2023 to January 2024 investigated MRSA in 150 patients with DFIs at Al-Manathira General Hospital in Al-Najaf, Iraq. *Staphylococcus aureus* isolates were diagnosed using the Vitek® 2 technique. Methicillin-resistant *S. aureus* isolates were detected by a phenotypic method depending on cefoxitin disk diffusion and confirmed by a genotypic method by PCR targeting the *mecA*, and then the *pvl* gene was investigated. The genotypic methods employed particularly primers targeting the *mecA* gene and *pvl* gene using the monoplex PCR technique. **Results:** The findings demonstrated that 95 (93.1%) of the 102 *S. aureus* isolates were resistant to cefoxitin, and according to the findings of the molecular study, only 96 (94.11%) isolates showed the *mecA* gene. Regarding the *pvl* gene, 97 (95.09%) of isolates produced positive findings. **Conclusions:** Diabetic foot infections exhibited a significantly high presence of the *pvl* gene within MRSA strains. These were confirmed using PCR, the gold standard method for detecting the *mecA* gene associated with methicillin resistance.

Keywords: Diabetic foot infections, MRSA, PVL gene, mecA gene, S. aureus.

Article Information

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INTRUDUCTION

Hearing problems are common neurological deficit, and diabetes mellitus is a common cause for these problems. The organ of Corti which is essential for hearing is at the risk of damage by high blood sugar [(1).] Hearing impairment affects the quality of life of diabetic patients, many researchers found hearing impairment in diabetic patients [(2).] Diabetes mellitus can cause sensorineural hearing loss (SNHL) because of neuropathy and microvascular complications of diabetes mellitus. The presence of comorbidities in diabetic patients such as hypertension and atherosclerotic vascular diseases, also can affect hearing [(3).] Some histopathological studies

revealed a neurological and vascular damage in the inner ear of diabetic patients [4]. It is well known now that increased oxidative stress has an important role in the development of long term complications of diabetes mellitus [5]. Diabetes-relatedhearing impairment is of gradual onset but progressive and bilateral sensorineural deficit, which affect higher more than lower frequencies[2]. The present study aims to study the relation of sensorineural hearing with diabetic mellitus in comparison to non-diabetic control.

PATIENTS AND METHODS

A cross-sectional study was conducted from August 2023 to January 2024 on 150 patients with diabetic foot infections (112 males and 38 females) at Al-Manathira General Hospital in Al-Najaf governorate. S. aureus isolates were diagnosed using the Vitek® 2 technique (Biomerieux, France) after bacterial cultures on mannitol salt agar and blood agar were incubated at 37°C for 18–24 hours [10].

Methicillin-resistant S. aureus was detected according to the CLSI 2020, and cefoxitin inhibition zones of 21 mm or less were considered indicative of MRSA[11].

The extraction of DNA was carried out using the Wizard® Genomic DNA Purification Kit (Promega, USA) and then assessed for purity and concentration using a nanodrop spectrophotometer.

The monoplex PCR technique was carried out using a master mix (Promega, USA), and specific primers described by (12, 13) for the macA gene and pvl gene were designed by Macrogen, Korea, as shown in Table 1, under the reaction conditions demonstrated in Table 2.

The Study Design

It is a prospective case-control study.

RESULTS

Out of 150 wound swabs from DFIs, 102 S. aureus isolates constituted 95 (93.1%) phenotypically methicillin-resistant S. aureus, depending on cefoxitin disc diffusion, as demonstrated in Figure 1.

A polymerase chain reaction (PCR) assay was employed to detect the mecA gene, which is a marker for methicillin resistance. Out of 102 S. aureus isolates, 96 (94.11%) detected the presence of the mecA gene, indicating a concerningly high frequency of MRSA, as shown in Figure 2.

Our findings in Figure 3 show a very high prevalence (95.09%) of the pvl gene among the MRSA isolates.

96 (94.11%) of the mecA gene of MRSA isolates harbored a pvl gene associated with the mecA gene, while one MRSA isolate harbored the pvl gene, and the mecA gene was absent, as demonstrated in Figure 4.

Figure 1. Shows Identifying methicillin-resistant S. aureus using cefoxitin disc diffusion technique.

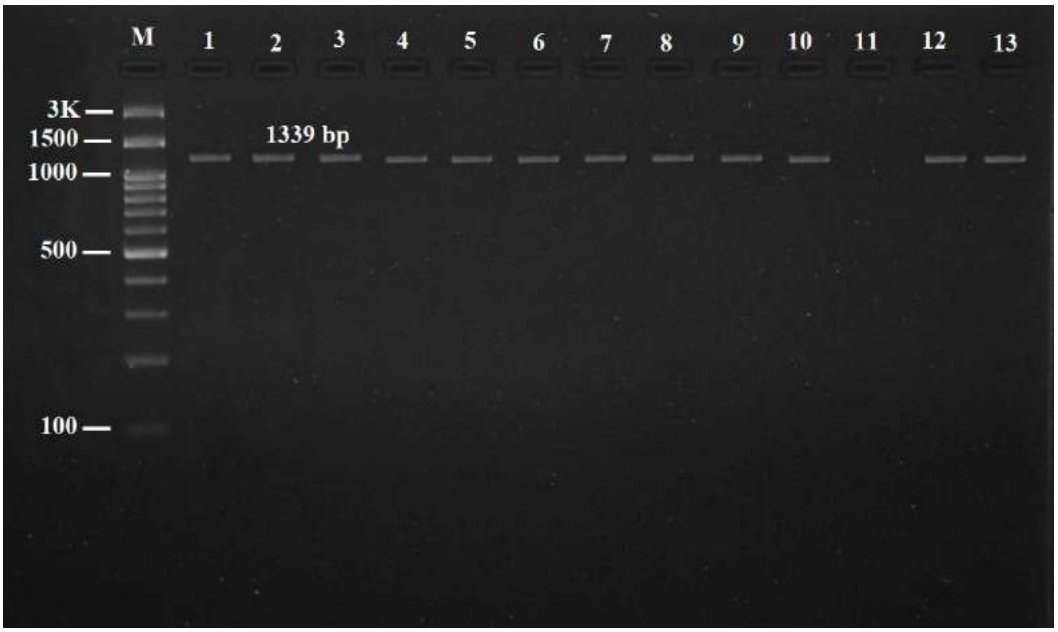

Figure 2. Visualized using agarose gel electrophoresis, PCR products from the mecA gene in MRSA isolates display a consistent band size of 1339 base pairs, with positive results present in lanes 1–10 and 12–13. Interestingly, the isolate in lane 11 lacks this band, indicating a negative mecA gene result. Line M: DNA markers (100–3000 bp) were considered controls.

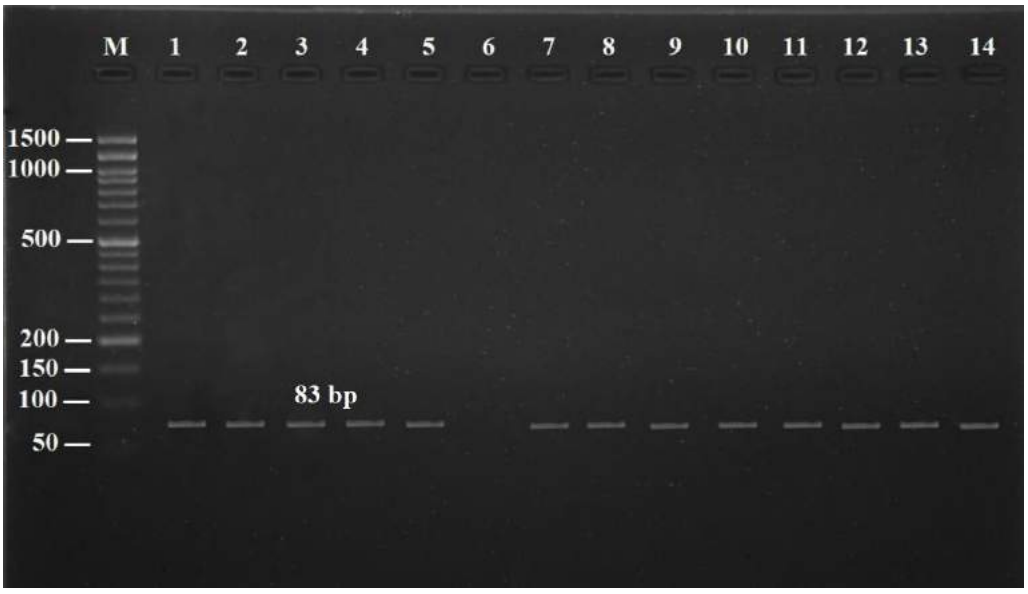

Figure 3. Visualized using agarose gel electrophoresis, PCR products from the PVL gene in MRSA isolates display a consistent band size of 83 base pairs, with positive results present in lanes 1–5 and 7–14. The isolate in lane 6 lacks this band, indicating a negative PVL gene result. Line M: DNA markers (50–1500 bp) were considered controls.

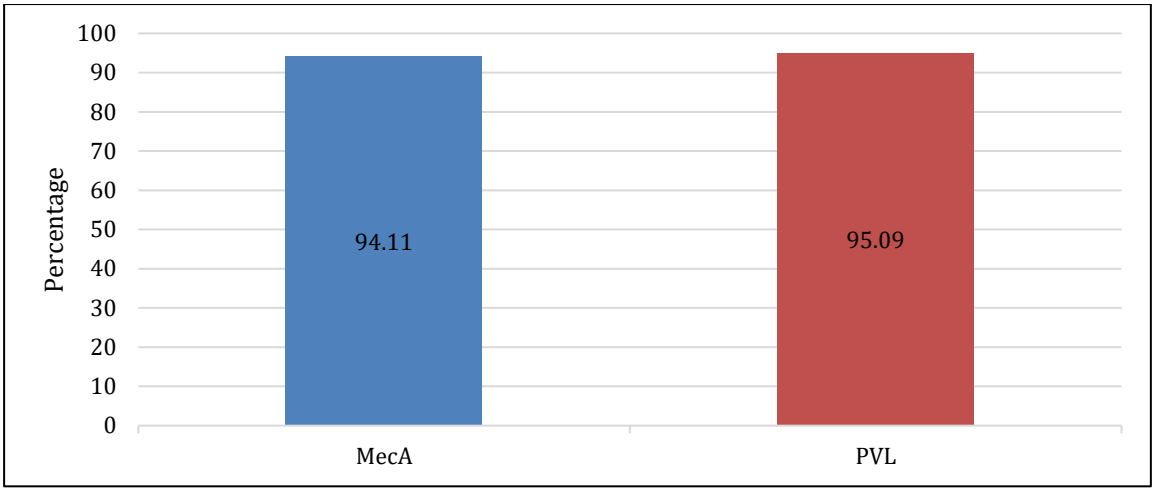

Figure 4. Percentage of mecA gene and PVL gene among MRSA isolates.

DISCUSSION

Staphylococcus aureus is a common infection in hospitals and communities. S. aureus infections, particularly MRSA, are a growing public health concern. The global epidemiology of MRSA infections is evolving rapidly [5]. This study evaluated 150 wound swabs from DFIs, of which 102 S. aureus isolates were identified from diabetic foot infections. This study is consistent with the findings of [14]. The findings indicated that of the 102 isolates of S. aureus, 95 (93.1%) exhibited resistance to the cefoxitin antibiotic. As shown in Figure 1. The results of this study are consistent with previous studies conducted by [2, 15]. The findings presented in this study contradict the findings published by [16, 17].

In the current study, PCR analysis confirmed the presence of the mecA gene in 96 of 102 isolates, highlighting a concerningly high prevalence of MRSA (94.11%) as shown in Figure 2. Our findings detected a mecA gene prevalence of 94.11% in S. aureus isolates, consistent with the high rates reported by (94% and 94.33%, respectively), (17,18). Ahrabi et al. reported a lower prevalence (54.54%), suggesting potential regional or methodological differences[19].

The PVL gene was detected in a remarkable 95.09% of the studied S. aureus isolates, further indicating its prevalence within the community, as shown in Figure 3. Panton-Valentine leukocidin (PVL), a significant virulence determinant of Staphylococcus aureus, is encoded by the LukS-PV and LukF-PV genes [20]. PVL production occurs in diverse S. aureus strains, including both MSSA and MRSA [21]. Our results are consistent with those of [22], who showed a high PVL prevalence in MRSA (100%). However, they differ from the results (26.6%), (49%), and (0%), respectively,[2,23,24].
Our research, as shown in Figure 4, revealed a very high co-occurrence of the pvl and mecA genes in all the S. aureus isolates we examined. This finding indicates that the pvl gene might play a role in increasing both the virulence and antibiotic resistance of S. aureus bacteria.
Panton-valentine leukocidin (PVL), a potent toxin, serves as a significant indicator for community-acquired MRSA, a strain often implicated in skin and soft tissue infections, including abscesses, furuncles, and cellulitis[2, 25]. MRSA isolates may be transmitted from community to hospital or hospital to community due to their high pathogenicity[26].

Evidence gathered from various countries suggests a concerning rise in the prevalence of the Panton-Valentine leukocidin (PVL) gene within methicillin-resistant Staphylococcus aureus (MRSA) isolates[2]. Our study identified a high prevalence of PVL in MRSA strains (75%), consistent with our study conducted by (27). D'Souza et al. found that PVL-positive isolates were prevalent among methicillin-resistant Staphylococcus aureus (MRSA) at a rate of 64%[28]. The prevalence of PVL is lower in various regions of the world, being much lower in areas like France, and the UK (5%, and 4.9%, respectively) [24]. These findings highlight the significant geographical variation in PVL incidence across different communities.

CONCLUSIONS

The current study showed a high prevalence of the pvl gene among MRSA isolates with diabetic foot infections. It serves as a community-associated MRSA marker. The PCR study is effective in detecting pvl and mecA genes.

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Multi-Organ Histopathological Changes in SARS COV2 Infection: A Systematic Review and Meta-analysis

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ABSTRACT

Background: *The World Health Organization has officially acknowledged the emergence of Coronavirus Disease 2019 (COVID-19), attributed to the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus, as a rapidly escalating global public health issue and declared it a pandemic. SARS-CoV-2 infection can lead to varied and multiorgan pathologies, with the most notable impacts occurring in the lungs (characterized by phases of diffuse alveolar damage, microthrombi, and bronchopneumonia), heart (involving lymphocytic myocarditis), kidney (resulting in acute tubular injury), and vasculature (involving microthrombi and deep vein thrombi).* ***Objectives:*** *To summarize, resolve contradiction and provide solid evidence on multiorgan histopathological changes caused by SARS-CoV2 infection.* ***Material and method:*** *Histological data obtained from autopsy and biopsy studies were gathered following the guidelines of the Preferred Reporting Items for Systematic Review (PRISMA). An extensive electronic search was conducted on databases such as PubMed, Science Direct, Scopus, and Google Scholar, covering the period from database inception to March 2022. The collected studies underwent a systematic literature search, and a thorough critical review was performed.* ***Result:*** *After excluding studies that did not meet the eligibility criteria, a total of 58 articles were included in the review. We estimate the histopathological findings of 13 organ. For the pool proportion of exudative, proliferative and fibrotic phase of diffuse alveolar damage of lung is (70.666%, 56.126% and 33.031%) respectively. For liver steotosis is 35.808%. For acute tubular injury of kidney is 74.872%. For adrenal cortical necrosis is 13.113%. For brain gliosis is 13.865%. For heart necrosis is 5.477%. For gastrointestinal tract the pool proportion of inflammatory cells infiltration is 6.171%. For placental infarction is 25.684%. For orchitis is 29.019%. For perivascular inflammation of skin is 35.176%. For lymphocytic depletion of white pulp of spleen is 69.204%. For hemophagiocytosis of lymph node is 7.022%. For bone marrow fibrosis is 8.473%.*

Conclusion: *COVID-19 is characterized as a multiorgan infection closely associated with a hyperinflammatory state, believed to initiate with diffuse alveolar damage and immuno-thrombotic microangiopathy. The extensive activation of the immune system and microvascular damage may contribute to indirect harm to other organs, although the direct impact of the virus on these tissues cannot be ruled out.*

Keywords: Covid 19, SARS-Cov2, Meta-Analysis and Review.

Article Information

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INTRUDUCTION

The global spread of the novel SARS-CoV-2 (severe acute respiratory syndrome coronavirus2) infection has prompted the World Health Organization to declare it a pandemic[1]. This virus easily transmits through droplets, fomites, or contact with the bodily fluids of infected individuals reaching another person's face, mouth, nose, or eyes. SARS-CoV-2 binds to Angiotensin-converting enzyme 2 (ACE2), highly expressed in the respiratory tract, initiating invasion into human cells. This invasion results in severe destruction and inflammation across various organs, potentially leading to fibrosis and affecting vascular supply. Clinical presentations primarily encompass fever, cough, fatigue, and shortness of breath, accompanied by less frequent symptoms such as headache, sore throat, and rhinorrhea. Approximately one-fifth of patients (20%) experience severe manifestations, including respiratory failure, multiorgan failure, and septic shock, necessitating intensive care[2,3,4].

The incubation period of covid19 range between 0–24 days[5]. COVID-19 predominantly affects the lungs and heart. In the lungs, the characteristic injury pattern is diffuse alveolar damage (DAD), representing a nonspecific interstitial pneumonia progressing through distinct phases. This pattern involves hyaline membranes in early stages and fibrosis in later stages, often with interstitial lymphocytic infiltrates featuring predominant CD4-positive T cells. Severe infections may display "acute fibrinous and organizing pneumonia (AFOP)," characterized by intra-alveolar fibrin balls without hyaline membranes. Other common findings include microvascular thrombi and hemorrhage. In the heart, a spectrum from mild interstitial chronic inflammation within the myocardium without necrosis to lymphocytic myocarditis with myonecrosis is observed. Additional organs involved in this disease are the liver, spleen, and kidney. The liver exhibits frequent sinusoidal dilatation with congestion and steatosis. The spleen typically shows lymphocytic depletion of the white pulp, leading to a decrease or absence of lymphoid follicles. Kidney involvement manifests as acute tubular necrosis (ATN), characterized by glomerular capillary thrombi, vacuolization, and dilatation of tubules[6].

MATERIAL AND METHOD

Histological data obtained from autopsy and biopsy studies adhered to Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) standards. A comprehensive electronic search spanning PubMed, Science Direct, Scopus, and Google Scholar, covering the period from the inception of databases from December 1st, 2021 to March 1st, 2022, was conducted, as illustrated in Figure 1.

Inclusion criteria encompassed articles that presented histopathological observations of organs in individuals diagnosed with COVID-19 following postmortem examinations or biopsies.

Exclusive Criteria: Articles that did not mention about histopathology of organs, unavailable full text studies on animal models and case report and reviews.

Statistical analysis involved the consolidation of data for each histopathological observation related to SARS-CoV-2 infection in different organs. Prevalence of event rates, presented as proportions with a 95% confidence interval, was calculated. Pooled proportions were determined through random-effects models, and heterogeneity was assessed using the Q statistic and I2. Using I-Squared test. Random effect model used when there is significant heterogeneity. In general, random effect model adopted to overcome heterogeneity between studies.

The lung: A total of 352 from 487 (72.2%) patients demonstrated exudative phase of diffuse alveolar damage. Upon meta-analysis, the data indicated a pooled proportion of exudative phase of 70.666%. 95% confidence interval (CI): 59.538 to 80.666 with notable heterogeneity observed among the studies (P = < 0.0001; I2 =86.10%) as showed in table 1 and figure 2.

A total of 205 from 487 (42%) patients demonstrated proliferative phase of diffuse alveolar damage. Upon meta-analysis, the data indicated a pooled proportion of proliferative phase of 56.126%. 95% confidence interval (CI): 37.322 to 74.059 with notable heterogeneity observed among the studies (P =< 0.0001; I2 =94.97%) as showed in table 2 and figure 3.

A total of 161 from 487 (33%) patients demonstrated fibrotic phase of diffuse alveolar damage. Upon meta-analysis, the data indicated a pooled proportion of fibrotic phase of 33.031%. 95% confidence interval (CI): 19.972 to 47.586 with notable heterogeneity observed among the studies (P =< 0.0001; I2 =91.67%) as showed in table 3 and figure 4.

The liver: A total of 168 from 516 (32.5%) patients demonstrate steotosis of the liver. Upon meta-analysis, the data indicated a pooled proportion of steotosis of 35.808%. 95% confidence interval (CI): 20.478 to 52.798 with notable heterogeneity observed among the studies (P =< 0.0001; I2 =92.78 %).

The Kidney: A total of 217 from 409 (53%) patients demonstrate acute tubular injury (ATI). Upon meta-analysis, the data indicated a pooled proportion of ATI of 74.872 %. 95% confidence interval (CI): 57.095 to 89.133 with notable heterogeneity observed among the studies (P =< 0.0001; I2 = 92.34%).

Adrenal gland: A total of 8 from 140 (5.7%) patients demonstrate cortical necrosis of the adrenal gland. Upon meta-analysis, the data indicated a pooled proportion of cortical necrosis of 13.113 %. 95% confidence interval (CI): 0.805 to 36.741 with notable heterogeneity observed among the studies (P =< 0.0001; I2 =89.40 %).

The brain: A total of 17 from 177 (9.6%) patients demonstrates gliosis. Upon meta-analysis, the data indicated a pooled proportion of gliosis of 13.865 %. 95% confidence interval (CI): 1.071 to 37.535 with notable heterogeneity observed among the studies (P < 0.0001; I2 =92.36%)

The heart: A total of 17 from 415 (4%) patients demonstrate necrosis in the heart. Upon meta-analysis, the data indicated a pooled proportion of necrosis of 5.477 %. 95% confidence interval (CI): 2.341 to 9.825 with notable heterogeneity observed among the studies (P = 0.0035; I2 =53.86 %) as showed in table 4 and figure 5.

Gastrointestinal tract (GIT): A total of 7 from 146 (4.7%) patients demonstrate inflammatory cells infiltration of GIT. Upon meta-analysis, the data indicated a pooled proportion of inflammatory cells infiltration of 6.171 %. 95% confidence interval (CI): 0.00698 to 22.460 with notable heterogeneity observed among the studies (P < 0.0001; I2 =85.24 %).

The placenta: A total of 16 from 109(14.6%) patients demonstrates placental infarction. Upon meta-analysis, the data indicated a pooled proportion of placental infarction of25.684 %. 95% confidence interval (CI): 0.346 to 71.178 with notable heterogeneity observed among the studies (P < 0.0001; I2 =94.95 %).

The testis: A total of 7 from 23(30.4%) patients demonstrate orchitis. Upon meta-analysis, the data indicated a pooled proportion of orchitis of29.019 %. 95% confidence interval (CI): 0.588 to 76.189 with notable heterogeneity observed among the studies (P = 0.0018; I2 =84.12 %).

Skin: A total of 41 from 99(41.4%) patients demonstrate perivascular inflammation in the skin. Upon meta-analysis, the data indicated a pooled proportion of perivascular inflammation of 35.176 %. 95% confidence interval (CI): 2.182 to 81.139 with notable heterogeneity observed among the studies (P < 0.0001; I2 =95.88 %).

Spleen: A total of 124 from 168(73.8%) patients demonstrate lymphocytic depletion of white pulp of the spleen. Upon meta-analysis, the data indicated a pooled proportion of lymphocytic depletion of white pulp of 69.204 %. 95 % confidence interval (CI): 30.300 to 96.482 with notable heterogeneity observed among the studies (P < 0.0001; I2 =95.61 %).

Lymph node (LN): A total of 4 from 94(4.2%) patients demonstrate absence of hemophagiocytosis in the LN. Upon meta-analysis, the data indicated a pooled proportion of hemophagiocytosis of7.022 %. 95 % confidence interval (CI): 0.121 to 23.118 with notable heterogeneity observed among the studies (P= 0.0019; I2 =73.71 %).

Bone marrow: A total of 12 from 79(15.1%) patients demonstrate fibrosis of bone marrow. Upon meta-analysis, the data indicated a pooled proportion of the fibrosis of 8.473 %. 95 % confidence interval (CI): 0.00105 to 31.320 with notable heterogeneity observed among the studies (P < 0.0001; I2 = 87.01%).

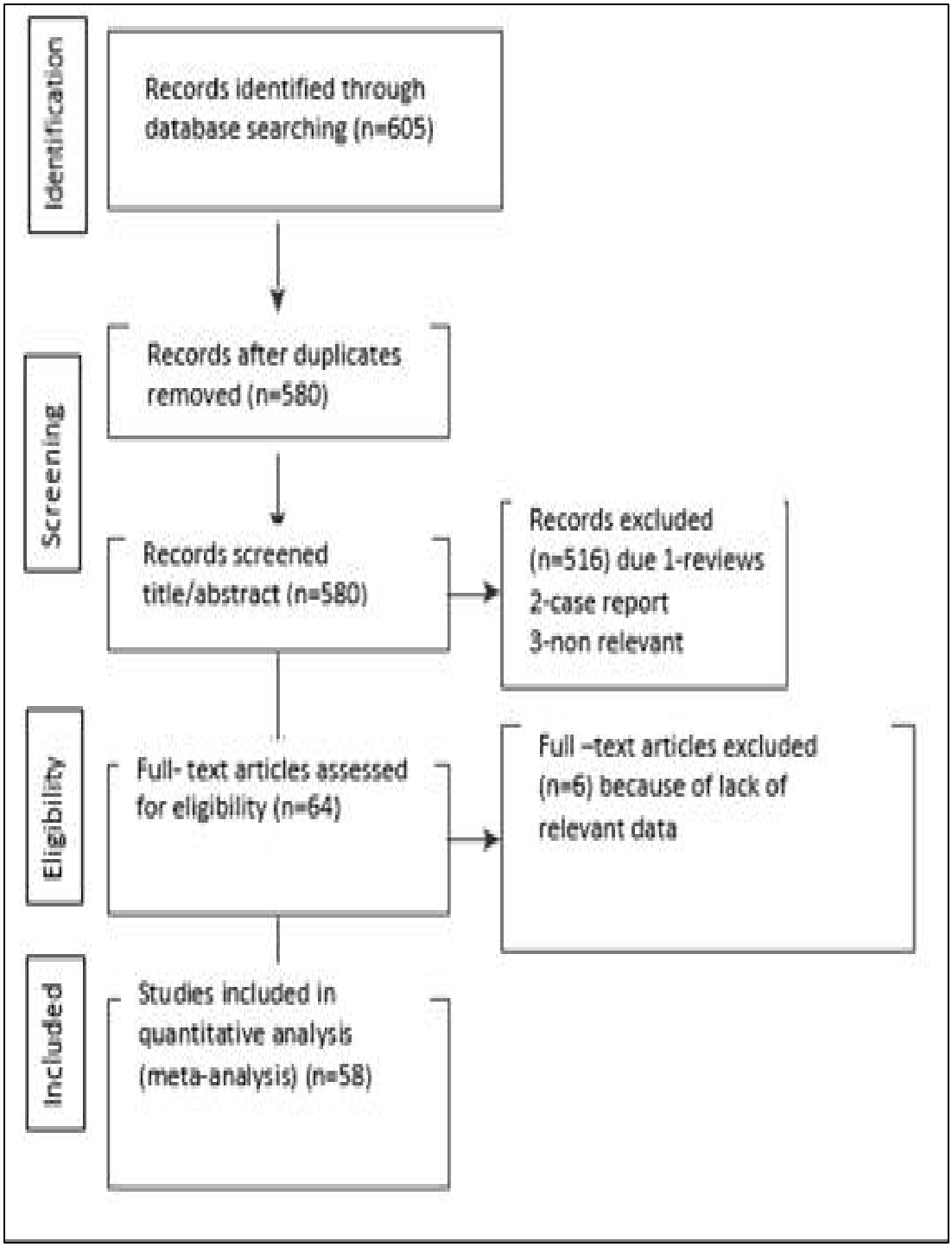


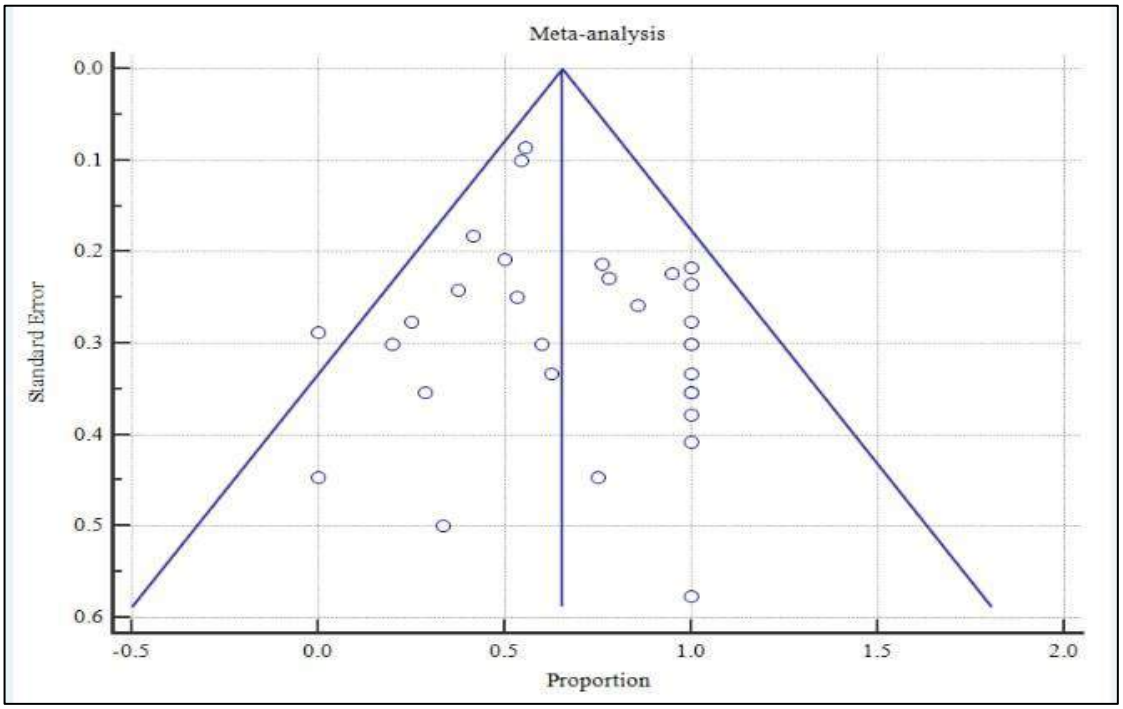
Figure 1. The PRISMA flow diagram, outlining the process of literature search and selection, adheres to the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-analyses) guidelines.

Table 1. Show how the Forest plots of exudative phase of DAD of coronavirus patients.

Study	Standard deviation	Proportion (%)	95% CI
DUAN et al., 2020	6	100.000	54.074 to 100.000
Núñez-Torrón et al., 2020	16	37.500	15.198 to 64.565
Bösmüller et al., 2020	4	75.000	19.412 to 99.369
Falleni et al., 2021	7	100.000	59.038 to 100.000
Pessolani et al., 2020	4	0.000	0.000 to 60.236
Rapkiewicz et al., 2020	7	100.000	59.038 to 100.000
Schurink et al., 2020	18	77.778	52.363 to 93.591
Wang et al., 2021	2	100.000	15.811 to 100.000
Recalde-Zamacona et al., 2020	10	100.000	69.150 to 100.000
Haberecker et al., 2021	15	53.333	26.586 to 78.733
Wong et al., 2021	8	100.000	63.058 to 100.000
Hirschbu¨ hl et al., 2021	19	94.737	73.972 to 99.867
Remmelink et al., 2020	17	100.000	80.494 to 100.000
Bryce et al., 2021	99	54.545	44.225 to 64.586
Diaz et al., 2021	11	0.000	0.000 to 28.491
Williams et al., 2021	5	100.000	47.818 to 100.000
Magro et al., 2020	12	100.000	73.535 to 100.000
Bradley et al., 2020	14	85.714	57.187 to 98.221
Merdji et al.,2021	22	50.000	28.221 to 71.779
Flikweert et al., 2020	7	28.571	3.669 to 70.958
Felix et al., 2021	12	25.000	5.486 to 57.186
Buja et al., 2021	3	33.333	0.840 to 90.570
Himware et al., 2021	29	41.379	23.524 to 61.064
Fox et al., 2020	10	20.000	2.521 to 55.610
Hanley et al., 2020	10	60.000	26.238 to 87.845
Duarte-Neto et al., 2020	10	100.000	69.150 to 100.000
Menter et al., 2020	21	76.190	52.834 to 91.782
Roden et al.,2020	8	62.500	24.486 to 91.477
Hooper et al., 2021	135	55.556	46.761 to 64.101
Prieto-Pérez et al., 2020	20	100.000	83.157 to 100.000
Total (fixed effects)	561	65.272	61.280 to 69.111
Total (random effects)	561	70.666	59.538 to 80.666

Test for heterogeneity

Q	208.6007
DF	29
Significance level	P < 0.0001
I^2 (inconsistency)	86.10%
95% CI for I^2	81.23 to 89.70



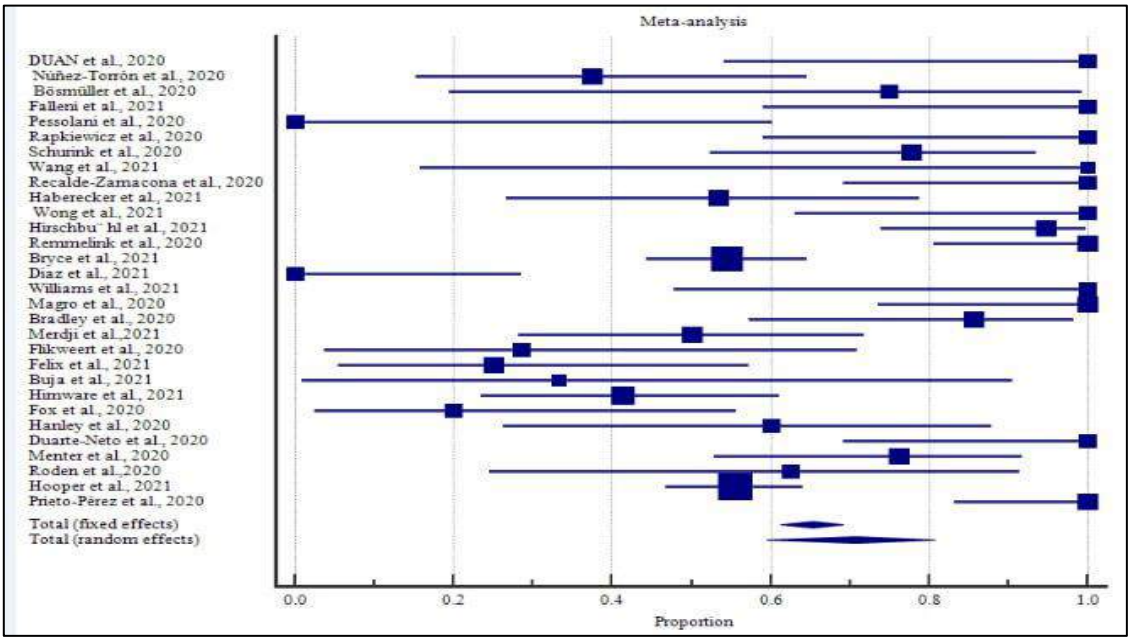


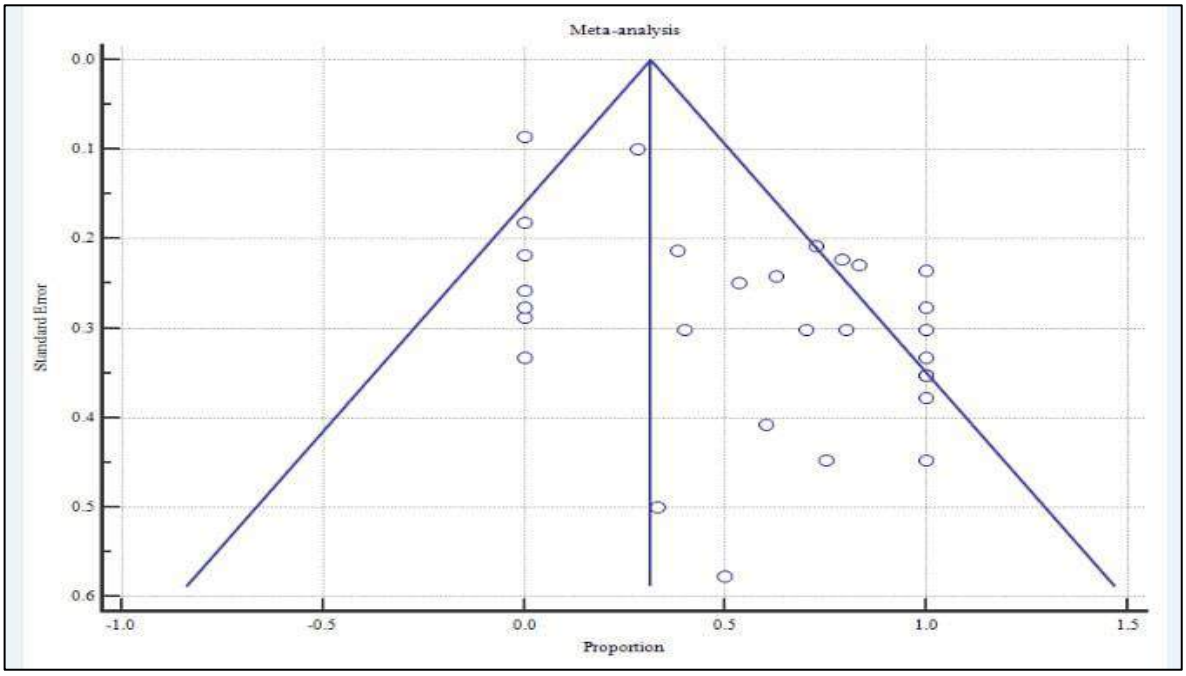
Figure 2. Show the Forest and funnel plots of exudative phase of DAD of coronavirus patients.

Table 2. Show the Forest plots of proliferative phase of DAD of coronavirus patients.

Study	Standard deviation	Proportion (%)	95% CI
DUAN et al., 2020	6	100.000	54.074 to 100.000
Núñez-Torrón et al., 2020	16	62.500	35.435 to 84.802
Bösmüller et al., 2020	4	100.000	39.764 to 100.000
Falleni et al., 2021	7	100.000	59.038 to 100.000
Pessolani et al., 2020	4	75.000	19.412 to 99.369
Rapkiewicz et al., 2020	7	100.000	59.038 to 100.000
Schurink et al., 2020	18	83.333	58.582 to 96.421
Wang et al., 2021	2	50.000	1.258 to 98.742
Recalde-Zamacona et al., 2020	10	100.000	69.150 to 100.000
Haberecker et al., 2021	15	53.333	26.586 to 78.733
Wong et al., 2021	8	100.000	63.058 to 100.000
Hirschbu¨ hl et al., 2021	19	78.947	54.435 to 93.948
Remmelink et al., 2020	17	100.000	80.494 to 100.000
Bryce et al., 2021	99	28.283	19.686 to 38.222
Diaz et al., 2021	11	0.000	0.000 to 28.491
Williams et al., 2021	5	60.000	14.663 to 94.726
Magro et al., 2020	12	100.000	73.535 to 100.000
Bradley et al., 2020	14	0.000	0.000 to 23.164
Merdji et al.,2021	22	72.727	49.778 to 89.271
Flikweert et al., 2020	7	100.000	59.038 to 100.000
Felix et al., 2021	12	0.000	0.000 to 26.465
Buja et al., 2021	3	33.333	0.840 to 90.570
Himware et al., 2021	29	0.000	0.000 to 11.944
Fox et al., 2020	10	70.000	34.755 to 93.326
Hanley et al., 2020	10	40.000	12.155 to 73.762
Duarte-Neto et al., 2020	10	80.000	44.390 to 97.479
Menter et al., 2020	21	38.095	18.107 to 61.565
Roden et al.,2020	8	0.000	0.000 to 36.942
Hooper et al., 2021	135	0.000	0.000 to 2.696
Prieto-Pérez et al., 2020	20	0.000	0.000 to 16.843
Total (fixed effects)	561	31.421	27.695 to 35.334
Total (random effects)	561	56.126	37.322 to 74.059

Test for heterogeneity

Q	576.0321
DF	29
Significance level	P < 0.0001
I^2 (inconsistency)	94.97%
95% CI for I^2	93.71 to 95.97



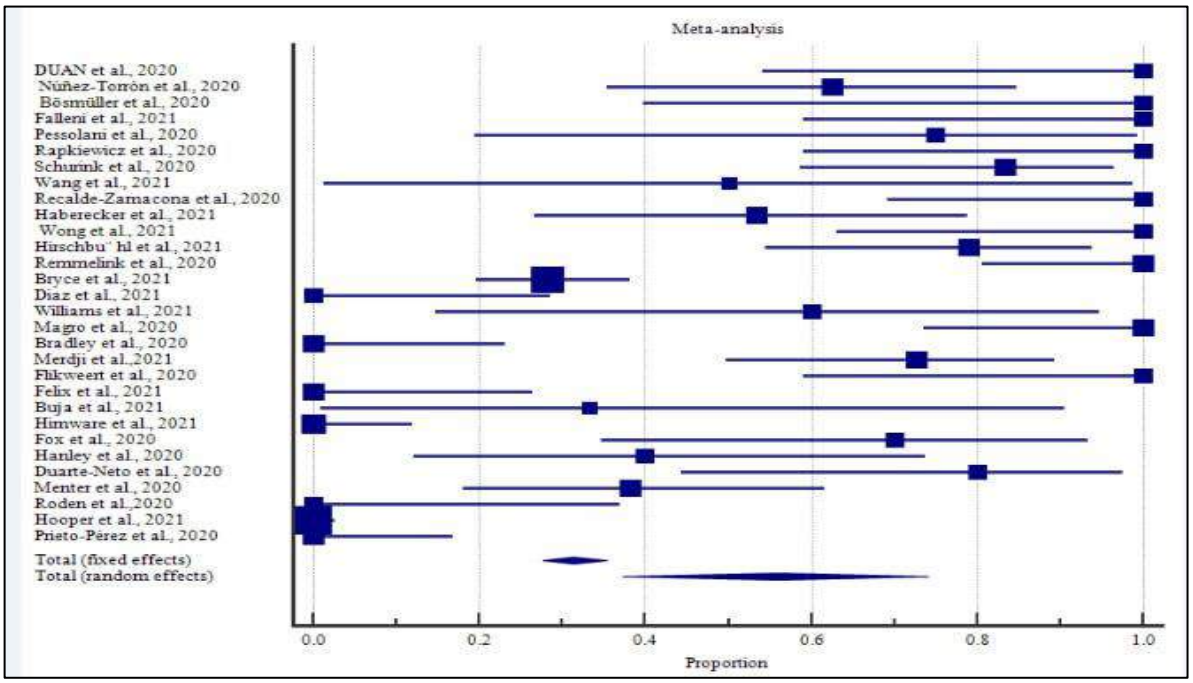


Figure 3. Show the Forest and funnel plots of proliferative phase of DAD of coronavirus patients.

Table 3. Show the Forest plots of fibrotic phase of DAD of coronavirus patients.

Study	Standard deviation	Proportion (%)	95% CI
DUAN et al., 2020	6	0.000	0.000 to 45.926
Núñez-Torrón et al., 2020	16	25.000	7.266 to 52.377
Bösmüller et al., 2020	4	50.000	6.759 to 93.241
Falleni et al., 2021	7	100.000	59.038 to 100.000
Pessolani et al., 2020	4	50.000	6.759 to 93.241
Rapkiewicz et al., 2020	7	0.000	0.000 to 40.962
Schurink et al., 2020	18	22.222	6.409 to 47.637
Wang et al., 2021	2	0.000	0.000 to 84.189
Recalde-Zamacona et al., 2020	10	100.000	69.150 to 100.000
Haberecker et al., 2021	15	53.333	26.586 to 78.733
Wong et al., 2021	8	100.000	63.058 to 100.000
Hirschbu¨ hl et al., 2021	19	5.263	0.133 to 26.028
Remmelink et al., 2020	17	58.824	32.925 to 81.556
Bryce et al., 2021	99	0.000	0.000 to 3.658
Díaz et al., 2021	11	27.273	6.022 to 60.974
Williams et al., 2021	5	20.000	0.505 to 71.642
Magro et al., 2020	12	100.000	73.535 to 100.000
Bradley et al., 2020	14	78.571	49.202 to 95.342
Merdji et al.,2021	22	45.455	24.386 to 67.790
Flikweert et al., 2020	7	14.286	0.361 to 57.872
Felix et al., 2021	12	0.000	0.000 to 26.465
Buja et al., 2021	3	33.333	0.840 to 90.570
Himware et al., 2021	29	0.000	0.000 to 11.944
Fox et al., 2020	10	10.000	0.253 to 44.502
Hanley et al., 2020	10	30.000	6.674 to 65.245
Duarte-Neto et al., 2020	10	0.000	0.000 to 30.850
Menter et al., 2020	21	0.000	0.000 to 16.110
Roden et al.,2020	8	12.500	0.316 to 52.651
Hooper et al., 2021	135	34.815	26.825 to 43.487
Prieto-Pérez et al., 2020	20	70.000	45.721 to 88.107
Total (fixed effects)	561	24.546	21.126 to 28.222
Total (random effects)	561	33.031	19.972 to 47.586

Test for heterogeneity

Q	348.1990
DF	29
Significance level	P < 0.0001
I^2 (inconsistency)	91.67%
95% CI for I^2	89.21 to 93.57

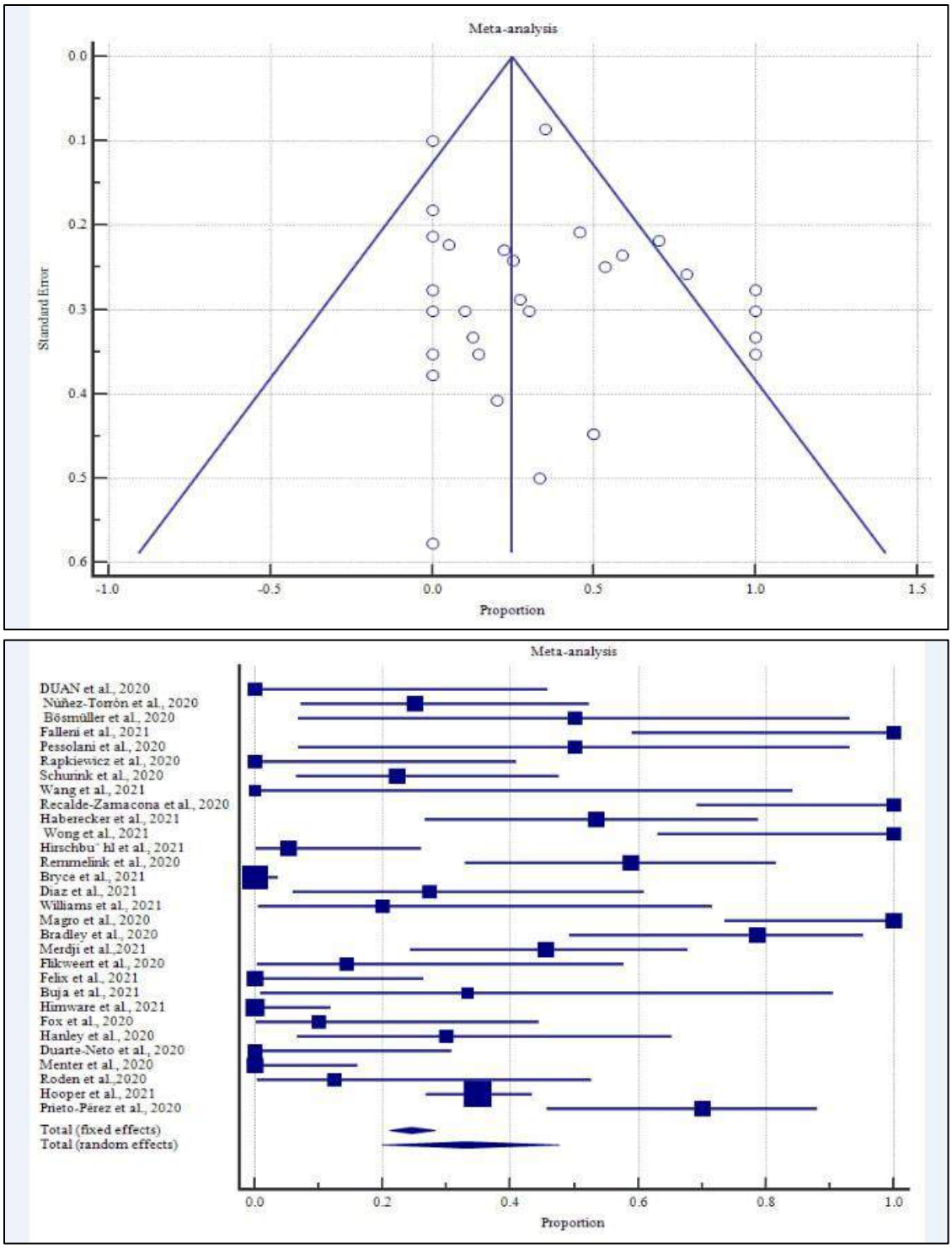


Figure 4 Show the Forest and funnel plots of fibrotic phase of DAD of coronavirus patients.

Table 4. Show the Forest plots of necrosis of the heart of coronavirus patients.

Study	Standard deviation	Proportion (%)	95% CI
Pessolani et al., 2020	3	33.333	0.840 to 90.570
Rapkiewicz et al., 2020	7	42.857	9.899 to 81.595
Schurink et al., 2020	21	0.000	0.000 to 16.110
Nikkhoo et al., 2021	25	0.000	0.000 to 13.719
Wang et al., 2021	1	100.000	2.500 to 100.000
Recalde-Zamacona et al., 2020	10	0.000	0.000 to 30.850
Haberecker et al., 2021	15	0.000	0.000 to 21.802
Wong et al., 2021	5	0.000	0.000 to 52.182
Remmelink et al., 2020	17	11.765	1.458 to 36.441
Bryce et al., 2021	97	0.000	0.000 to 3.732
Magro et al., 2020	12	0.000	0.000 to 26.465
Bradley et al., 2020	14	7.143	0.181 to 33.868
Buja et al., 2021	3	0.000	0.000 to 70.760
Fox et al., 2020	10	0.000	0.000 to 30.850
Hanley et al., 2020	9	11.111	0.281 to 48.250
Duarte-Neto et al., 2020	10	0.000	0.000 to 30.850
Menter et al., 2020	21	4.762	0.120 to 23.816
Hooper et al., 2021	135	5.185	2.110 to 10.392
Total (fixed effects)	415	3.834	2.233 to 6.100
Total (random effects)	415	5.477	2.341 to 9.825

Test for heterogeneity

Q	36.8426
DF	17
Significance level	P = 0.0035
I^2 (inconsistency)	53.86%
95% CI for I^2	21.30 to 72.95

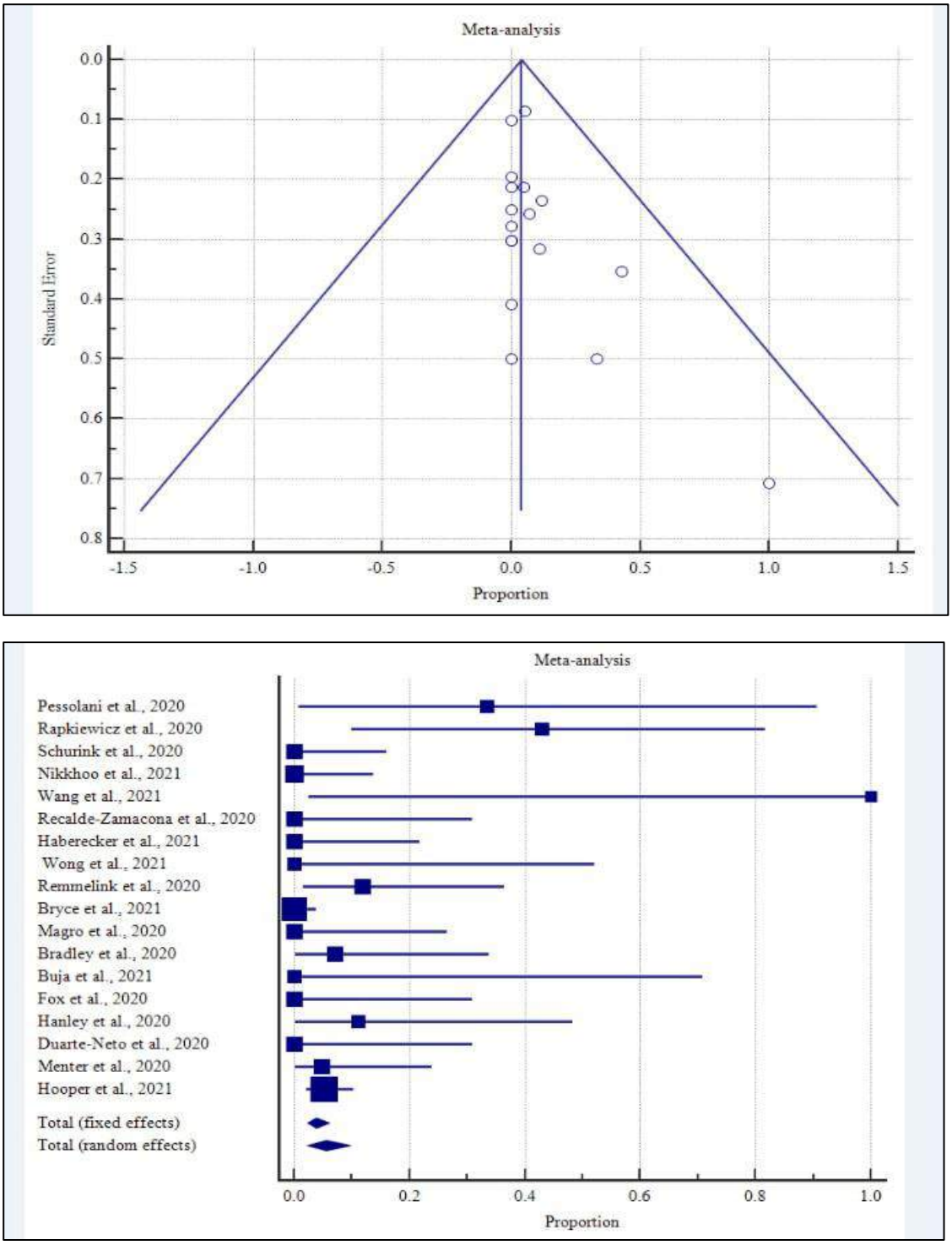


Figure 5. Show the Forest and funnel plots of necrosis of the heart coronavirus patients.

DISCUSSION

In our investigation, we presented key observations pertaining to COVID-19 tissues. We examined studies that documented microscopic (histopathological) data obtained from various organs through both biopsies and autopsies. Post-mortem evidence offers crucial information that contributes to an enhanced comprehension of the pathophysiology of COVID-19 infection. This understanding aids clinicians in identifying optimal and effective treatments to mitigate mortality. Autopsy series reveal the involvement of multiple systems in COVID-19, with respiratory symptoms and findings predominantly observed.

Our study is the first meta-analysis study including histopathological changes of 13 organs with Covid19 (lung, liver, kidney, adrenal gland, brain, heart, GIT, placenta, testis, skin, spleen, lymph node and bone marrow).

Lung histopathological findings in 561 patients show DAD (exudative phase, proliferative phase and fibrotic phase) which agreed with systemic review of (Hariri et al.) [7], (Pandey et al.)[8] and (Pannone et al.)[9]. The pool proportion of exudative phase of DAD is 70.666%, proliferative phase is 56.126%, fibrotic phase is 33.031% , there is only single meta-analysis that shows the proportion of DAD without dividing it in to three phases[10].

Liver histopathological findings in 516 patients show steotosis which agreed with systemic review of (Idalsoaga et al.)[11]

The pool proportion of steotosis is 35.808%, which disagree with (Díaz et al.)[12] which show it 55.1%[13].

Kidney histopathological findings in 409 patients show acute tubular injury which is agreed with systemic review of (Jeyalan et al.)[13].

The pool proportion of ATI is 74.872% which disagree with (Shao et al., Yang et al., Silver et al., and Oliveira et al.) which show (0.10%,0.123%,0.28%,0.12%) respectively [14] [15] [16] [17].

Adrenal histopathological findings in 140 patients show cortical necrosis which agree with the systemic review of (Kanczkowski et al.)[18].

The pool proportion of cortical necrosis is 13.113%, there is no data to compare with it

Brain histopathological findings in 177 patients show gliosis which agree with systemic review of (Pajo et al.)[19].

The pool proportion of gliosis is 13.865%, there is no data to compare with it.

Heart histopathological findings in 415 patients show necrosis which are agree with systemic review of (Almamlouk et al.)[20], (Maiese et al.)[21] and (Roshdy et al.)[22].

The pool proportion of necrosis is 5.477% there is no data to compare with it.

GIT histopathological findings in 146 patients show inflammatory cells infiltration which agree with a systemic review of (Singh et al.)[23] and, (Deshmukh et al.) [24].

The pool proportion of inflammatory cells infiltration is 6.171%, there is no data to compare with it.

Placental histopathological findings in 109 patients show placental infarction which agree with systemic review of (Gesaka et al.)[25], (Sharps et al.)[26], (Almohammadi.)[27] and (Ghazi et al.)[28].

The pool proportion of placental infarction is 25.684%, there is no data to compare with it.

Testicular histopathological findinds in 23 patients show orchitis which agree with systemic review of (Kloping et al.)[29] and (Sengupta et al.)[30].

The pool proportion of orchitis is 29.019%, there is no data to compare with it.

Skin histopathological findings in 99 patients show perivascular inflammation which agree with systemic review of (Nobari et al.)[31] and (Rongioletti et al.)[32].

The pool proportion of perivascular inflammation is 35.176%, there is no data to compare with it.

Splenic histopathological findings in 168 patients show lymphocytic depletion of white pulp which agree with systemic review of (Hammoud et al.)[33], (Malik et al.)[34] and (Octavius et al.)[35].

The pool proportion of lymphocytic depletion of white pulp is 69.204%, there is no data to compare with it.

Lymph node histopathological findings in 94 patients show hemophagiocytosis which agree with systemic review of (Vasquez-Bonilla et al.)[36], and (Octavius et al.)[35].

The pool proportion of hemophagiocytosis is 7.022%, there is no data to compare with it.

Bone marrow histopathological findings in 79 patients show fibrosis which disagree with systemic review of (Octavius et al.)[35], (Vasquez-Bonilla et al.)[36] and (Menezes et al.)[37]

The pool proportion of fibrosis is 8.473%, there is no data to compare with it.

CONCLUSION

COVID-19, characterized by a myriad of symptoms, is increasingly recognized as a systemic disease. Although it primarily affects the respiratory system, evidence of SARS-CoV-2 has been found in various organs, accompanied by organ damage.

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Estimate the Prevalence of Depression in Type 2 Diabetes Patients

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ABSTRACT

Background: *Diabetes is a chronic disease characterized by high blood sugar levels, resulting from insulin resistance and the destruction of pancreatic beta cells. This disease is associated with several serious complications that are sometimes life-threatening to patients. One of these complications is that it can depression accompanies patients with type 2 diabetes, as it is a Major Depressive Disorder (MDD), and the most important symptoms are social isolation, sleep disturbances, lack of appetite, loss of desire, and passion for daily activities. Opinions differed about the origin of the emergence of this psychological disorder in terms of the involvement of multiple factors, including internal, and some of them are external to causing this disorder. In this study, we will shed light on one of the complications associated with diabetes, which is depression.*

Method and Results: *This cross-sectional study included the selection of 128 individuals diagnosed with type 2 diabetes from the Diabetes and Endocrinology Center located in Najaf, Iraq from August 2023 to October 2023. The study included 43 men and 85 women. The ages of the participants ranged from 30 to 75 years. Patients were randomly selected during their visit to the center. Results: 31(24.2%) individuals did not manifest any symptoms of depression 75.78% suffered from depression, most of the depressed people were women, and the difference between the sexes was statistically significant p-value (0. 002).*

Conclusion; *Our study highlights a striking connection between type 2 diabetes and depression, with a significantly higher incidence of depression in diabetic patients. Additionally, the fact that females are more likely to suffer from depression compared to males also calls for further research into other factors that could potentially exacerbate mental health issues in type 2 diabetes women.*

Keywords: Diabetes Mellitus, Insulin Resistance, Depression.

Article Information

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INTRUDUCTION

Diabetes is defined, according to the World Health Organization, as high levels of blood glucose resulting in long-term harm to the heart, blood vessels, eyes, kidneys, and nerves.

More than 90% of instances of diabetes mellitus are classified as type 2 diabetes mellitus (T2DM), which is a medical disorder insufficient release of insulin by the β-cells in the pancreatic islets, together with the presence of tissue insulin resistance (IR) and inadequate insulin secretion. The compensatory insulin secretory response is insufficient[(1,2,3)]. T2DM was initially characterized as a constituent of metabolic syndrome in the year 1988. Previously referred to as non-insulin-dependent

DM, is the prevailing manifestation of DM. T2DM arises due to the interplay of many risk factors, including genetic predisposition, environmental influences, and behavioral variables[4].

The prognosis for patients with both depression and T2DM is worse than for patients with either illness alone. Comorbid depression and T2DM are associated with a higher risk of complications and death[5]. These Two disorders are associated, with depression serving as a risk factor for diabetes and diabetes serving as a risk factor for depression in the future[6]. There are substantial health and financial consequences associated with both MDD and T2DM, and estimates show increased expenses in both high- and low-income nations[7]. More commonly than would be predicted by chance, people with T2DM and depression share a common etiology, which is generally thought to be either shared genetic or environmental factors**.** Numerous population-based studies have conclusively demonstrated that individuals diagnosed with type 2 diabetes exhibit higher rates of both depression prevalence and incidence when compared to individuals without diabetes[8]**.** Diabetes and depressive disorders are connected, both of which are quite common worldwide and contribute significantly to the global disease burden[9,10].

Major depressive disorder (MDD) is a psychiatric condition defined by a profound feeling of melancholy, encompassing a range of symptoms influenced by factors such as biochemical, environmental, genetic, and other related factors .Various psychological issues can impede an individual's capacity to engage in productive work. Problems in sleeping, studying, eating, and deriving pleasure from previously enjoyable activities [11, 12, 13].

MDD is influenced by several factors, and there exists a diversity of perspectives on the nomenclature and categorization of these causal elements. Depression can be attributed to two distinct categories of variables: endogenous factors, which involve internal inflammatory reactions or abnormalities in nerve transmission, and external factors, encompassing stressors related to daily life, loss, and related circumstances[13]. The etiology and pathophysiology of MDD are associated with a range of factors, such as psychological stress, immunological activation, changes in endogenous opioids, and genetic predisposition[14].

Study design

This is a cross-sectional study that included the selection of 128 individuals diagnosed with type 2 diabetes from the Diabetes and Endocrinology Center located in Najaf, Iraq from August 2023 to October 2023.

METHODS

In this study, informed consent was obtained directly from the patients. In addition, participants underwent depression screening using the Hamilton Depression Scale. This scale consists of multiple-choice questions, with each question having a specific score. At the end of the test, each patient's final score was calculated to determine if they suffer from depression and the severity of their depression, and a comprehensive interview was conducted with each patient. The study included 43 men and 85 women. The ages of the participants ranged from 30 to 75 years. Patients were randomly selected during their visit to the center.

Inclusion criteria: Patients with type 2 diabetes were diagnosed by performing basic tests to diagnose diabetes, such as fasting blood sugar and HbA1C, and confirming the diagnosis by the center's specialist.

Exclusion criteria: Type 1 diabetes patients**,** Pregnant women with gestational diabetes**,** and pre-diabetic patients**.**

RESULTS

The Hamilton Depression Rating Scale, a standardized instrument for assessing the severity of depression, was used to assess the depressive symptoms of 128 study participants. the study found a high prevalence of depression among patients with diabetes mellitus (DM), with over three-quarters (75.78%) experiencing symptoms,32 (33%) was detected to have mild depression, 33(34.02%) moderate, 17(17.52%) sever, and 15(15.46%) very severe depression. In contrast, a smaller proportion 31 (24.2%) did not report any depression.

Tab (2) below illustrates some of the main characteristics of the DM patients with and without depression, DM patients with depression had 30(30.9%) individuals under 50 years old and 67(69.1%) individuals <50 years and older, while the DM patients without depression had 9(29.0%) individuals under 50 years old and 22(71.0%) individuals <50 years and more, the p-value was (0.516) indicating no statistically significant difference between the two groups. Similarly, the mean age of patients with and without depression did not differ significantly.

The mean age for those without depression was 53.70±9.93years, and for those with depression, it was 53.64±8.90years, with a p-value of (0.975). Notably, women were more likely to be depressed than men, and this difference was statistically significant (p-value=0.002).DM without depression 31 patients, 18(58.1%) were male and 13(41.9%) were female, DM with depression97 patients, male 25(28.5%), female 72(74.2%), There was no statistically significant difference in diabetes duration between the two groups.

The DM depressive group included 63 (64.9%) patients who had diabetes for less than 10 years, 26 (26.8%) patients who had diabetes for 10-15 years and 8(8.2%) patients with diabetes for 15 years or more. The non-depressive group had 14(45.2%) patients with diabetes for less than 10 years, 14(45.2%) patients with diabetes for 10-15 years, and 3(9.7%) patients with diabetes for 15 years or more, the p-value was (0.128). The marital status of the participants did not show any significant difference between the two groups.

The depressive group had 29(93.5%) married patients and 2(6.5%) widows, the non-depressive group had 95(97.9%) married patients and 2(2.1%) widows, the p-value was (0.247). Our study revealed no significant association between educational level and depression among the participants. The non-depressive group had 21(67.7%) illiterate patients, 1(3.2%) patient who had completed primary school graduate, 4(12.9%) patients who had completed secondary school graduate, and 5(16.1%) patients who had higher education graduates. The depressive group had 67 (75.3%) Illiterate patients, no patients who had completed primary school, 17(19.1%) patients who had completed secondary school, and 5(5.6%) patients who had completed higher education, the p-value was (0.086).

Outlines the mean routine screenings for type 2 diabetes. Diabetic patients without depression had a mean FBS of (222.28±128.93 mg\dl), while those with depression had a mean FBS of (211.25±102.28 mg\dl) with a p-value (0.687). The mean HbA1c of non-depressive patients was (8.52±3.10 mmol\dl), whereas those with depression had a mean of HbA1c (8.83±3.13 mmol\dl) with a p-value (0.741). However, the difference in these values between the two groups was not statistically significant.

This study found no significant link between a family history of diabetes and the risk of developing the disease. seven (22.6%) of the non-depressive group's individuals had a positive genetic component, whereas 24 (77.4%) had a negative one, of the participants in the depressed group, 22 (22.7%) had a positive genetic component and 75 (77.3%) had a negative genetic factor. The p-value found was (0.991). Additionally, there was no significant

difference in the two groups' adherence to treatment. Of the individuals in the non-depressive group, 28 (90.3%) took their medication regularly, while 3 (9.7%) did not. Nine participants (9.3%) in the depressive group did not take their medication as prescribed, whereas 88 participants (90.7%) did. The obtained p-value was (0.594).

Table (1) Classification of the severity of depression according to the Hamilton scale.

Diabetic Patients	
DM patients without depression	31 (24.2%)
DM patients with depression	97 (75.78%)
Total	128
Hamilton Depression Rating Scale (HAM-D)	**NO.**
Mild depression	32(33%)
Moderate depression	33(34.02%)
Severe depression	17(17.52%)
Very severe depression	15(15.46%)
Total	97

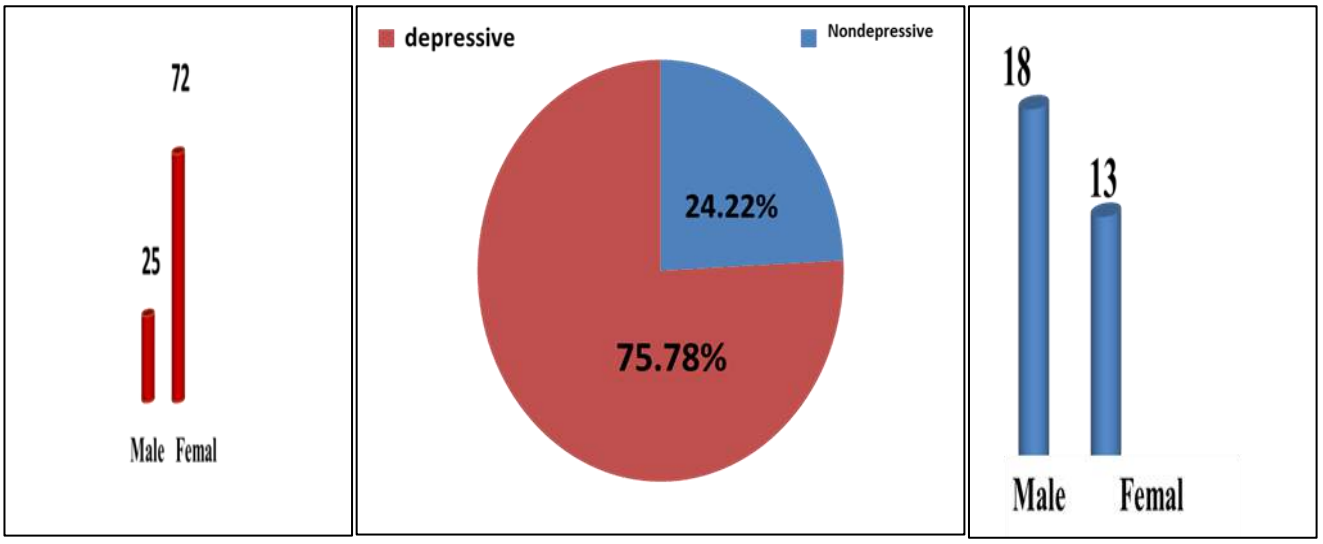

Figure 1: Prevalence of depression in diabetic patients according to sex (N=128).

Table (2): Comparison of Socio-demographic and clinical Parameters between diabetic patients with and without depression.

Patients' Sociodemographic characteristics			Depression		Total	P value
			No	Yes		
Age group (Year)	Mean±SD		53.70±9.93	53.64±8.90	53.6641	**0.975**
	<50	Count	9	30	39	**0.516**
		%	29.0%	30.9%	30.5%	
	50 and more	Count	22	67	89	
		%	71.0%	69.1%	69.5%	
Sex	Male	Count	18	25	43	**0. 002**
		%	58.1%	25.8%	33.6%	
	Female	Count	13	72	85	
		%	41.9%	74.2%	66.4%	
Onset of disease (years)	<10	Count	14	63	77	**0.128**
		%	45.2%	64.9%	60.2%	
	10-15	Count	14	26	40	
		%	45.2%	26.8%	31.3%	
	15 and more	Count	3	8	11	
		%	9.7%	8.2%	8.6%	
Social	Married	Count	29	95	124	**0.247**
		%	93.5%	97.9%	96.9%	
	Widowed	Count	2	2	4	
		%	6.5%	2.1%	3.1%	
Level of Education	Illiterate	Count	21	67	88	**0.086**
		%	67.7%	75.3%	73.3%	
	Primary school	Count	1	0	1	
		%	3.2%	0.0%	0.8%	
	Secondary school	Count	4	17	21	
		%	12.9%	19.1%	17.5%	
	Higher	Count	5	5	10	
		%	16.1%	5.6%	8.3%	
Family history	Negative	Count	24	75	99	**0.991**
		%	77.4%	77.3%	77.3%	
	Posative	Count	7	22	29	
		%	22.6%	22.7%	22.7%	
Treatment	Regular	Count	28	88	116	**0.594**
		%	90.3%	90.7%	90.6%	
	Unregular	Count	3	9	12	
		%	9.7%	9.3%	9.4%	

Table (3): Comparison of routine test value for diabetic Patients with and without depression.

Parameters	Mean ±SD		
	Non-depressive	Depressive	P value
FBS mg\dl	222.28±128.93 (n=21)	211.25±102.28 (n=67)	0.687
HbA1C mmol\dl	8.52±3.10 (n=15)	8.83±3.13 (n=38)	0.741

DISCUSSION

The study's initial objectives were to determine the prevalence of depression among type 2 diabetic patients. So all study participants had their depression diagnosed using the Hamilton test, and it was revealed that 97 of type 2 diabetics, or over 75%, had depression in varying degrees, ranging from mild to moderate to severe and very severe, and 31 or 24.2% they do not experience depression, this study is in line with Das [15],Kawada[16],Kalantari [17], Khaledi[18], Wang[19], a that have shown that people with type 2 diabetes are two or three times more likely than other people to experience depression. One such study suggests that patients with type 2 diabetes may be susceptible to several internal and environmental factors that might be responsible for this issue. Numerous investigations and perspectives on this subject have been conducted. Because the research populations were diverse and lived in varied environments, it is to be expected that the percentages would range from one study to the next. This is what Alajmani [20] demonstrated in his research and demonstrated the difference in the prevalence of depression among diabetic patients according to the environment and living conditions of the groups participating in the study.

Concerning age, the present study found the mean age of diabetics with depression (53.64±8.90) was somewhat the same as the mean age of those without depression (53.70±9.93). Additionally, a majority of depressed individuals in this study were 50 and over, and there was no statistically significant difference in this finding, the present results are consistent with Bruce [21], which demonstrated that there was no statistically significant difference between the two groups and that the average age of depressed individuals was lower than that of non-depressed individuals and contradict with Lloyd [22]. Based on what was mentioned previously, we can say perhaps the age factor may have a smaller effect when compared to the other factors that have a greater effect on creating depression.

Our study found a significant difference in depression rates between males and females with type 2 diabetes (p-value = 0.002). A significantly higher number of women 72(74.2%) experienced depression compared to men 25 (25.8%). This aligns with previous research by Yang[23], which obtained similar and statistically significant results that showed that the prevalence of depression in women with type 2 diabetes was higher than in men in different regions of the world. There are many factors, such as continuous hormonal changes in women and other physiological factors that contribute to increasing the possibility of depression in women compared to men. Suggesting that sex is an important factor to consider when evaluating mental health in this population.

Patients were categorized into three groups based on the duration of their diabetes: less than 10 years, from 10 to 15 years, and more than 15 years. Among these groups, the majority of patients who were suffering from depression had a diabetes duration of less than 10 years, while those without depression had a diabetes duration ranging from less than 10 years to 15 years. After conducting a statistical analysis, the difference was not considered statistically significant with a p-value of (0.128). These results agree with Ahmad [24].

The majority of participants in this study, both those experiencing depression and those who were not (non-depressed 93.5%, depressed97.9%), were married. Only 3.1% of participants (non-depressed 6.5%, depressed 2.1%) were widows, and the difference between the two groups was not statistically significant (p-value=0.247). This finding aligns with Al-Mohaimeed [25]and Al-Ozairi [26] who also found no significant difference in the marital status of participants.

The study shows that illiterates, those with secondary school, and those with higher education were suffer from depression more than those with primary school; this finding is in line with Mushtaque [27] since it was demonstrated that the degree of education significantly contributes to the development of depression in people with diabetes. It is possible that some patients provided inaccurate or incomplete information about their education level, while others may have chosen not to disclose this information due to embarrassment. In this case, It is important to distinguish between health awareness and educational background because it is not always the case that those with less education will not be aware of health awareness.

Participant's positive family history of diabetes was (nondepressed 77.4%, depressed 77.3%), and the p-value (0.991), the difference did not indicate statistical significance, and this is consistent with Kim [28], which reached a result similar to what this study reached and lagged with Wu [29]who found that a family history of diabetes has an important relationship with depression. Regularity in taking diabetes treatment was studied for the participants in this study, and more than 90% of both depressed and non-depressed groups were regular in taking treatment, and the p-value (0.594), which was not statistically significant and agrees with Lunghi [30], differs with Mendes [31]. Adherence to treatment alone may not be enough to control the disease. The patient needs to have a special diet and a healthy lifestyle in addition to medication adherence to achieve this. The study involved routine screening for diabetes in both the depressed and non-depressed groups. The mean of FBS for the depressed group (211.25±102.28) and the non-depressed group (222.28±128.93) were compared, and the results showed no statistically significant difference between the two groups p-value (0.687). Although the study agrees with Albekairy [32] and disagrees with Mallorquí-Bagué [33]. The HbA1c levels were, DM with the depressed group (8.83±3.13) and DM nondepressed (8.52±3.10), but the difference was not significant p-value (0.741). Although the study agrees with Mansori [34], it differs from Amelia & Yunanda[35]

CONCLUSION

Our study highlights a striking connection between type 2 diabetes and depression, with a significantly higher incidence of depression in diabetic patients. Additionally, the fact that females are more likely to suffer from depression compared to males also calls for further research into other factors that could potentially exacerbate mental health issues in type 2 diabetes women.

RECOMMENDATIONS

In-depth studies are crucial to explore the multifaceted link between type 2 diabetes and depression. This will help us understand the

underlying mechanisms and potential contributing factors. It is also very important to create special departments and cadres in the specialized centers for diabetics to provide psychological support to patients and follow up on the condition. Psychological treatment of patients and including this matter in the treatment plan for diabetics in general.

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Role of Cathepsin G in Rheumatoid Arthritis Diagnosis and Disease Activity Evaluation

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ABSTRACT

Background: *"Rheumatoid arthritis (RA) is an autoimmune disease that affects approximately 1% of the global population and results in chronic synovitis and joint destruction." It is believed that elevated serum level of cathepsin G (CTSG), a serine protease, contribute to RA pathogenesis and exacerbate disease activity. However, the data about the potential role of CTSG in the diagnosis of RA and disease activity evaluation are limited. The objective of this research is to determine whether CTSG could serve as a potential biomarker for the diagnosis and evaluation of RA activity.* ***Methods:*** *One hundred and thirty-two patients with inflammatory arthritis from the Rheumatology Department at Al-Sader Medical City in Al-Najaf City participated in this cross-sectional study from July 2023 to September 2023. The study included 121 females and 11 males, ranging in age from 18 to 70 years. The level of CTSG and anti-citrullinated peptide antibodies (ACPA) in the serum was assessed using ELISA. Other routine tests were evaluated, including erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and rheumatoid factor (RF).* ***Results:*** *The mean of CTSG was significantly lower in RA patients (110.53 pg/ml ± 49.959) than in those with other types of inflammatory arthritis (132.65 pg/ml ± 30.199). According to "DAS-28 ESR" and "DAS-28 CRP", the study found no significant difference in CTSG levels across the four disease activity groups (P = 0.585, P = 0.823, respectively). Additionally, CTSG had a significant negative correlation with diabetes mellitus and treatment intake in newly diagnosed RA (P = 0.009, P = 0.041, respectively). This study is the first to evaluate CTSG as an RA diagnostic tool, showing a sensitivity of 70.1% and a specificity of 60.0% at a cut-off value of ≤133.33 pg/ml.* ***Conclusions:*** *The study results suggest that CTSG has potential as a diagnostic biomarker for RA when used alongside other clinical and laboratory criteria. However, it should not be solely relied upon for evaluating RA activity.*

Key words: Rheumatoid Arthritis , Cathepsin G , DAS-28 ESR, DAS-28 CRP, Disease activity.

Article Information

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INTRUDUCTION

Rheumatoid arthritis (RA) is a chronic autoimmune disorder that causes inflammation and affects the synovial joints, leading to joint destruction and deformity [(1)]. The cause of RA is unknown[(2)]. This condition can affect people of all ages, but it is more common in women between the third and fifth decades of life [(3)].

Early diagnosis and treatment are critical for a better prognosis, as well as preventing irreversible joint damage and disability. In fact, up to 90% of patients can avoid or significantly delay disease progression with early intervention. Late diagnosis of RA and lack of treatment can cause severe damage to multiple tissues and organs, leading to high disability, reduced quality of life, and mortality [(4).]

The RA occurrence rate is consistently around 0.5–1.0% across the world [3]. In Iraq, a study conducted between 2014 and 2019 reported increased RA incidence from 1.1 in 2014 to 1.7 in 2019, with a cumulative risk of 10.0 [5]. Rheumatoid arthritis is a complicated condition that exhibits several clinical symptoms and varying therapeutic responses. However, as researchers have gained a better understanding of the underlying pathogenesis of the disorder, there has been a growing interest in identifying biomarkers that could help diagnose and monitor its progression at various stages [6]. "Biomarkers play a crucial role in guiding the clinical and therapeutic management of all phases of RA because they can help predict disease development in individuals at risk, improve diagnosis by closing the serological gap, provide prognostic information for treatment choices, assess treatment responses and outcomes, and monitor disease activity and progression" [7].

When comparing RA diagnostic criteria, the significant role of biomarkers can be seen [6]. Rheumatoid factor is the only biomarker for the "American College of Rheumatology (ACR) 1987 criteria " [8]. Four serological tests (ACPA, RF, ESR, and CRP) are used in the "American College of Rheumatology and the European League Against Rheumatism (ACR/EULAR) 2010 criteria " for the diagnosis of RA [4,8].

There are certain limitations to the biomarkers that are frequently used to diagnose RA. For example, RF is found in 80% of patients with RA, but it can also be found in healthy individuals and those with other diseases [9]. The ACPA test is more accurate, but both ACPA and RF still fail to detect 20-25% of RA-negative patients. Moreover, ESR is not specific and can be affected by various factors [10]. Furthermore, CRP levels can be normal in 40% of RA patients and elevated in others diseases [11]. Despite some progress in including ACPA in the updated criteria, there remains a clear need for new biomarkers in the diagnosis of RA [12].

Cathepsin G (CTSG) is a serine protease that is produced in the bone marrow and stored in azurophilic neutrophil granules. It's can also be found in other myeloid cells. When activated, CTSG is released into the extracellular space and plays a role in degrading chemokines and extracellular matrix proteins, as well as regulating pro-inflammatory cytokines. It has been linked to chronic inflammatory diseases and was found to be highly active in the synovial fluid of rheumatoid arthritis patients [13,14]. Cathepsin G is thought to be involved in inflammation and immune reactions by facilitating immune cell movement [14], making it a potential biomarker or therapeutic target for autoimmune diseases [15].

The research aimed to determine the potential of CTSG as a biomarker for diagnosis rheumatoid arthritis and distinguishing it from other types of inflammatory arthritis, as well as investigate the role of CTSG in assessing RA activity.

Samples Collection and Methods

A cross-sectional study was conducted on 132 patients with inflammatory arthritis. In this study, all participants were pioneers in the Rheumatology Department at Al-Sader Medical City in Al-Najaf City from July 2023 to September 2023.The study involved 107 patients had RA and 25 patients who had other types of inflammatory arthritis such as systemic lupus erythematosus (15 patients), Sjögren's syndrome (3 patients), Behçet's disease (2 patients), polymyalgia rheumatica (2 patients), psoriatic arthritis (1 patient), palindromic rheumatism (1 patient), and monoarticular arthritis (1 patient). The study included 121 females and 11males participants, ranging in age from 18 to 70 years. Based on the inclusion criteria, patients with other inflammatory arthritis and those diagnosed with RA by a physician using the "2010 ACR/EULAR" diagnostic criteria for RA were included. However, the study excluded patients who were under 18 or over 70 years old, as well as those

with any other autoimmune diseases, central nervous system diseases, acute local inflammation, wounds, recent surgeries, cancer, chronic infections, or immunodeficiency diseases.

The participants were randomly selected and divided into two groups. The first group consisted of patients with RA classified into various disease activity levels, "remission, mild, moderate, or severe, based on their disease activity score (DAS-28 ESR and DAS-28 CRP)". The second group included patients with other types of inflammatory arthritis. The patients completed a questionnaire providing information on their name, age, gender, BMI, contraceptive pill use, the family history of RA, as well as any history of chronic diseases like diabetes mellitus, hypertension, and other relevant details.

During the patient examination, a specialist evaluated the number of tender and swollen joints used to determine the disease activity score. In addition, serum levels of CTSG and ACPA were measured in patients with inflammatory arthritis using the ELISA kit from Sunlong, China. The study also measured the ESR using the Westergren method, assessed RF using the sandwich immunodetection method from Boditech, Korea, and measured CRP using a particle-enhanced immune-turbidimetry assay from Cobas, Germany.

STATISTICAL ANALYSIS

The data was analyzed using Statistical Package of Social Science (SPSS) software version 21 (San Diego, California, USA). Continuous variables were presented as means and SD, while categorical variables were expressed as frequency and percentage. The chi-square test was used to determine the significance of categorical variables. Pearson and Spearman correlations were used to measure the correlation between nominal and ordinal scales. An independent t-test and an ANOVA test were used to measure serological parameters between study groups and assess the significance level of different clinical and laboratory parameters. Median ± IQR was reported when variance was not found. Furthermore, ROC curves were used to evaluate the diagnostic utility of CTSG, RF, and ACPA. Sensitivity, specificity, PPV, and NPV were calculated for inflammatory arthritis patients. A significance level of $P<0.05$ was considered statistically significant, while $P<0.01$ was deemed highly significant.

RESULTS

A cross-sectional study was conducted on 132 patients with inflammatory arthritis, including 107 patients with RA and 25 patients with other types of inflammatory arthritis. The age mean and SD (46.85 year ±10.564) of patients with RA were significantly higher than those of patients with other types of inflammatory arthritis (39.48 year ±12.460), indicating a significant difference (P = 0.003) between the two groups. However, RA patients had higher BMI means and SD (29.93 kg/m2 ±5.424) than those with other types of inflammatory arthritis (27.40 kg/m2 ±5.874); this difference was statistically significant (P = 0.04). Furthermore, the smoking index means and SD of RA patients (15.24±14.629) and those of patients with other types of inflammatory arthritis (40.00±0.0) did not differ significantly (P = 0.141). Also, there were no significant differences between the two groups of patients in terms of sex (P = 0.999), hypertension (P = 0.099), smoking (P = 0.152), or contraceptive pill use (P = 0.744). However, a significant difference was observed (P = 0.005) between the two patient groups

according to diabetes mellitus, as shown in Tab.1.

Moreover, the median (IQR) of ESR level was higher in RA patients [38.00 mm/hr (30.00)] than the median (IQR) [30.00 mm/hr (32.50)] of patients with other types of inflammatory arthritis. However, the difference between these two groups was not significant. However, the median (IQR) of CRP, RF, and ACPA levels were higher in RA patients [5.49 mg/L (9.31), 18.00 IU/mL (32.00), 12.896 U/ml (5.042), respectively] than the median (IQR) [1.68 mg/L (4.46), 8.00 IU/mL (4.00), 5.884 U/ml (2.572), respectively] of patients with the other types of inflammatory arthritis. There was a significant difference (P = 0.01, P = 0.001, and P = < 0.001, respectively) in these levels between the two groups of patients. On the other hand, the mean and standard deviation (SD) of CTSG level were lower in patients with RA (110.53 pg/ml ± 49.959) compared to those with other types of inflammatory arthritis (132.65 pg/ml ± 30.199). There was a significant difference between the two groups of patients (P = 0.006), as shown in Tab.1. There was no significant difference (P = 0.727) in CTSG level between RA patients with a disease duration of less than six months (113.379 pg/ml ±37.064) and those with more than six months (109.520 pg/ml ±53.461). Rheumatoid arthritis patients with a good response to treatment had a higher mean of CTSG (113.41 pg/ml ± 50.155) than those with a poor response (102.27 pg/ml ± 55.288), but the difference was not significant (P = 0.311). Similarly, untreated newly diagnosed RA patients had a higher mean of CTSG (123.00 pg/ml ± 22.68) compared to those with long-term regularly treated RA (113.52 pg/ml ± 54.03). However, the two groups had no significant difference (P = 0.55). In addition, untreated newly diagnosed RA patients had a significantly (P = 0.031) higher mean of CTSG (123.00 pg/ml ± 22.68) compared to those with regularly treated newly diagnosed RA (97.30 pg/ml ± 40.019), as shown in Tab. 2.

According to the DAS-28 ESR and DAS-28 CRP indices, there was no statistically significant difference in CTSG level among the four disease activity groups (P = 0.585 and P = 0.823, respectively), as shown in Tab.3. Additionally, there was no significant correlation between CTSG and RA activity according to DAS-28 ESR and DAS-28 CRP (R = -0.011, P = 0.911; R = -0.032, P = 0.742, respectively), as shown in Fig.1 and Fig.2.

However, CTSG had a significant negative correlation with diabetes mellitus and treatment intake in newly diagnosed RA (P = 0.009, P = 0.041, respectively). There was no significant correlation with other factors such as age, sex, hypertension, smoking index, BMI, family history, disease duration, regularity of treatment intake, response to treatment, untreated newly diagnosed RA/treated long-term RA, and contraceptive pill use (P = 0.598, P = 0.518, P = 0.727, P = 0.992, P = 0.973, P = 0.865, P = 0.316, P = 0.531, P = 0.263, P = 0.194, P = 0.835, respectively), as shown in Tab.4. As well, CTSG showed no significant correlation with hemoglobin level, WBC, or platelet count [(P = 0.765), (P = 0.199), and (P = 0.95), respectively], as shown in Tab.5.

Furthermore, CTSG had a non-significant correlation with ESR, CRP, RF, and ACPA (P > 0.05). On the other hand, ESR had a statistically significant correlation with CRP (P < 0.001). Furthermore, CRP had a low positive correlation with RF but was statistically insignificant (P = 0.059). However, RF had a highly statistically significant correlation with ACPA (P < 0.001), as shown in Tab.6.

The study evaluated the diagnostic performance of CTSG compared to RF and ACPA in distinguishing between patients with RA and other types of inflammatory arthritis using the Receiver Operator Characteristic (ROC) Curve, as shown in Fig.3. The cut-off value for CTSG was determined to be ≤133.33

pg/ml, and the AUC of CTSG was 0.656. Additionally, ACPA showed excellent diagnostic performance with an AUC of 0.813, which was highly significant (P < 0.001). Rheumatoid factor also had good diagnostic performance, with an AUC of 0.743, which was highly significant. Cathepsin G has a sensitivity of 70.1%, almost equal to the sensitivity of RF (72.0%) and lower than the sensitivity of ACPA (85.0%). CTSG has a specificity of 60.0%, lower than the specificities of RF (84.0%) and ACPA (80.0%), as shown in Tab.7.

Table 1: Demographic and clinical characteristics of the Inflammatory Arthritis Patients (n= 132)

Characteristic	RA patients (n=107)	Other inflammatory arthritis patients (n=25)	Characteristic
Demographics			
Age, mean (year) ±S.D.	46.85±10.564	39.48±12.460	0.003**
Sex [females/males (% of females)]	98/9 (91.6%)	23/2 (92.0%)	0.999
BMI, mean (kg/m2) ±S.D.	29.93±5.424	27.40±5.874	0.04*
Hypertension [Hypertensive/Non-hypertensive (% of Hypertensive)]	42/65 (39.3%)	7 /18 (28.0%)	0.099
Diabetes mellitus [Diabetic/Non-diabetic (% of Diabetic)]	17 /90 (15.9%)	1 /24 (4.0%)	0.005**
Smoking [Smoker/Non-smoker (% of Smoker)]	10 /97 (9.3%)	1 /24 (4.0%)	0.152
Smoking index, mean pack per year ±S.D.	15.24±14.629	40.00±0.0	0.141
Contraceptive pill use [user/Non-user (% of Contraceptive pills user)]	6 /91 (6.2%)	1 /21 (4.5%)	0.744
Clinical data			
ESR level (mm/hr), median (IQR)	38.00 (30.00)	30.00 (32.50)	0.657
CRP level (mg/L), median (IQR)	5.49 (9.31)	1.68 (4.46)	0.01*
RF level (IU/mL), median (IQR)	18.00 (32.00)	8.00 (4.00)	0.001**
ACPA level (U/ml), median (IQR)	12.896 (5.042)	5.884 (2.572)	<0.001**
CTSG level (pg/ml), mean ±S.D.	110.53± 49.959	132.65± 30.199	0.006**

BMI body mass index, ESR erythrocyte sediment rate, CRP C-reactive protein, RF rheumatoid factor, ACPA anti-citrullinated peptide antibody, CTSG cathepsin G, IQR interquartile range, S.D standard deviation *: significant difference, **: highly significant difference.

Table 2: Comparison of CTSG According to the Disease Duration and Features of Treatment Intake		
Characteristic	CTSG mean ± SD (pg/ml)	P-value
Less than 6 months (n=28)	113.379±37.064	0.727
More than 6 months (n=79)	109.520±53.461	
Good response to treatment (n= 57)	113.41 ± 50.155	0.311
Poor response to treatment (n= 38)	102.27 ± 55.288	
Untreated newly diagnosed RA (n=12)	123.00 ± 22.68	0.55
Long-term regularly treated RA (n=54)	113.52 ± 54.03	
Untreated newly diagnosed RA (n=12)	123.00 ± 22.68	0.031*
Regularly treated newly diagnosed RA (n=13)	97.30 ± 40.019	
CTSG cathepsin G. *: significant difference		

Table 3: CTSG comparison among RA patients' disease activity groups using DAS-28 ESR and DAS-28 CRP				
DAS-28 ESR of RA patients (n=107)				
CTSG level (pg/ml), mean ±S.D.				
Remission (n =2)	Low activity (n =2)	Moderate activity (n =33)	High activity (n =70)	P-Value
149.61±22.237	80.13±51.942	109.95±39.846	110.55±54.594	0.585
DAS-28 CRP of RA patients (n=107)				
CTSG level (pg/ml), mean ±S.D.				
Remission (n = 6)	Low activity (n = 6)	Moderate activity (n = 61)	High activity (n = 34)	P-Value
97.01±49.416	123.59±36.756	111.63±46.993	108.64±57.942	0.823
CTSG cathepsin G, DAS28 disease activity score, ESR erythrocyte sediment rate, CRP C-reactive protein.				

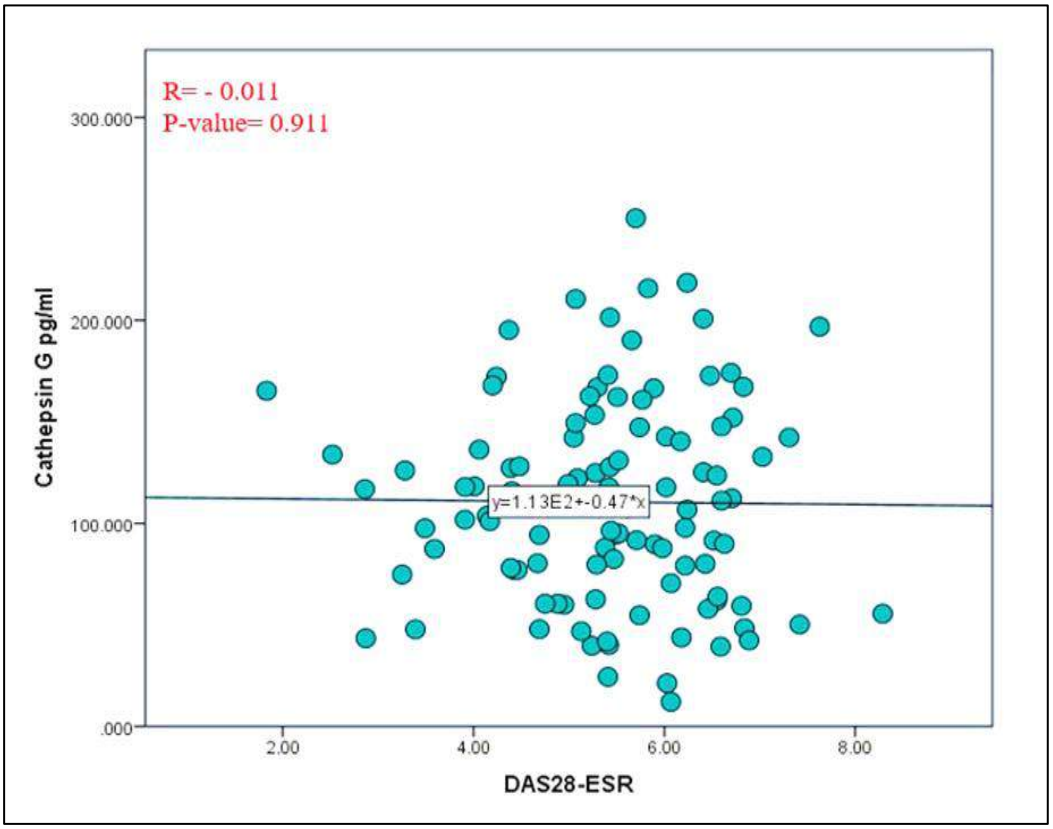

Figure 1: Correlations of CTSG with RA Activity According to DAS-28 ESR.

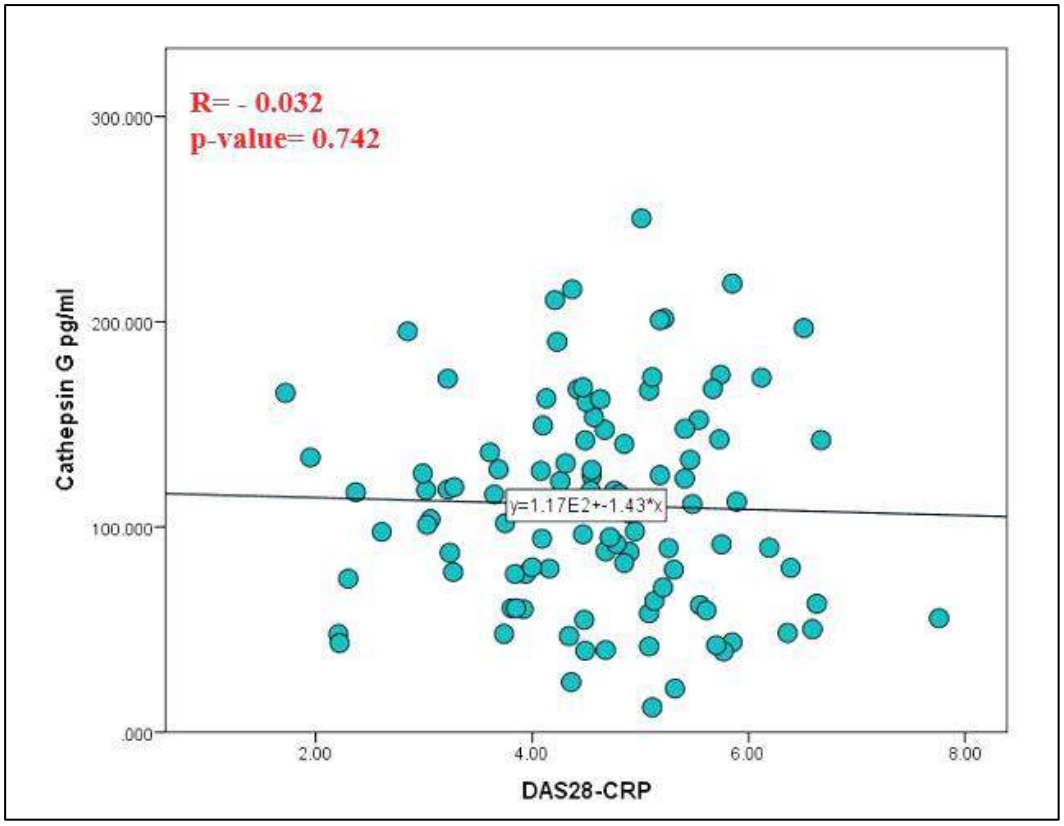

Figure 2: Correlations of CTSG with RA Activity According to DAS-28 CRP.

Table 4: Correlations of Demographic Characteristics, BMI and Features of Treatment Intake with CTSG Values in the RA Patients		
Characteristic of RA Patients		CTSG
Age (years)	r	-0.052
	P	0.598
Sex	r	-0.063
	P	0.518
Hypertension	r	-0.034
	P	0.727
Diabetes Mellitus	r	-0.252
	P	0.009**
Smoking index, (pack per year)	r	0.001
	P	0.992
BMI	r	0.003
	P	0.973
Family History	r	-0.017
	P	0.865
Disease Duration	r	-0.097
	P	0.316
Regularity of treatment intake	r	-0.061
	P	0.531
Response to Treatment	r	0.115
	P	0.263
Untreated newly diagnosed RA/ treated long-term RA	r	-0.137
	P	0.194
Untreated, newly diagnosed/Regularly treated, newly diagnosed RA	r	-0.410
	P	0.041*
Contraceptive pill use	r	0.021
	P	0.835
CTSG cathepsin G, *BMI* body mass index, *r* correlation coefficient, *P* P-value. *: Significant, **: highly significant		

Table 5: Correlations of Haematological Parameters with CTSG Values in the RA Patients		
Characteristic of RA patients		CTSG
Haemoglobin level (mg/dl)	r	-0.029
	p	0.765
WBC Count (x 10^9/liter)	r	-0.125
	p	0.199
Platelets Count (x 10^9/liter)	r	-0.006
	p	0.95
CTSG cathepsin G, *WBC* white blood cell, *r* correlation coefficient, *P* P-value		

Table 6: Correlations between Biomarkers in the RA patients						
Parameters		CTSG	ESR (mm/h)	CRP (mg/L)	RF (IU/mL)	ACPA(U/ml)
CTSG	P	1				
	r	-				
ESR (mm/h)	r	-0.056	1			
	P	0.566	-			
CRP (mg/L)	r	0.05	0.537	1		
	P	0.609	<0.001**	-		
RF (IU/mL)	r	-0.096	0.097	0.183	1	
	P	0.328	0.318	0.059	-	
ACPA (U/ml)	r	0.073	0.061	0.006	0.549	1
	P	0.453	0.534	0.949	<0.001**	-
CTSG cathepsin G, *ESR* erythrocyte sediment rate, *CRP* C-reactive protein, *RF* rheumatoid factor, *ACPA* anti-citrullinated peptide antibody, *r* Correlation Coefficient, *P* P-value. **: highly significant						

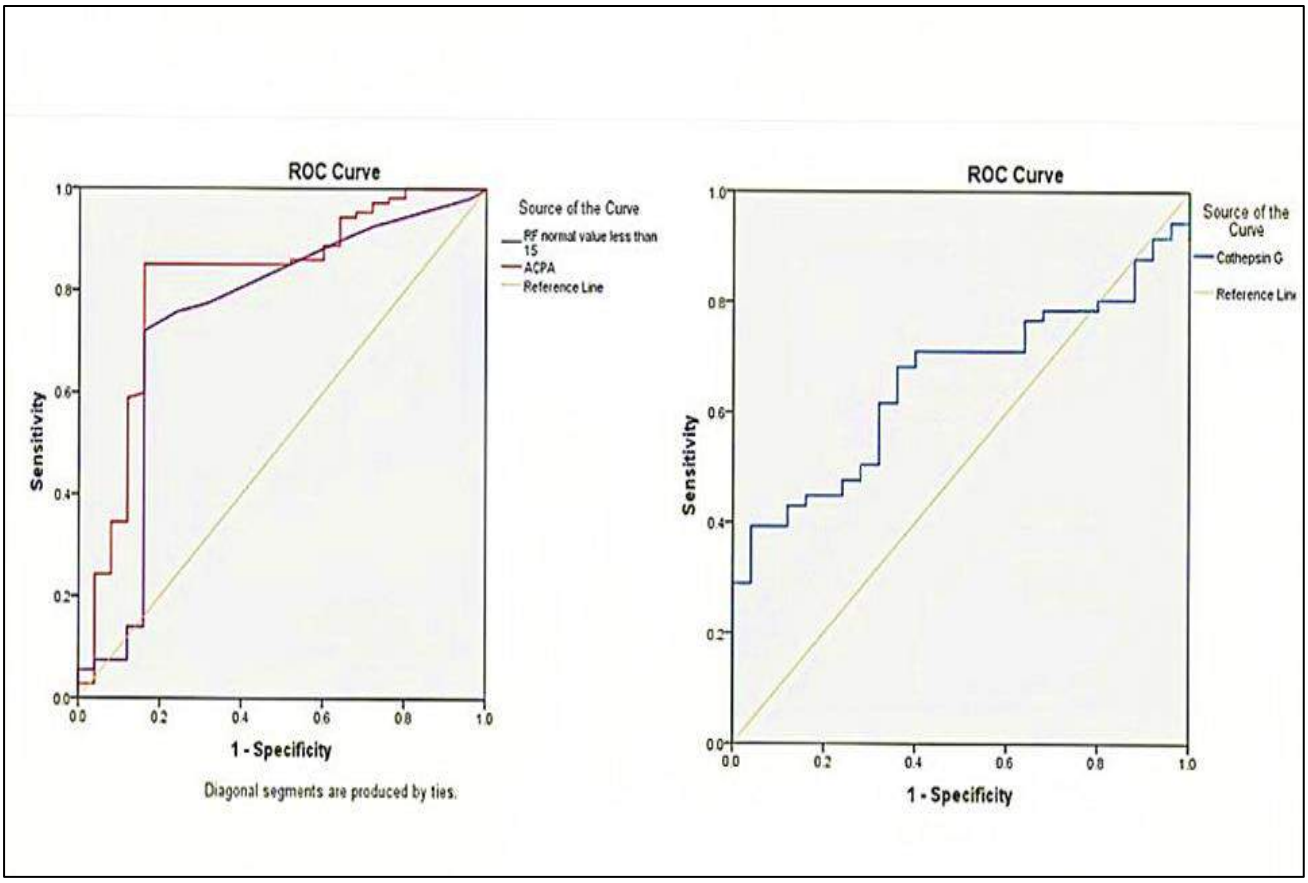

Figure 3: The Diagnostic Performance of RF, ACPA, and CTSG.

Table 7: Receiver Operator Characteristic (ROC) Curve for Biomarkers of Patients with Inflammatory Arthritis

Characteristic	CTSG	RF	ACPA
AUC	0.656	0.743	0.813
SE	0.050	0.063	0.053
Sig.	0.015*	<0.001**	<0.001**
95% confidence interval	0.559-0.754	0.619 -0.867	0.709 -0.917
Optimal cut-point value (pg/ml)	133.33	12.500	9.920
Sensitivity (%)	70.1%	72.0%	85.0%
Specificity (%)	60.0%	84.0%	80.0%
PPV (%)	88.2%	71.96%	85.04%
NPV (%)	31.9%	84%	80%
Diagnostic effectiveness (accuracy)	68.18%	74.24%	84.09%
Youden's index	0.3	0.56	0.65

CTSG cathepsin G, *RF* rheumatoid factor, *ACPA* anti-citrullinated peptide antibody *AUC* area under the curve, *SE* standard error *Sig* significant, *PPV* positive predictive value, *NPV* negative predictive value. *: Significant difference, **: highly significant difference

DISCUSSION

The current study found that the mean level of CTSG was significantly lower in patients with RA compared to patients with other types of inflammatory arthritis. Although previous studies have shown a correlation between CTSG levels and many other inflammatory arthritis or autoimmune diseases, such as psoriatic arthritis (PsA) and systemic lupus erythematosus (SLE), there have been no studies specifically comparing CTSG levels between RA and other types of inflammatory arthritis.

Popova-Belova et al. [16] found significantly higher CTSG levels in patients with PsA compared to a control group of gouty arthritis patients and healthy controls. Kida et al. [17] found that CTSG is the main antigen for anti-neutrophil cytoplasmic antibodies in systemic SLE. They reported that patients with active SLE displayed considerably higher levels of CTSG antibodies in their serum than those with inactive SLE or healthy controls. These high levels decreased rapidly after corticosteroid therapy. Patients with other types of inflammatory arthritis have higher levels of CTSG compared to those with RA. These high levels are potentially due to CTSG's contribution to the development of autoimmune disorders characterized by cytokine production and neutrophil infiltration in the joints. However, additional studies are required to understand CTSG's involvement in different types of inflammatory arthritis.

The relatively small sample size of patients with other types of inflammatory arthritis compared to those with RA may contribute to the lower levels of CTSG in RA patients. Furthermore, in the present study, SLE patients dominated the other inflammatory arthritis group. As previously mentioned, CTSG is known to impact SLE and is linked to disease activity. Despite this lower level of CTSG in RA patients, Gao et al. [15] found that the synovial fluid of RA patients has a higher level of CTSG than that of healthy controls, which leads this study to suspect a

higher level of CTSG in those patients compared to healthy controls. It is also believed that this increase in CTSG level may be due to the release of a protease enzyme by neutrophils during inflammation [18].

Cathepsin G can change and activate molecules like cytokines, chemokines, and cell surface receptors that are important for the immune system and the inflammatory response [14]. It can also attract more monocytes to the inflammation site. In RA, immune cells such as monocytes and neutrophils infiltrate and lead to inflammation of the synovial tissue that lines the joints [19]. These cells release CTSG and other pro-inflammatory cytokines, which lead to inflammation and joints damage [20]. The high level of CTSG may participate in the RA pathogenesis by recruitment cells to the affected joints and initiating an inflammatory response [14]. It may also be used as an indicator for RA diagnosis and progression. However, additional researches are necessary to clarify the role and effects of CTSG in the pathogenesis of RA.

The current study revealed that RA patients with disease duration less than six months had slightly higher CTSG levels compared to those with disease duration more than six months. However, the difference did not reach statistical significance. According to the researcher's knowledge, there are no studies available to compare this result. Rheumatoid arthritis patients with disease duration less than six months have slightly higher CTSG levels, may due to increased inflammatory immune response in the early stages of RA that stimulates the recruitment and activation of neutrophils in the joints [21]. As part of the immunological response, neutrophils produce and release CTSG [18]. The low levels of CTSG in RA patients with disease duration more than six months may be related to long-term use of medications for RA, which can reduce inflammation and slow disease progression. This may result in decreased CTSG levels.

The current study did not find a significant difference in CTSG level based on response to treatment in RA patients. According to previous study conducted by Miyata et al. [19] , CTSG levels have been found to be affected by RA treatment and decrease after treatment, suggesting that it may be a potential biomarker of response to treatment.

The current study found there was no statistically significant difference in the levels of CTSG between patients with long-term, regularly treated RA and those with newly diagnosed, untreated RA. According to the researcher's knowledge, there are no studies available to compare this result. However, the levels of CTSG were slightly higher in the untreated, newly diagnosed RA group compared to the regularly treated long-term RA group. This increase can be attributed to excessive inflammation and neutrophil infiltration in the joints, which leads to the release of CTSG from various immune cells and neutrophil granules. Cathepsin G also attracts monocytes and regulates immune responses, thus increasing inflammation and joint damage [14].

The study found that regularly treated newly diagnosed RA patients had significantly lower CTSG levels than untreated newly diagnosed RA patients. The reason behind this significantly lower level of CTSG was explained previously. This finding suggests that CTSG could serve as a new diagnostic biomarker or treatment target for RA. This idea is supported by previous studies that found the activity of human leukocyte CTSG can be inhibited by anti-rheumatic drugs. As a result of this finding, the authors recommend CTSG inhibition as a potential therapeutic approach for treating autoimmune diseases such as RA [19,22, 15].

To the best of our knowledge, this is the first study to evaluate the role of CTSG in RA activity. The findings of this study revealed a non-significant association between CTSG and disease activity (according to the DAS-28 ESR and DAS-28 CRP indices). Additionally, a study by Popova-Belova et al. [16] found that CTSG did not show strong associations with disease activity in patients with PsA. Another study by Ruge et al. [23], who work on other types of cathepsins (S and L), also found no significant association between these cathepsins and RA activity, as characterized by DAS-28 ESR. The level of serum cathepsins cannot precisely indicate the level of inflammation present in the joints [24].

The current study showed that CTSG had a significant negative correlation with diabetes mellitus. The findings differ from the study which reported increased CTSG in patients with Type 1 diabetes [25]. Furthermore, this study has shown a negative correlation between CTSG and treatment intake in newly diagnosed RA patients. The elevated CTSG levels observed in RA patients could be attributed to joint inflammation triggering the release of CTSG by immune cells like neutrophils and monocytes as part of the inflammatory process [14]. Conversely, RA medications that reduce immune responses and inflammation may have an impact on the lower levels of CTSG in regularly treated, newly diagnosed RA patients. These results indicate that CTSG might hold potential as a biomarker for monitoring treatment outcomes in diagnosed RA patients and as a target for RA therapy.

This is the first study that used a receiver operator characteristic (ROC) curve analysis to measure the diagnostic value of CTSG and its usefulness in differentiation of RA from other types of inflammatory arthritis. According to the results, CTSG has demonstrated reliable diagnostic performance with an AUC of 0.656 at a cut-off value of ≤133.33 pg/ml, a sensitivity of 70.1%, and a specificity of 60.0%. Cathepsin G had lower accuracy, sensitivity, and AUC than ACPA and RF; the specificity was similar to RF but lower than ACPA.

CONCLUSION

In conclusion, the findings showed that CTSG could be a useful diagnostic biomarker for RA when combined with other clinical and laboratory criteria but is unreliable for RA activity evaluation.

Ethical Approval

Before commencing the study project, the ethical committee of the College of Medicine at the University of Kufa had approved. The Rheumatology Department of Al-Sadr Medical City also granted permission, and the patient's consent was obtained to conduct a questionnaire and collect a blood sample.

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Admission Pattern and Treatment Outcome in Pediatric Intensive Care Unit in Al Zahraa Teaching Hospital, Iraq, Najaf

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ABSTRACT

Background: *The Pediatric Intensive Care Unit (PICU) represents a specialized healthcare setting dedicated to providing critical care for infants, children, and adolescents facing severe and life-threatening medical conditions. The rate of pediatric mortality in the Pediatric Intensive Care Unit (PICU) varies worldwide, reflecting the diverse healthcare landscapes and socioeconomic factors influencing pediatric critical care outcomes. The objective of this study was to describe the pattern of admission and outcome of patients who received intensive care.*

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Patients and methods: *A retrospective study in which records of admissions (from 1st of January 2019 to 31 December 2023) were obtained from the PICU patient's file in Al Zahraa Teaching, Najaf, Iraq. Data used from the records included age, sex, residency, address, source of admission, diagnosis, duration of stay in the unit, need for intubation and mechanical ventilation, and patient outcome.* ***Results:*** *A total number of 1501 patients were admitted to the PICU during the study period. The patients were referred mainly from the Emergency Room 47% followed by the neonatal intensive care unit 21%. The case fatality rate was 54.1%. the main cause of death was RDS, sepsis, meningitis, and congenital heart diseases (29.2, 8.5, 6.6, and 6.6% respectively). 47.9% of PICU deaths occurred in patients admitted from the ER followed by NICU 24.2%. (P value 0.043, OR 1.082, 95% CI 1.002-1.168). The mean age of dead PICU patients was 15.697±32.79 months versus 23.301±39.91 months for discharged outcome (P value 0.000). Hospital stay length mean for PICU dead patients was 5.1±3.8 days versus 6.173±3.8 days for discharged patients (P value 0.000). That means the smaller age patients carry a higher risk of death in PICU, also the situation with fewer hospital stays days.*

Conclusions: *This study showed the case fatality rate was high (54.7%.). The most common causes of admission and death were RDS (22.7% and 29.2% respectively). The highest percentage of death occurs in the infant age group (78.1%). Neither gender nor residency represents a risk factor for PICU patient's death but endotracheal intubation does. The fewer days of hospital stay carry a higher risk factor for death.*

Keywords: PICU, NICU, Mortality, Outcome, Cause of death.

Article Information

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INTRUDUCTION

The Pediatric Intensive Care Unit (PICU) represents a specialized healthcare setting dedicated to providing critical care for infants, children, and adolescents facing severe and life-threatening medical conditions. Essential for managing a diverse range of cases, including respiratory distress, cardiac emergencies, neurological disorders, and traumatic injuries, the PICU serves as a vital component within the broader healthcare system [(1)]. Research plays a pivotal role in enhancing the care provided in Pediatric Intensive Care Units (PICUs) and ultimately contributes to improving pediatric mortality outcomes. [(6)]. Translating research findings from the Pediatric Intensive Care Unit (PICU) into clinical practice is a critical step in improving patient outcomes. Successful translation involves collaboration among healthcare professionals, administrators, and researchers to overcome barriers and facilitate the adoption of proven interventions [(2)]. Continuous education and training programs ensure that healthcare providers are informed about the latest evidence and can apply it in real-world patient care [(3)].

Aims of the Study

The objective of this study was to describe the pattern of admission and outcome of patients who received intensive care.

PATIENTS AND METHODS

A retrospective study in which records of admissions (from 1st of January 2019 to 31 December 2023) were obtained from the PICU records in Al Zahraa Teaching Hospital for child health and maternity. This hospital is a tertiary care center in Al Najaf city, Iraq. It is equipped with a six-bedded modern PICU, which admits pediatric patients from both medical and surgical subspecialties for immediate critical postoperative care. PICU records of all admissions, transfer out, discharges, and deaths were used for this study. Data used from the records included age, sex, residency, address, source of admission, diagnosis, duration of stay in the unit, need for intubation and mechanical ventilation, and patient outcome.

Ethical approval was given by the Ethics Committee of the pediatrics department.

The data obtained were entered into the Statistical Package for Scientific Solutions (SPSS) version 28.0 spreadsheet and analyzed. Means, standard deviations, percentages, and ranges were used as appropriate to describe continuous variables

RESULTS

A total number of 1501 patients were admitted to the PICU in Al Zahraa Teaching Hospital for Child Health and Maternity in a period between 1st of January 2019 to 31 December 2023. See Figure 1.

The patient's age ranges from 1 to 288 months with an average of 19.2 ± 36.4 months. They had stayed in PICU for an average of 5.6 ± 3.48 days ranging from 30 to 1 day. About Forty percent of patients who admitted to PICU were lived outside Al Najaf city. Other Socio-demographic variables are shown in Table 1.

The main cause of PICU admission was respiratory distress syndrome (RDS), 22.7% followed by pneumonia 7.8%. Other causes are shown in Table 2 in sequences.

The most common body system involved in patients who had been admitted to PICU was the Respiratory system 47.7% followed by surgical cases 14.1%. Other systems involved are shown in Figure 2.

The main hospital department that referred most of the patients to the PICU was the Emergency Room 47% followed by neonatal intensive care unit 21%. Other department percentages are shown in Figure 3.

The case fatality in Al Zahraa Teaching Hospital is 54.7%. About 54% of PICU patients required endotracheal intubation and mechanical ventilation. The case fatality in Al Zahraa Teaching Hospital is 54.7%. About 54% of PICU patients required endotracheal intubation

and mechanical ventilation. 64.4% of intubated patients died versus 41.8% discharged (P value 0.000, OR 0.403 95%CI 0.325-0.501). Other patient's characteristics pose no risk factor for death as shown in Table 4.

Respiratory distress syndrome (RDS) followed by sepsis represents the main cause of death in PICU, 29.2% and 8.5% respectively. (P value 0.000, OR 1.058, 95%CI 1.042-1.074). Other diagnoses of PICU patients with their frequencies and percentages are stated in Table 5. , 54.3% of deaths were due to diseases of the respiratory tract followed by the central nervous system at 12.9%. (P value 0.630, OR 0.986, 95% CI 0.932-1.044). Other systems involved are described in table 6.

47.9% of PICU deaths occurred in patients admitted from the emergency room (ER) followed by neonatal intensive care unit (NICU) 24.2%. (P value 0.043, OR 1.082, 95% CI 1.002-1.168). Other referral wards and their contribution to PICU deaths are shown in Table 7., The mean age of dead PICU patients was 15.697±32.79 months versus 23.301±39.91 months for discharged outcome. (P value 0.000). Hospital stay length also represents a risk factor for PICU patient's death as shown in Table 8.

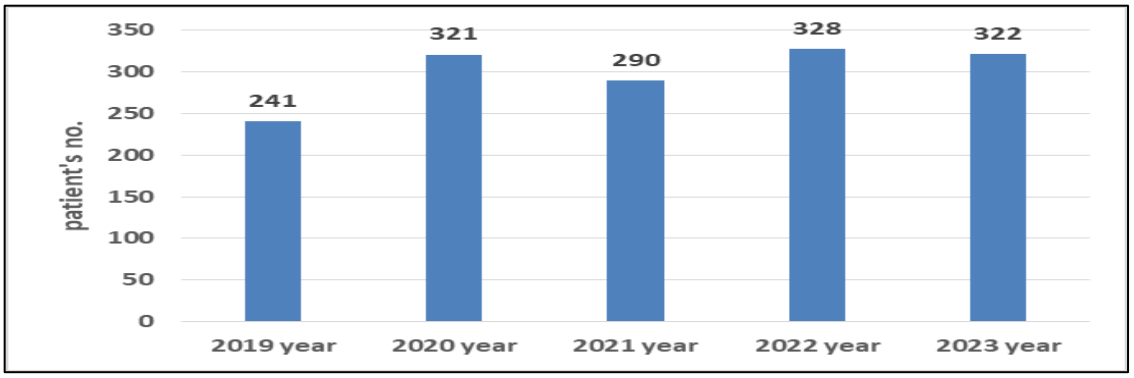

Figure 1: Number of patients admitted to PICU for each year.

Table 1: Socio-demographic variable of PICU patients.

Age group (months)	Patient no.	%
< 12	1109	73.9
13 to 59	192	12.8
60 to 120	168	11.2
> 120	32	2.1
Gender		
Male	828	55.2
Female	673	44.8
Residency		
Rular	842	56.1
Urban	659	43.9
Address		
Najaf	876	58.4
Diwaniyah	303	20.2
Samawah	224	14.9
Hila	54	3.6
Baghdad	36	2.4
Others	8	0.5
Total	1501	100

Table 2: The causes of PICU admission.

Diagnosis	Frequency	Percent
RDS	340	22.7
Pneumonia	117	7.8
CHD	99	6.6
Sepsis	94	6.3
Meningitis	81	5.4
Intussusception	79	5.3
DKA	67	4.5
Renal Failure	61	4.1
Status Epilepticus	50	3.3
Pneumothorax	49	3.3
Diaphragmatic Hernia	46	3.1
Surgical Cases	45	3.0
ICH	40	2.7
Metabolic	34	2.3
Unknown	30	2.0
HIEP	28	1.9
TEF	23	1.5
Electrical Shock	22	1.5
Poisoning	20	1.3
Hepatic Failure	20	1.3
Severe Dehydration	20	1.3
Scorpion Bite	18	1.2
COVID-19	16	1.1
Status Asthmaticus	16	1.1
GBS	14	0.9
Submersion	14	0.9
CRRT	11	0.7
Gastroschisis	10	0.7
Omphalocele	9	0.6
Burn	9	0.6
HBV	6	0.4
Croup	5	0.3
Tetanus	4	0.3
Drug Poisoning	2	0.1
Miscellaneous	2	0.1
Total	1501	100.0

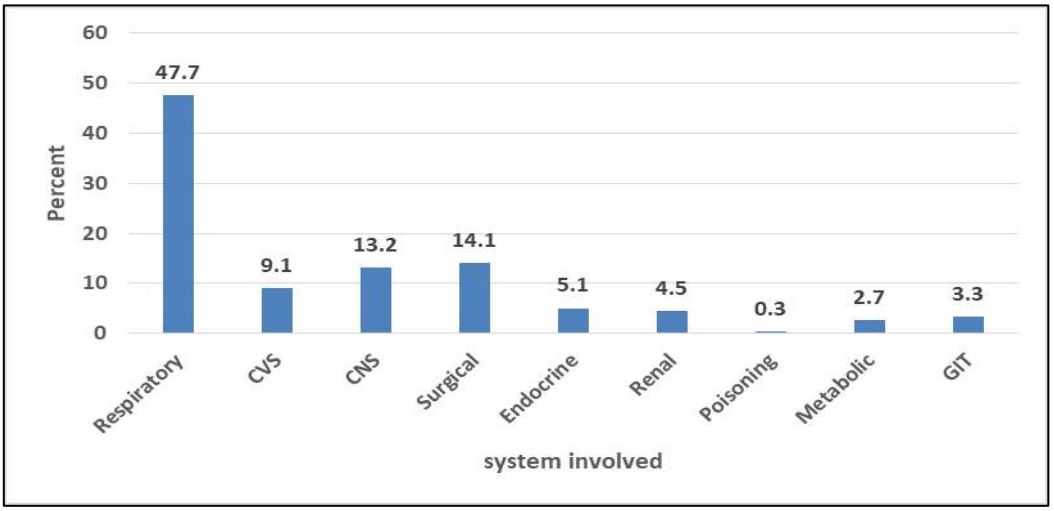

Figure 2: System involved in patients admitted to PICU.

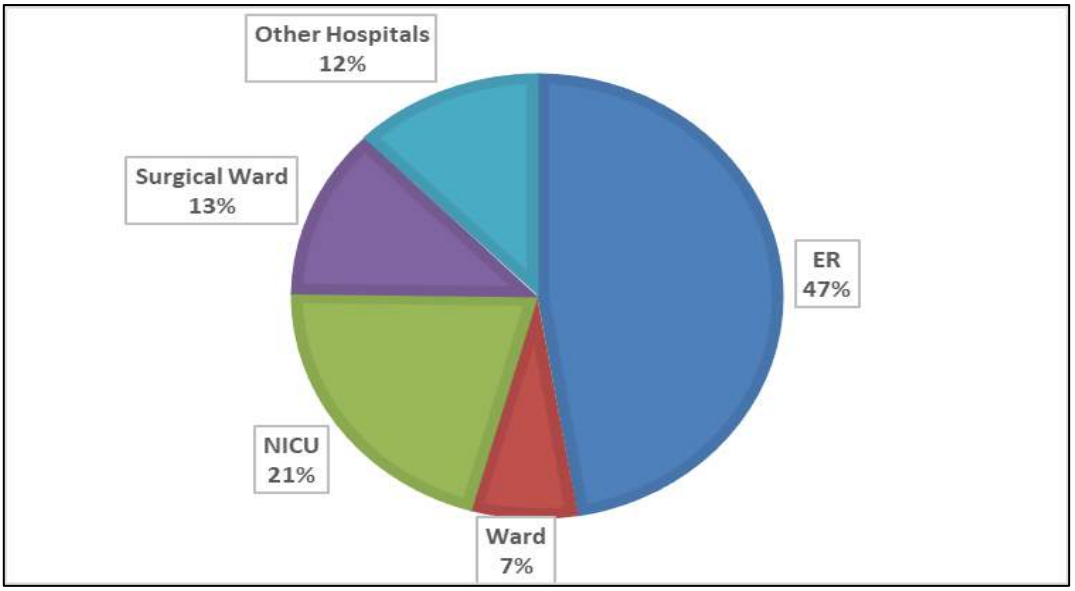

Figure 3: the main hospital departments that referred patients to PICU.

Table 3: The outcome of patients admitted to PICU.

Variables		Frequency	Percent
ET	No	687	45.8
	Yes	814	54.2
Fate	Dead	821	54.7
	Discharged	680	45.3
Total		1501	100.0

Table 4: Socio-demographic variable and outcome of patients in PICU.

		Fate				P value	OR	95% CI	
		Dead		Discharged					
		N	%	N	%			Lower	Upper
Gender	Male	442	53.8	386	56.8	0.197	0.859	0.683	1.082
	Female	379	46.2	293	43.2				
Residency	Rular	471	57.4	370	54.5	0.298	1.130	0.898	1.422
	Urban	350	42.6	309	45.5				
ET	NO	292	35.6	395	58.2	0.000	0.403	0.325	0.501
	Yes	529	64.4	284	41.8				
Age Groups (months)	< 12	641	78.1	468	68.9	0.004	1.253	1.077	1.458
	13 to 59	99	12.1	93	13.7				
	60 to 120	66	8	101	14.9				
	> 120	15	1.8	17	2.5				

Table 5: Diagnosis of patients admitted to PICU and their outcome.

	Fate						P value	OR	95% CI	
	Dead		Discharged		Total					
Diagnosis	N	%	N	%	N	%			Lower	Upper
Unknown	30	3.7	0	0	30	2	0.000	1.058	1.042	1.074
Meningitis	54	6.6	27	4	81	5.4				
RDS	239	29.2	101	14.9	340	22.7				
Pneumothorax	16	2	33	4.9	49	3.3				
CHD	54	6.6	45	6.6	99	6.6				
Sepsis	70	8.5	24	3.5	94	6.3				
Surgical Cases	15	1.8	30	4.4	45	3				
GBS	12	1.5	2	0.3	14	0.9				
Renal Failure	42	5.1	19	2.8	61	4.1				
Status Epilepticus	13	1.6	37	5.4	50	3.3				
Pneumonia	51	6.2	65	9.6	116	7.7				
DKA	21	2.6	46	6.8	67	4.5				
Poisoning	13	1.6	7	1	20	1.3				
TEF	16	2	7	1	23	1.5				
Intussusception	39	4.8	40	5.9	79	5.3				
Diaphragmatic Hernia	18	2.2	28	4.1	46	3.1				
Omphalocele	2	0.2	7	1	9	0.6				
Metabolic	28	3.4	6	0.9	34	2.3				
Electrical Shock	7	0.9	15	2.2	22	1.5				
ICH	22	2.7	18	2.7	40	2.7				
Burn	4	0.5	5	0.7	9	0.6				
HBV	2	0.2	4	0.6	6	0.4				
Scorpion Bite	1	0.1	17	2.5	18	1.2				
Submersion	6	0.7	8	1.2	14	0.9				
Hepatic Failure	5	0.6	15	2.2	20	1.3				
HIEP	19	2.3	9	1.3	28	1.9				
COVID-19	6	0.7	10	1.5	16	1.1				

Gastroschisis	1	0.1	9	1.3	10	0.7				
Status Asthmaticus	3	0.4	13	1.9	16	1.1				
Drug Poisoning	2	0.2	0	0	2	0.1				
Tetanus	4	0.5	0	0	4	0.3				
Severe Dehydration	2	0.2	18	2.7	20	1.3				
Croup	2	0.2	3	0.4	5	0.3				
CRRT	0	0	11	1.6	11	0.7				

Table 6: Body systems involved and PICU patient outcomes.

System Involved	Fate				P value	OR	95% CI	
	Dead		Discharged				Lower	Upper
	N	%	N	%				
Respiratory	446	54.3	269	39.6	0.630	0.986	0.932	1.044
CVS	65	7.9	72	10.6				
CNS	106	12.9	92	13.5				
Surgical	89	10.8	122	18				
Endocrine	27	3.3	49	7.2				
Renal	40	4.9	28	4.1				
Poisoning	3	0.4	2	0.3				
Metabolic	33	4	7	1				
GIT	12	1.5	38	5.6				

Table 7: Referral wards to PICU and patient outcomes.

Referral Ward	Fate				P value	OR	95% CI	
	Dead		Discharged				Lower	Upper
	N	%	N	%				
ER	393	47.9	317	46.7	0.043	1.082	1.002	1.168
Ward	56	6.8	49	7.2				
NICU	199	24.2	114	16.8				
Surgical Ward	72	8.8	114	16.8				
Other Hospitals	101	12.3	85	12.5				

Table 8: hospital stay and patient age in relation to their outcome.

	fate	N	Mean	Std. Deviation	P value
hospital stay (days)	Dead	821	5.107	3.8	0.000
	Discharged	676	6.173	3.8	
Age (months)	Dead	821	15.697	32.79	0.000
	Discharged	677	23.301	39.91	

DISCUSSION

This study looked at treatment outcomes and associated factors in pediatric patients admitted to the PICU. The PICU is a special unit that focuses on the care of critically ill patients. To achieve a positive outcome, all aspects of patient management must be thoroughly understood. [4]A pediatric intensive care unit's goal is to prevent death by closely monitoring and treating critically ill children who are considered to be at higher risk of death.

During the study period (from 2019 till 2023) a total number of 1501 patients had been admitted to PICU. This admission rate was less than that described by Parasher et al [5] who counted 2810 patients admitted into PICU in Udaipur district of Rajasthan in India from January 2016 to December 2020. However, their PICU was of ten beds and the city has a higher population number. The highest percentage of patients age group admitted to PICU was infants (73.9%), this goes with Parasher et al [5], Edae et al [6], and Gresh et al [7].

Of PICU patients, 55.2% were male versus 44.8% female. Male predominance also has been described by Parasher et al [5], Edae et al [6], and Abhulimhen-Iyoha [17 8]. Male gender is a known risk factor for many diseases. [9] Rural residents resemble 56.1% versus 53.9% of urban residents. This finding is against Dendir et al [10] who count 53.3% urban versus 46.7% rural residents. A total percent of 43.6 PICU patients were outside Najaf city, many of them from rural areas in which the health care standards are below optimum.

The main cause of PICU admission was respiratory distress syndrome (RDS), 22.7% followed by pneumonia, congenital heart diseases, and sepsis, 7.8%, 6.6%, and 6.3% respectively. Parasher et al [5] describes a 39.2% of admission were due to surgical diseases followed by pneumonia 17.3% and sepsis 15.2%. Edae et al [6] show that 14.2% of patients were admitted due to acute kidney injury followed by meningitis and pneumonia, 12.3% and 11.5% respectively. This difference may be attributed to the presence of assisted ventilation facilities within neonatal intensive care in these hospitals which is not the situation in ours that required referral of neonates with RDS to PICU. According to the above-mentioned findings, logically respiratory system is the most involved system in PICU patients accounting for 47.7%. Tazebew et al [11], also describe that the respiratory system is the mainly involved system in PICU patients in Addis Ababa, the capital city of Ethiopia but with a lower percentage (10%). Unlike Abhulimhen-Iyoha [8] who stated that 41.1% of PICU patients in India were admitted due to cardiovascular diseases.

By far, the emergency room (ER) was the main referral hospital ward to PICU with 47% followed by NICU at 21%. This is quite logical and supported by Edae et al [47] who found that 52.3% of PICU patients were referred from ER. The case fatality in Al Zahraa Teaching Hospital PICU was 54.7%. this mortality rate is lower than that found by Dendir et al [10] (71.8%) and much higher than that found by Parasher et al [14 5] and Abhulimhen-Iyoha [8], 2.1% and 2.4% respectively. I think that adding the NICU critically ill patients to the PICU may augment its mortality rate as the highest death in the pediatric age group is during the neonatal period.

54.2% of PICU patients required intonation and mechanical ventilation, again this is logical because the main system involved was the respiratory. This is close to Dendir et al [10] findings, of 43.4%, and against Tazebew et al [11] who count only 10% of PICU patients who need mechanical ventilation.

Neither gender nor residency resembles a risk factor for death in PICU patients (P value 0.197, OR 0.859, 95% CI 0.683-1.082, P value 0.298, OR 1.130, 95% CI 0.898-1.422 respectively). These findings are in agreement with Edae et al [6], Abhulimhen-Iyoha [17 8]., and Tazebew et al [11]. We have found that 64.4% of intubated

PICU patients, were died (P value 0.000, OR 0.403, 95% CI 0.325-0.501). This came in agreement with Dendir et al [10] who found that 61% mortality rate in PICU intubated patients. The infant age group resembles 78.1% of the case mortality rate in comparison to other age groups in PICU patients. A closer mortality rate for this age group was also described by Parasher et al [5] and Edae et al [6].

We have found that the main cause of death in PICU patients was RDS followed by sepsis, meningitis, congenital heart disease, and pneumonia (29.2%, 8.5%, 6.6%, 6.6%, and 6.2% respectively). According to Parasher et al [5], 38% of PICU deaths were due to sepsis, followed by meningitis (32.2%), congenital heart disease (29.9%), and pneumonia (22%). If we omit the burden of NICU-referred patients we can match the results of the leading cause of death. According to the above-mentioned results, the main system involved in PICU death was the respiratory system (54.3%) followed by the central nervous system, surgical cases, and cardiovascular systems (12.9%, 10.8%, and 7.9 respectively).

Out of total PICU death; 47.9% occurred in patients admitted from the emergency room (ER) followed by neonatal intensive care unit (NICU) 24.2%. (P value 0.043, OR 1.082, 95% CI 1.002-1.168). both of these wards deal with critically ill patients and resemble the main referral source for PICU admission. Edae et al [6] have found that 52% of PICU patients were admitted from ER, however, NICU contributed to only 1.2%.

The mean age of dead PICU patients was 15.697±32.79 months versus 23.301±39.91 months for discharged outcome (P value 0.000). Hospital stay length mean for PICU dead patients was 5.1±3.8 days versus 6.173±3.8 days for discharged patients (P value 0.000). That means the smaller age patients carry a higher risk of death in PICU, also the situation with fewer hospital stays days. Tazebew et al [11] state that the majority of the children (84.2%) stayed in the ICU for less than seven days.

CONCLUSIONS

In conclusion, this study showed the case fatality rate was high (54.7%.). The most common causes of admission and death were RDS (22.7% and 29.2% respectively). The highest percentage of death occurs in the infant age group (78.1%). Nearly half of PICU cases were referred from ER (47%) followed by NICU (21%). Neither gender nor residency represents a risk factor for PICU patient's death but endotracheal intubation does. The fewer days of hospital stay carry a higher risk factor for death

RECOMMENDATIONS

1. Minimizing the death rate in Pediatric Intensive Care Units (PICU) involves a multifaceted approach that includes:
   A. Improving Quality of Care: Implementing evidence-based clinical guidelines, continuous staff education, and training can enhance patient care.
   B. Early Recognition and Management: Prompt identification and treatment of critical conditions can improve outcomes. This includes the use of early warning systems and rapid response teams.
   C. Regular Audits and Feedback: Conducting regular audits of PICU practices and outcomes, followed by feedback, can help identify areas for improvement.
2. Respiratory support by mechanical ventilation should be settled within the NICU to minimize the burden upon the PICU.
3. More focus should be directed toward the main cause of PICU death namely RDS, by revision of standard management consequences and staff training.

Limitations

Paperwork with patient's files is time-consuming with missing information. An electronic database may give more accurate and easy-to-access patient information.

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Causes of Neonatal Re-admission in 24 hours after Cesarean Section

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ABSTRACT

Background: According to the American Academy of Pediatrics (AAP), a mother who gives birth to a healthy, term baby should stay in the hospital for as long as necessary to detect any possible issues and give caregivers time to get the child (and family) ready for release. The infant's risk of readmission may be heightened by diagnostic mistakes, or misdiagnosis, and inadequate assessment by healthcare personnel. In order to assess the frequency and cause of hospital readmissions within the first twenty-four hours of life, this study will examine the local pattern of neonatal readmissions in healthy infants.

Patients and methods: This cross-sectional study included 33 neonates who had a c-section birth, after being discharged, and readmission within 24 hours of delivery. The following newborn characteristics were examined: gestational age, birth weight, gender, duration of initial hospital stay (LOS), infant feeding method, and the type of ward from which the neonate was released following the initial evaluation. The following factors related to pregnancy and delivery were evaluated: advanced maternal age (>35 years), low socioeconomic status, low maternal education, high birth order (>2), joint family, rural area residents, primigravida, premature rupture of membranes (PROM), chorioamnionitis, preeclampsia, diabetes, psychosocial issues, thyroid disorders, and epidural/spinal anesthesia.

Results: Sixteen patients out of 33 readmitted neonates were male. the rate of neonatal readmission was 1.34%. Their average birth weight and gestational age were 2918.2 ± 619.2 gm and 36.8 ± 21.72 weeks respectively. They spent 2.62 ± 1.83 hrs. and readmitted at 7.82 ± 5.35 hrs. intervals. The main causes of readmission were vomiting and poor feeding. Regarding the maternal factors; epidural anesthesia and low education was the most significant risk factors.

Conclusions: An important component of the quality-of-care consequences of existing discharge rules and procedures may be revealed by this study. We come to the conclusion that vomiting and poor feeding are the main causes of hospital readmission in the first 24 hours of life, necessitating a proper intervention in the early hours of life. The early development of efficient lactation should be the focus of efforts to lessen digestive issues.

Keywords: Neonatal Readmission, Hospital Stay, Early Discharge.

Article Information

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INTRUDUCTION

According to the American Academy of Pediatrics (AAP), a mother who gives birth to a healthy-term baby should stay in the hospital for as long as necessary to detect any possible issues and give caregivers time to get the child (and family) ready for release. Diagnostic mistakes, or misdiagnosis, and inadequate assessment by healthcare personnel may heighten the infant's risk of readmission. [1]. Over the past 20 years, there has been a noticeable decline in the average length of hospital stay for women and babies. This decrease can be attributed to the hospital's bed capacity limitations as well as the desire of families to lower hospital stay costs.[2] Iraq's "Early Discharge" (ED) policy is primarily motivated by a shortage of hospital beds rather than budgetary limitations because the country's healthcare system is essentially free. There are 1.3 hospital beds in Iraq for every 1,000 people. Here, 2.9 beds is the worldwide mean. [3] A readmission occurs when a patient is admitted to the same hospital or another hospital within 30 days of being discharged.[4] Over the last ten years, readmission rates for term-born babies have been less than 1.3% in the US and less than 8% worldwide. The literature has examined factors that may have an effect on readmission of neonates. These include readmission-influencing variables related to mothers, newborns, institutions, and healthcare providers. [5]

Many studies examined the causes of neonatal readmission. In 2013, Young et al conducted a study in the USA and found that 5308 out of 296,114 births of health discharge neonates were readmitted to the hospital within 28 days, i.e., (1.8%). Feeding problems were the cause for the majority of readmissions (41%), followed by jaundice (35%).[6] A study by Alsulami and Al Saif [7] conducted at King Abdulaziz Medical City (KAMC), Riyadh, Saudi Arabia, between 2010 and 2011, showed that 947 out of 16,844 neonates (1.34%) were either readmitted to the hospital or had visited the emergency room (ER) within 7 days from discharge. Moreover, jaundice was the most common cause of readmission/ER visits (38%).(8). No study has assessed the etiology of neonatal readmissions in Iraq. Normal vaginal deliveries of newborns are released from most public sector hospitals as soon as the mother and child are stable, whereas cesarean deliveries usually result in the mother and child being released on the second day following birth. To assess the frequency and cause of hospital readmissions within the first 24 hours of life, the current study examined the local pattern of neonatal readmissions in healthy infants.

PATIENTS AND METHODS

This cross-sectional study was carried out between December 1, 2022, and June 1, 2023. The study included 33 neonate patients

The Study Design

It is a cross-sectional study.

Exclusion criteria

1. Newborns who were deemed healthy and free of prenatal or postnatal issues at the time of discharge.
2. A c-section birth result.
3. After being discharged, readmission within 24 hours of delivery.
4. Unexpected visit to the hospital's emergency room or NICU.

Neonatal patients delivered in another hospital were an exclusion criterion. The investigation did not include mothers with a history of preterm birth, infants with intrauterine growth retardation (IUGR), congenital malformations, birth asphyxia, or respiratory distress.

Retrospective file reviews of the hospital's medical records were used to gather clinical information on both the mother and the child. Three files per readmission were examined: one from the readmission of the infant, one from the admission of the newborn's birth, and one from the admittance of the mother's The following

newborn characteristics were examined: gestational age, birth weight, gender, duration of initial hospital stay (LOS), infant feeding method, and the type of ward from which the neonate was released following the initial evaluation.

The following factors related to pregnancy and delivery were evaluated: advanced maternal age (>35 years), low socioeconomic status, low maternal education, high birth order (>2), joint family, rural area residents, primigravida, premature rupture of membranes (PROM), chorioamnionitis, preeclampsia, diabetes, psychosocial issues, thyroid disorders, and epidural/spinal anesthesia delivery.

Data collection

Using Excel from Microsoft Office 2019, all information was gathered and examined. All neonatal and maternal variables' means, standard deviations, maximums, minimums, and frequencies were computed. The scientific committee of the pediatrics division at the University of Kufa's faculty of medicine gave the study its ethical blessing.

RESULTS

During the study period, a total number of 33 neonates were enrolled in the study as their readmission criteria fit the study's inclusion criteria. The average birth weight of enrolled patients was 2918.2 ± 619.2 gm, ranging from 4800 to 2000 gm. Other neonatal criteria were listed in Table 1. Sixteen patients out of a total of 33 readmitted neonates were male, Figure 1.

The number of cesarean sections that had been done in Al Zahraa teaching hospital in the study period (i.e., between the 1st of September 2022 till the 1st of June 2023 were 3118 cesareans, of which 657 neonates had been admitted directly to NICU due to various medical problems. So, 2461 cesareans produce healthy neonates that considered for home discharge with their mothers was 2461, so the rate of neonatal readmission was 1.34%. The causes of neonatal readmission within 24 hours of birth were as the following: About half of thirty-three readmitted neonate patients were exclusively breastfed. About 90% of readmitted neonate patients were initially discharged to shared hospital wards. Many maternal risk factors had been studied that may be related to neonatal readmission. All of the thirty-three readmitted neonate mothers were delivered by spinal anesthesia. Other risk factors are stated in Figure 4. Each presumed maternal risk factor was studied in comparison with the length of initial hospital stay, Table 2.

All the thirty-three readmitted neonates were discharged well to home. Twenty-two patients out of a total number of 33 enrolled neonates were discharged initially from NICU within a period of less than 2 hours, Figure 6.

Table (1): The neonatal criteria of readmitted patients.

Variables	Mean	Std. Deviation
GA (wks.)	36.82	1.72
Birth Weight (gm)	2918.18	619.2
Length of Initial Stay (hrs.)	2.62	1.83
Interval Discharge and Readmission (hrs.)	7.82	5.35
APGAR score 1 min	6.6	1.1
APGAR score 5 min	8.7	0.8

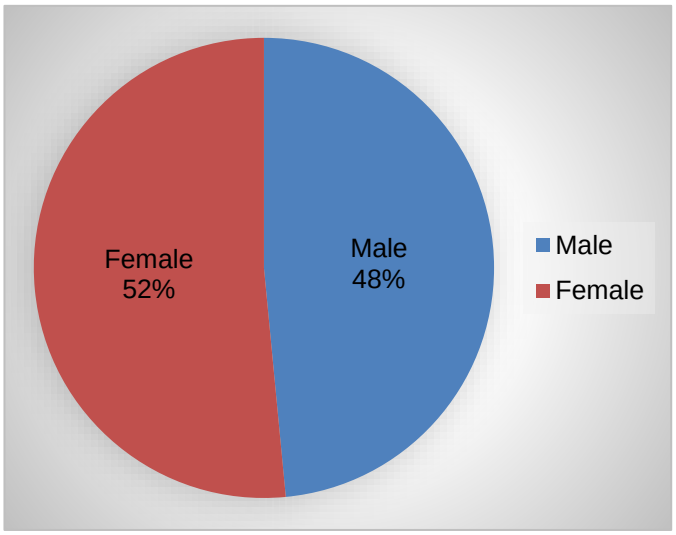

Figure 1: Sex distribution of enrolled patients.

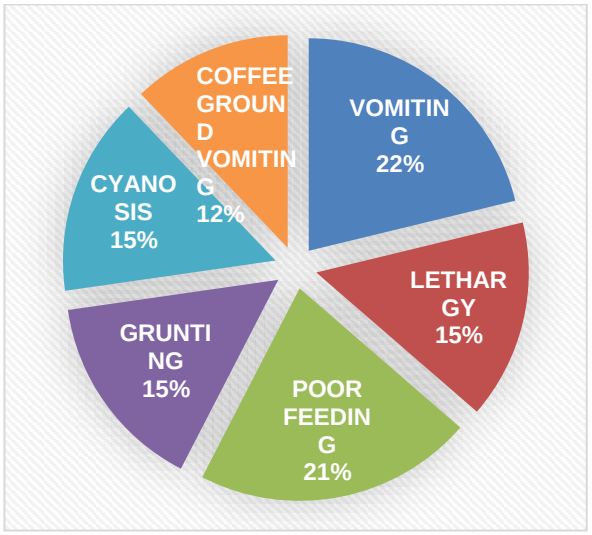

Figure 2: causes of neonatal readmission.

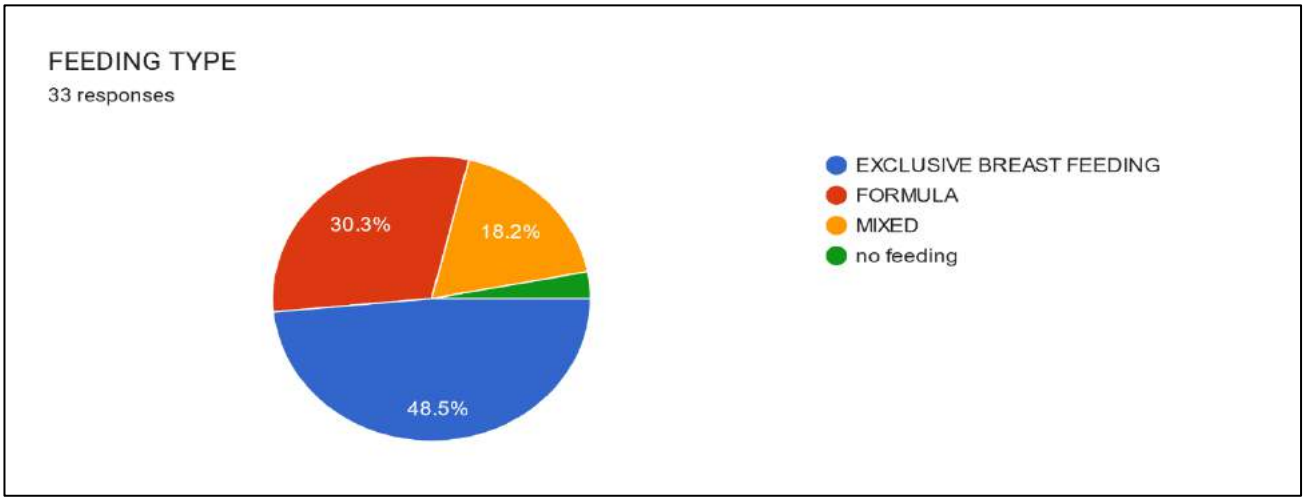

Figure 3: Type of feeding in the enrolled patients.

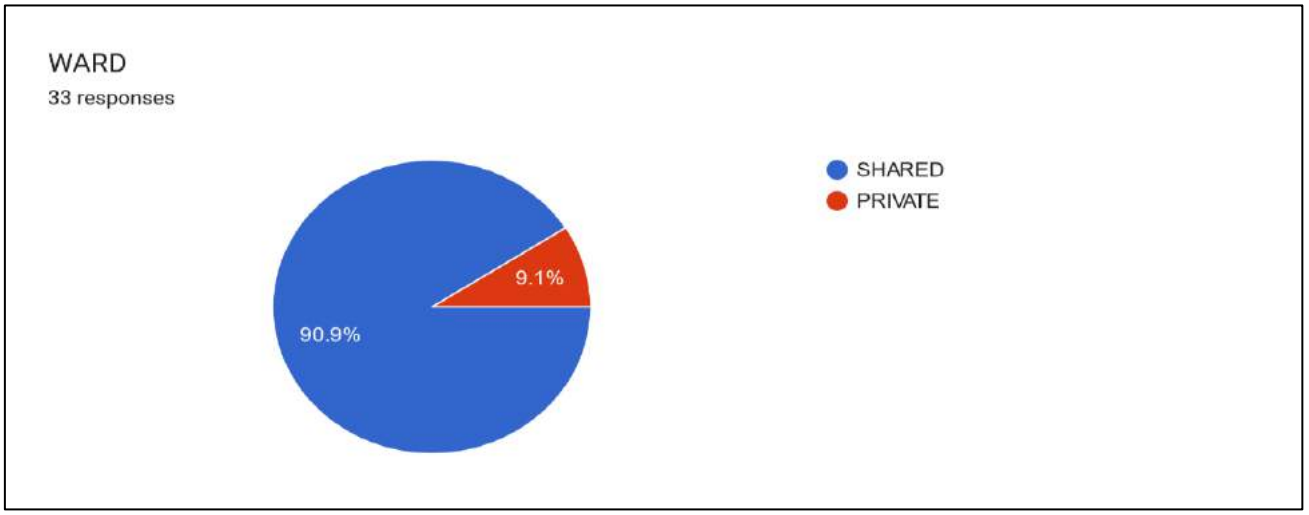

Figure 4: Type of discharged hospital ward.

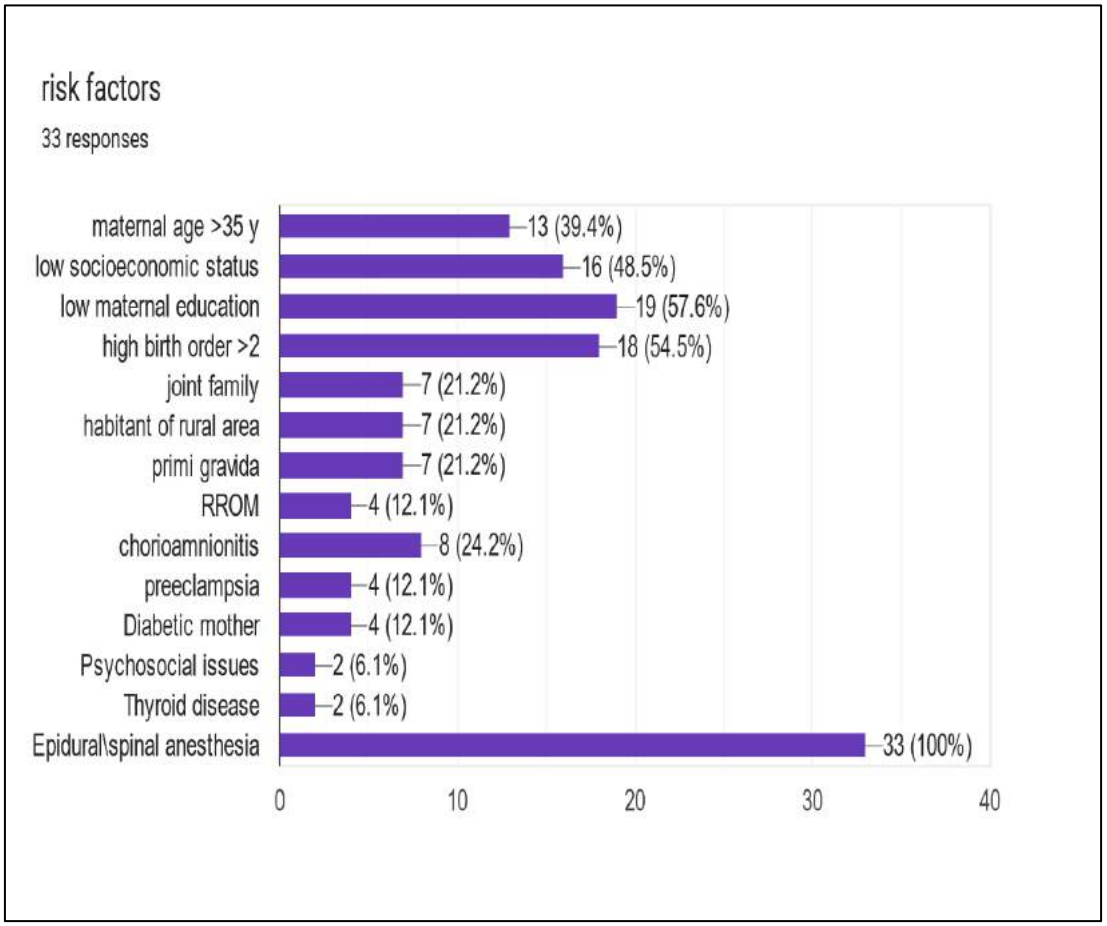

Figure 5: Maternal risk factors that may be associated with neonatal readmission.

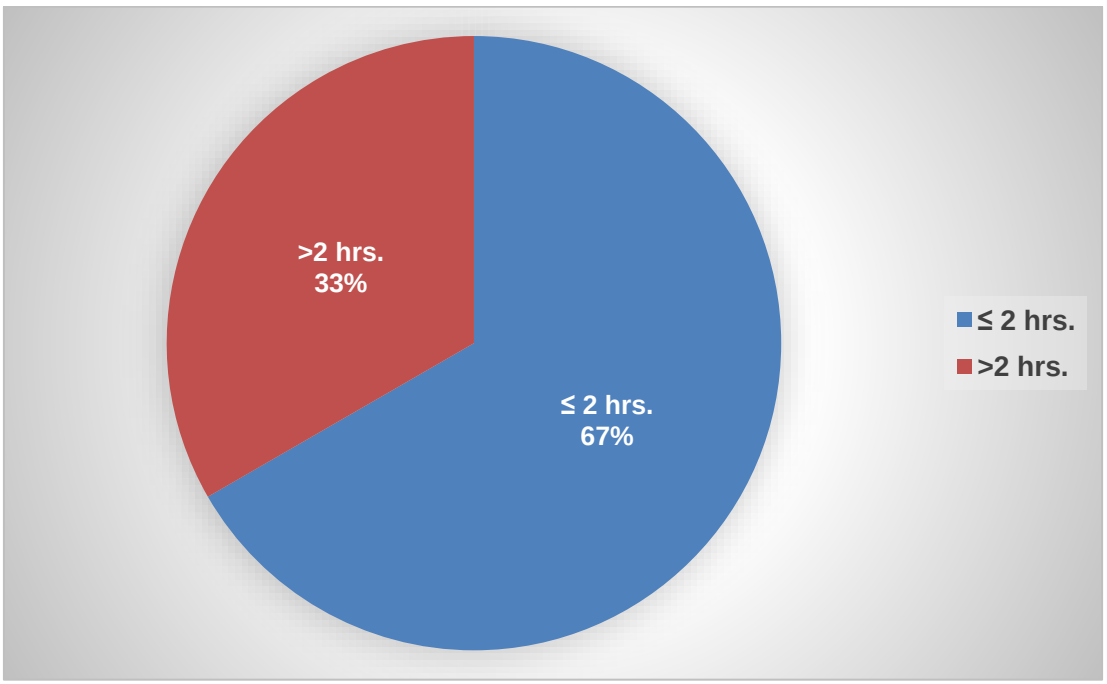

Figure 6: the percent of early vs late initial NICU discharge.

Table 2: Comparison of maternal risk factors with the initial hospital stay.

		Length of stay groups		Total	Odd ratio	P value	95% CI
		≤2 hrs.	>2 hrs.				
Sex	Male	11	5	16	1.2	1.000	0.281 – 5.124
	female	11	6	17			
Ward	Private	3	0	3	1.579	0.534	1.2 – 2.07
	Shared	19	11	30			
Advanced maternal age	No	13	7	20	0.825	1.000	0.185 – 3.676
	yes	9	4	13			
Low socioeconomic status	No	12	6	18	1.000	1.000	0.234 – 4.278
	yes	10	5	15			
low maternal education	No	11	3	14	2.667	0.278	0.55 – 12.79
	yes	11	8	19			
high birth order	No	10	5	15	1.000	1.000	0.234 – 4.278
	Yes	12	6	18			
joint Family	No	18	7	25	2.571	0.391	0.5 – 13.22
	Yes	4	4	8			
Habitants of rural area	No	16	9	25	0.593	0.687	0.098 – 3.57
	Yes	6	2	8			
Primi gravida	No	18	9	27	1.000	1.000	1.53 – 6.53
	Yes	4	2	6			
PROM	No	19	10	29	0.633	1.000	0.058 – 6.9
	Yes	3	1	4			
Chorioamnionitis	No	18	7	25	2.57	0.391	0.5 – 13.22
	Yes	4	4	8			
Preeclampsia	No	19	10	29	0.633	1.000	0.058 – 6.9
	Yes	3	1	4			
Diabetes	No	19	10	29	0.633	1.000	0.058 – 6.9
	Yes	3	1	4			
Psychosocial issues	No	20	11	31	0.645	0.542	0.497 – 0.83
	Yes	2	0	2			
Thyroid Diseases	No	21	10	31	2.1	1.000	0.12 – 37.1
	Yes	1	1	2			
Feeding Type	No feed	1	0	1		0.665	
	EBF	10	6	16			
	BF	6	4	10			
	MF	5	1	6			

DISCUSSION

The healthcare institution is greatly impacted by readmissions or rehospitalizations, which result in a strain on staff, resources, expertise, tools, and procedures. The first hospital stay after delivery offers a chance to learn how to nurse, become a better parent, and spot any early newborn health issues. Many of these abilities, nevertheless, take time to develop; for example,

well-established breastmilk, which is linked to a lower risk of jaundice, often takes three or four days to build and is less likely to occur in situations where early discharge occurs. [9] The ever-growing demands on hospital resources and patient turnover are in competition with this time-sensitive service. [10, 11] The goal of the current study was to evaluate the outcomes, causes, risk factors, and readmission rate of neonates who were released from tertiary care institutions in Najaf City, Iraq.

The rate of neonatal readmission in Al Zahraa Teaching Hospital in our study was 1.34%, this rate is lower than that found by Bawazeer, et al [12], 2.1%. however, Bawazeer, et al studied neonatal readmission during 28 days (neonatal period) which is not the situation in our study (first 24 hours of life). Habib H. S. [13], give a neonatal readmission rate close to our findings, 1.3%. Bayoumi et al [10] found a higher neonatal readmission rate (10.1%). This controversy may be justified by different assessments of discharge readiness following childbirth in these study centers.

The period of time from the time of birth till the patient is released to go home is referred to as the initial hospital stay. In our study, this equated to 2.62±1.83 hours. When compared to US standards, which generally recommend regular newborn discharge 24 hours after vaginal delivery and 72 hours following cesarean delivery, this is a fairly short period of time. Nonetheless, during the past ten years, the average duration of stay in the hospital following birth for healthy, full-term infants in the US has decreased significantly. Many managed care companies are trying to speed up this trend by enacting policies that demand an earlier release for full-term newborns without evident health issues, all in an effort to control the cost of medical treatment. [14–16]

The main causes of neonatal readmission during the first 24 hours in our study were: poor feeding (22%), vomiting (21%) cyanosis, grunting, lethargy (15% for each), and coffee ground vomiting, (12%). According to Farahat [17], the main cause of neonatal readmission was hyperbilirubinemia followed by suspected neonatal sepsis (84.2% and 10.5% respectively. This is quite logical and highly anticipated because this study considered readmission during the first 28 days of life which is not the situation in our study. Bawazeer, et al [12] studied early neonatal readmission (less than 7 days) and found that the main causes of readmission were: jaundice (54.8%), suspected sepsis (11.5%), and gastrointestinal tract disorders (6.4%). Brown et al [18] had correlate the length of the Initial Hospital Stay with the causes of neonatal readmission and found that apnea was the most common cause of neonatal readmission in correlation with a shorter period of Initial Hospital Stay, followed by birth defects and feeding and gastrointestinal problems.

Neonatal and maternal risk factors have been studied to anticipate the probability of them as potential risk factors for readmission. Regarding neonatal risk factors, our study found that 52% of readmitted neonates were female versus 48% male. This variable was statistically insignificant to be regarded as a potential risk factor for neonatal readmission. This is against Bawazeer, et al [12], who found that the readmitted neonates were mainly male (54.7%) in comparison to (45.3%) females with a statistically significant P value (0.01). This difference could be attributed to big differences in the population sample size (33 neonates in our study versus 570 in Bawazeer, et al study). The mean Birth Weight of readmitted neonates was 2918.18±619.2 gm. That is so close to Bawazeer, et al [12] findings (3000±50) gm and lower than that for Jarrett et al, [19] (3387±575 gm). Again, different sample sizes may attribute this variance. Sixteen out of a total of 33 readmitted neonates (48.5%) had started exclusive breastfeeding, 10 (30%) neonates fed by the bottle, 6 (18.2%) by mixed feeding and lastly, one neonate (3%) did not commence

feeding at all. This variable was insignificant as a potential neonatal readmission risk factor (P value 0.665). Jarrett et al, [19] reported that breasts 69%, artificial (bottle, syringe) 10%, breast and artificial 20%, and No documentation 1%. The lower percentage of exclusive breastfeeding in our center notifies poor counseling skills about the importance of exclusive breastfeeding.

Many maternal variables had been evaluated in our study searching for their significance as neonatal readmission risk factors; these variables were: advanced maternal age (>35 years), low socioeconomic status, low maternal education, high birth order (>2), joint family, rural area residents, primigravida, premature rupture of membranes (PROM), chorioamnionitis, preeclampsia, diabetes, psychosocial issues, thyroid disorders, and epidural/spinal anesthesia. No one of these maternal variables was statistically significant as a risk factor for neonatal readmission during the first 24 hours of life. This is the same in Jarrett et al, [51] study, however; he studies only preeclampsia and maternal diabetes mellitus. Bawazeer, et al [44] studied more maternal diseases such as anemia, obesity, and asthma but they also did not record a statistically significant odd ratio. Farahat [49], gets a significant odd ratio regarding maternal variables (odd ratio 1.985, 95% CI 0.962 -0.097, P value 0.05) but he studies all the maternal risk factors in one scope i.e., obstetric complications.

Another variable that was neither neonatal nor maternal was the ward type to which the neonate with his mother had been discharged for. About 90% of neonates were discharged to shared wards in comparison to only 10% to private ones. The odd ratio points toward the possible contribution of this variable as a risk factor for neonatal readmission.

CONCLUSIONS

An important component of the quality-of-care consequences of existing discharge rules and procedures may be revealed by this study. We come to the conclusion that vomiting and poor feeding are the main causes of hospital readmission in the first 24 hours of life, necessitating a proper intervention in the early hours of life. The early development of efficient lactation should be the focus of efforts to lessen digestive issues.

RECOMMENDATIONS

1) To support and inform nursing mothers through medical professionals.
2) To design a systematic strategic plan for the infants who were released within two hours; this includes altering the post-discharge care routines, such as early follow-up or home visits from medical personnel.
3) Future prospective studies on the factors that influence newborn readmission are urged as they may aid in the early detection and evaluation of these factors prior to discharge.
4) As a final point, we propose that by adhering to the proper national guidelines, the morbidity and mortality caused by early neonatal discharge can be significantly reduced and managed.

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Knowledge and Attitude of The Medical Staff Toward Poliomyelitis and Polio Vaccination Campaign: A Cross–Sectional Study in Al-Najaf Al -Ashraf City–Iraq

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ABSTRACT

Background: *Medical staff needs to understand factors that affect poliomyelitis vaccination because their attitudes about vaccination are highly associated with rates of childhood vaccination if medical staff do not view a specific vaccine as necessary or acceptable, vaccination campaigns can be ineffective.* ***Aim of the study:*** *To assess the knowledge and attitudes of the medical staff toward poliomyelitis and polio vaccination campaigns.* ***Patients and methods:*** *A cross-sectional study conducted from 1st of January 2019 to 29th February 2020, and included (332) medical staff from randomly selected hospitals and primary health centers. Data were collected using a questionnaire designed for the study, which consists of three parts: socio-demographic and personal information, knowledge, and attitude. for knowledge, each correct answer scored 1 while the wrong one scored 0. For attitude, each "agree" answer scored 1 while "disagree" or "I don't know" scored 0. Statistical analyses were done using SPSS version 26 and a P- value ≤0.05 was considered Statistically significant.* ***Results:*** *The study included 332 medical staff: 143 (43.1%) doctors, 77 (23.2%) dentists, and 112 (33.7%) pharmacists. The mean knowledge score was higher among doctors than dentists and pharmacists (18.22, 15.38, and 15.67, respectively, p = 0.0001). As age increased, the mean knowledge score also increased (20.22 for 50-59 years old vs. 14.81 for 23-29 years old, P = 0.0001). The attitude score followed the same trend, being higher among doctors and older age groups (2.97 vs. 2.68 and 2.82, P = 0.0001) and higher for the older age group (3 vs. 2.82, P = 0.0001).* ***Conclusions:*** *The mean knowledge and attitude scores of doctors are significantly higher than dentists and pharmacists and for the older age group.*

Keywords: Attitude, Knowledge, Polio Campaign, Poliomyelitis.

Article Information

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INTRUDUCTION

Poliomyelitis (polio) is a highly contagious viral disease caused by the poliovirus that most commonly affects children under 5 years of age. It is a serious problem in large parts of the developing world and continually poses threat to the child population with critical concerns for social and economic development [(1)].

Poliomyelitis is mainly transmitted via the fecal-oral route and through person-to-person contact with infected secretions from the nose and mouth. Approximately 95% of individuals infected with polio show no apparent symptoms, while another 4%–8% experience minor, non-specific symptoms such as sore throat, fever, nausea, and vomiting, which are common to many viral illnesses [(2)].

There are two rounds per year for the polio vaccine campaign, each round consists of two campaigns one month apart, the first campaign runs in March and the second one usually runs in October and the target group includes all the children from birth to five years. Polio vaccine is one of the most important vaccinations imposed on children .It is therefore included in the compulsory vaccination program in all countries, it is essential for the prevention of polio, which affects the nervous system and the life of the person in general [3].

Improving the immunization-related knowledge and attitudes of medical staff is vital. Enhanced knowledge about the risks and benefits of vaccination can significantly influence the success of immunization campaigns. Medical staff plays a critical role in child immunization and have a positive impact on parents' decisions regarding vaccinations. Therefore, medical staff needs to understand the factors that influence polio vaccination. Their attitudes towards vaccination are closely linked to children's vaccination rates. If medical staff do not perceive a particular vaccine as necessary or acceptable, vaccination campaigns may become ineffective. Barriers to immunization include misinformation about vaccine-preventable diseases, concerns about adverse effects, and misunderstandings about the development of diseases post-vaccination. Gaps in medical staff's knowledge about adverse effects and contraindications often lead to decreased immunization rates [4 5 6].

The medical staff is considered as an important member of society to fight endemic diseases like polio. The role of medical staff is exceptional for improving access to healthcare and health-seeking behavior [7]. Medical staff forms an important part of immunization campaigns this is expected to increase vaccination coverage. Furthermore, the medical staff is also responsible for ensuring the effectiveness of polio vaccines by adhering to cold chain guidelines [8]. They are the source of information to the parents and the community about the importance and benefits of vaccination. Therefore, the medical staff needs to have the proper knowledge and positive attitudes toward polio vaccination as it is crucial to the success of immunization campaigns. Furthermore, assessment of knowledge and attitudes is an ongoing process that serves as an educational diagnosis among medical staff to assess changing beliefs and behaviors over time [9]. So that this study aimed to assess the knowledge and attitude of the medical staff toward poliomyelitis and polio vaccination campaigns.

PATIENTS AND METHODS

The Study Design: A cross - sectional study from 1st January 2019 to 28th February 2020.

Setting: Primary health care centers and Hospitals in Al - Najaf city - Iraq.

Subjects: By using a simple random sampling, four major hospitals.

Controls: Seventeen primary health care centers were selected. The participants were selected conveniently.

Instruments

Data were collected by a questionnaire constructed by the researchers and derived from previous articles on medical staff knowledge and attitudes toward the poliomyelitis vaccination campaign (10 7) the questionnaire was reviewed by a panel of five experts in the field with an experience of more than five years. It includes full Socio-demographic and personal information and an assessment of medical staff knowledge and attitude toward the poliomyelitis and polio vaccination campaign. The data were collected by direct interviews with the medical staff.

Scoring: Regarding knowledge, each correct answer was given a score of 1, and each incorrect answer was given a score of 0. For attitude, each 'Agree' response was given a score of 1, while 'Disagree' or 'I don’t know' responses were given a score of 0."

Sample size calculation

The sample size estimation was according to the following equation

Sample size $= \frac{Z1-\alpha/2^2 P(1-P)}{d^2}$ [11]

Where Z1-α/2 equals 1.96 at 95% confidence intervals, d was decided to be 5%, and the expected proportion of the population with good knowledge about the polio vaccination Campaign based on previous studies in Pakistan is 70% of good knowledge, so that the minimal sample size equals332.

Pilot Study: Before collecting information, a two-week pilot study was conducted in Al - Sader Medical City from first to 14th. March 2019 on 20 medical personnel who were excluded from the study sample. It aimed to test the validity and reliability of the questionnaire and estimate the time needed for the collection of data, in addition to find any other difficulties. Modifications were made to the questionnaire according to the pilot study.

Ethical approval

The study was approved by the Iraqi Council for medical specialization, an official agreement from al Najaf – al Ashraf general health directorate and verbal consent was obtained from all participants. the data were kept confidentially.

STATISTICAL ANALYSIS

The collected data were summarized and analyzed statistically using the statistical package for the social science (SPSS) software. Mean and SD for continuous variables, t-test was used to compare the mean knowledge and attitude score between males and females, and ANOVA test was used to compare mean knowledge score among different occupations and age groups. Frequency and percentage illustrated categorical data; chi-square test was used to find associations between categorical variables. P-value ≤0.05 was considered Statistically significant.

RESULTS

The current study consists of 332 medical staff. They were distributed as follows: 143 (43.1%) doctors, 77 (23.2%) dentists, and 112 (33.7%) pharmacists. Regarding sex distribution, 134 (40.4%) were males and 198 (59.6%) were females. The mean age is 32.5±6.3 years with a minimum of 23 years and a maximum age of 55 years, the details of the socio-demographic features of the medical staff are shown in Table 1. The mean knowledge score was significantly higher among doctors than dentists and pharmacists (18.22, 15.38 and 15.67, respectively) P= 0.0001. As the age increases, the mean knowledge score increases (20.22 for 50-59 years old vs 14.81 for 23-29 years old) P= 0.0001. The attitude score followed the same role as it was higher among doctors (2.97 vs 2.68 and 2.82 with P = 0.0001) and older age group staff (3 vs 2.82 with a P = 0.0001). There was no significant difference in the knowledge and attitude regarding sex, as shown in Table 2.

Regarding the percentage of correct answers to knowledge questions, in general, the doctors had a higher percentage of correct answers and this is significant in questions about the causative agent, the main route of transmission, other possible routes of transmission, the routes of vaccine administration, immunization through a campaign to prevent poliomyelitis if poliomyelitis vaccine can cause paralysis, Poliomyelitis vaccination can be given with other vaccines in the same time, lack of immunization against polio is a major risk factor of poliomyelitis, More than one dose of vaccine required for complete protection, poliomyelitis drops vaccine can be given to a child with a mild illness, vomiting, and diarrhea are not

contraindicated to oral poliomyelitis vaccination, Poliomyelitis can cause the death of the patient and inactivated poliomyelitis vaccine (IPV) can't cause poliomyelitis, as shown in Table 3. For the attitude of the medical staff toward the polio campaign, 326 (98.2 %) supported immunization, with doctors being (100 %) agreed than others but with no significant relationship, while 309 (93.1 %) accepted immunization, which was significantly higher in doctors (100 %) than others.

Of the total sample, 305 (91.9 %) preferred immunization which again was significantly higher in doctors (97.2 %) than others, as shown in Table 4.

Table (1): Socio-demographic features of the sample (N= 332).

Feature		No.	%
Age group (years)	23-29	102	30.7
	30-39	184	55.4
	40-49	37	11.1
	50-59	9	2.7
Sex	Male	134	40.4
	Female	198	59.6
Occupation	Doctor	143	43.1
	Dentist	77	23.2
	Pharmacist	112	33.7
Address of work place	Primary health care centers (PHC)	222	66.9
	Hospitals	110	33.1
Marital Status	Married	263	79.2
	Unmarried	69	20.8
Number of children	No	87	26.2
	1-3	196	59.0
	4+	49	14.8

Table (2): The mean knowledge and attitudes scores according to sex, occupation, and age group.

Variables	Subgroup	Knowledge mean scores	P value	Attitudes mean scores	P value
Sex	Male	16.81	0.99	2.84	0.9
	Female	16.80		2.82	
Occupation	Doctor	18.22	0.0001	2.97	0.0001
	Dentist	15.83		2.79	
	Pharmacist	15.67		2.68	
Age group (years)	23-29	14.83	0.0001	2.82	0.0001
	30-39	17.45		2.83	
	40-49	18.22		2.81	
	50-59	20.22		3.00	

Table 3: The distribution of medical staff knowledge about poliomyelitis and poliomyelitis vaccination campaign.

Item name	Doctors N=143	Dentists N=77	Pharmacists N=112	Total	P value
Poliomyelitis is a viral disease	140 (97.9%)	64 (83.1%)	103 (92%)	307 (92.5%)	0.002*
The main route of transmission through feco- oral route	134 (93.7%)	49 (63.6%)	76 (67.9%)	76 (67.9%)	0.0001*
Route of vaccine administration through oral and injectable route	48 (33.6%)	17 (22.1%)	41 (36.6%)	106 (31.9%)	0.01*
What is the target age group for the Poliomyelitis Vaccination Campaign (0-5 years)	133 (93.0%)	67 (87.0%)	102 (91.1%)	302 (91.0%)	0.3
The target group includes children Who are all children regardless of immunization status	129 (90.2%)	64 (83.1%)	88 (78.6%)	281 (84.6%)	0.08
Immunize your child through a campaign, to prevent poliomyelitis	84 (58.7%)	34 (44.2%)	36 (32.1%)	154 (46.4%)	0.0001*
All children should be vaccinated in the campaign	136 (95.1%)	71 (92.2%)	110 (98.2%)	317 (95.5%)	0.1
Paralysis in Poliomyelitis is not curable	120 (83.9%)	53 (68.8%)	86 (76.8%)	259 (78.0%)	0.1
Poliomyelitis is preventable	142 (99.3%)	72 (93.5%)	105 (93.8%)	319 (96.1%)	0.1
Poliomyelitis can also be transmitted through contaminated food and water by the feces of an infected person	128 (89.5%)	64 (83.1%)	91 (81.3%)	283 (85.2%)	0.04*
poliomyelitis vaccine can cause paralysis	123 (86.0%)	66 (85.7%)	72 (64.3%)	261 (78.6%)	0.0001*

Immunization is the most effective way of preventing poliomyelitis	123 (86.0%)	59 (76.6%)	86 (76.8%)	268 (80.7%)	0.1
Poliomyelitis vaccination can be given with other vaccines at the same time	140 (97.9%)	64 (83.1%)	93 (83.0%)	297 (89.5%)	0.0001*
Lack of immunization against poliomyelitis is a major risk factor for poliomyelitis	127 (88.8%)	72 (93.5%)	103 (92.0%)	302 (91.0%)	0.001*
More than one dose of vaccine is required for complete protection	140 (97.9%)	56 (72.7%)	91 (81.3%)	287(86.4 %)	0.0001*
Healthy children also need vaccination	134 (93.7%)	71 (92.2%)	99 (88.4%)	304 (91.6%)	0.1
Poliomyelitis drops vaccine can be given to children with a mild illness	87 (60.8%)	38 (49.4%)	52 (46.4%)	177 (53.3%)	0.0001*
Vomiting and diarrhea are not contraindicated by oral Poliomyelitis vaccination	54 (37.8%)	23 (29.9%)	18 (16.1%)	95 (28.6%)	0.0001*
Poliomyelitis can cause the death of the patient	77 (53.8%)	41 (53.2%)	47 (42.0%)	165 (49.7%)	0.01*
Inactivated poliomyelitis vaccine (IPV) can't cause poliomyelitis	83 (58.0%)	31 (40.3%)	44 (39.3%)	158 (47.6%)	0.005*
The poliomyelitis campaigns are necessary	136 (95.1%)	72 (93.5%)	108 (96.4%)	316 (95.2%)	0.5
If the child completes vaccination on a card regularly, it is not sufficient, and need a vaccination campaign	74 (51.7%)	26 (33.8%)	50 (44.6%)	150 (45.2%)	0.1
Repeated vaccination in poliomyelitis campaign can't cause vaccination overdose	114 (79.7%)	47 (61.0%)	55 (49.1%)	216 (65.1%)	0.0001

Table 4: Distribution of attitude questions in the studied sample.

Attitude	The variables	Group			Total	p
		Doctors N=143	Dentists N=77	Pharmacists N=112		
Do you support the immunization activity against Poliomyelitis in the campaign	Agree	143(100%)	75(97.4%)	108(96.4%)	326(98.2%)	0.1
	Disagree	0 (0.0%)	2 (2.6%)	2 (1.8%)	4 (1.2%)	
	I don't know	0 (0.0%)	0 (0.0%)	2 (1.8%)	2 (0.6%)	
Do you accept the immunization with Poliomyelitis vaccination campaign	Agree	143(100%)	70(90.9%)	96 (85.7%)	309(93.1%)	0.0001
	Disagree	0 (0%)	6 (7.8%)	14 (12.5%)	20 (6.0%)	
	I don't know	0 (0.0%)	1 (1.3%)	2 (1.8%)	3 (0.9%)	
Do you prefer immunization with a vaccination campaign	Agree	139(97.2%)	70(90.9%)	96 (85.7%)	305(91.9%)	0.02
	Disagree	4 (2.8%)	5 (6.5%)	11 (9.8%)	20 (6.0%)	
	I don't know	0 (0.0%)	2 (2.6%)	5 (4.5%)	7 (2.1%)	

DISCUSSION

Poliomyelitis (polio) is a highly infectious viral disease; that most commonly affects children under the age of 5 years. [1]. To our knowledge, this is one of the few studies to assess medical staff knowledge and attitude towards the polio vaccination campaign. The results revealed that doctors had a significantly higher mean knowledge score than dentists and pharmacists. This finding is consistent with previous studies conducted in Nigeria, Pakistan, and India [12 13 14]. This disparity might be attributed to the fact that polio is more extensively covered in the curriculums of medical schools compared to dentistry and pharmacy schools, highlighting a gap in the teaching of this important subject.

In the current study, medical staff aged 50 - 59 years; were found to have a higher mean knowledge score than younger medical staff, this finding is similar to studies conducted in Pakistan and Saudi Arabia [13] [15]. This could be explained by the older the medical staff, the more the experience with poliomyelitis and polio vaccination campaigns. In contrast to a study conducted in Nigeria [16]., which showed that a high percentage of medical staff with good knowledge had few years of employment. our findings indicate otherwise. These differences could be attributed to variations in the medical school curricula, postgraduate training programs, and sample characteristics.

Regarding sex, there was no significant difference between males and females in the mean knowledge score, while a study conducted in Pakistan [13] reported a higher percentage of males than females had good knowledge this might be explained by similar academic achievement in males and female medical staff in Iraq and both have participated in regular and obligatory training courses designed by the ministry of health in Iraq.

For the attitude, there was a significant relationship between medical staff attitude and occupation where doctors had significantly higher mean attitude scores than dentists and pharmacists, this difference might be related to the lack of educational programs about the benefits of poliomyelitis vaccination campaigns for these groups and less contribution to the vaccination and campaign activities for dentists and pharmacists. This agreed with a study conducted in Pakistan where the doctors had significantly higher mean attitude scores than other groups of medical staff. [13].

With increasing age, the mean attitude score significantly increases, which is agreed with a study conducted in India [14]. This could be explained by the older medical staff the more experience with poliomyelitis and polio vaccination campaigns. In contrast to a study conducted in Pakistan where the younger medical staff had a positive attitude this might be attributed to the health system in Pakistan [13].

Again, there was no significant difference between males and females in the mean score of attitudes this might be due to both males and females being afraid of mistakenly conserved vaccines. The results from previous studies regarding attitude and sex are controversial as a study in Saudi Arabia showed that the positive attitude was associated with the female sex and the most likely reason for this discrepancy because the females regard the vaccine as an important issue to enhance their child health as well as prevention of disease; however, education program still needs to improve the information [17].

Opposite to it, a study conducted in Pakistan showed that the positive attitude was associated with the male sex than females. The most likely reason for this discrepancy might be due to the male more attending the conferences about the recent intensification of the polio education program in Pakistan. [13]. A study conducted in Malaysia found that the attitude of medical staff both males and females was poor and negative toward the vaccination in campaigns due to geographical factors and deprived equipment. [18].

This study also shed light on the areas where knowledge gaps were identified such as vomiting and diarrhea are contraindications to an oral poliomyelitis vaccination (71.4 %) and there is no possibility of death with polio (50.3%) this result was agreed with a study in Pakistan [19]. As (65.3 %) of the participants answered that vomiting and diarrhea are contraindicated to oral poliomyelitis vaccination and (36.9 %) there is no possibility of death with polio and disagreed with a study in Saudi Arabia [20], as the vomiting and diarrhea are contraindicated to oral poliomyelitis vaccination (19 %) and there is no possibility of death associated with polio (24%).

In the current study the percentage of medical staff that refused to immunize their child through campaign was (53.6%), and the rate of the participants that answered the question (Poliomyelitis is not preventable) was (3.9 %), and (8.4%) responded that the healthy child does not need vaccination, this result disagreed with a study in Saudi Arabia that identified the percentage of medical staff that refused to immunize their child through campaign was (12.8%), poliomyelitis is not preventable (20.3%), healthy child did not need vaccination (3.7%) [20], and about polio is not a viral disease (7.5%), lack of immunization against poliomyelitis is not a major risk factor of polio (9%) the main route of transmission is not through feco- oral route (32.1%), Paralysis in Poliomyelitis is curable (22%), Immunization is not the most effective way of preventing poliomyelitis (19.3%) and these results also disagreed with a study in Pakistan that identified poliomyelitis is not a viral disease (3.8%), lack of immunization against polio is not a major risk factor of polio (19.2%) the main route of transmission is not through the fecal-oral route (18.6%), Paralysis in Poliomyelitis is curable (68.1%), immunization is not the most effective way of preventing poliomyelitis (5.1%).[19]. These findings might be due to a lack of

information about the administration of the polio vaccine, the contraindications, and fear of the expiration of the vaccine these causes might have far-reaching implications as it could be a contributing factor towards the failure of immunization campaigns [19 20].

CONCLUSIONS

The knowledge and attitude of doctors are higher than those of dentists and pharmacists. There is no difference between males and females in the knowledge and attitude. Additionally, the knowledge and attitude are higher among the older age group.

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Effect of Depressive symptoms on Weight among Adults at Al-Najaf Province: A case-control Study

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ABSTRACT

Background: *Obesity and major depression disorder are two of the most common diseases in the world. They are also big problems because they have a very strong effect on people's health and well-being, but also because of their high morbidity and mortality rates and socioeconomic effect.*

Aim of the study: *This study to study the association between obesity and depression, and to explore the link between body mass index and socio-demographic characteristics.*

Patients and methods: *A case-control study utilized a random sample of 300 subjects (100 obese group and 200 normal weight group) attending diabetes and endocrinology center at Al- Najaf province. All subjects have been taken randomly from adult individuals with age range between (18-60) years in Al-Najaf province. The data were collected from October to the February. The obtained data analyzed by Version 28 of the Statistical Package for the Social Sciences (SPSS).*

Results: *The current study found a significant association between obesity and depression, indicates that those with moderate/severe depression scores are more likely to be obese than those with mild depression or no depression (odds ratio = 2.306 times, 95% CI: 1.360-3.910). With (p = 0.002). The relationship between obesity and socio-demographic factors, this study found positive association between obesity and exercise (p = 0.001) and family history of obesity (p = 0.037), while the other characteristics were not significant.*

Conclusions: *The current study concluded that those who had moderate or severe depression symptoms were more likely for developing obesity than those who had a mild or no depression symptoms.*

Keywords: Depression, obesity and case-control study.

Article Information

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INTRUDUCTION

Obesity and major depressive disorder are two of the most prevalent health issues globally, and they pose serious challenges from both an individual and societal perspective. Not only that, but these two diseases are becoming increasingly common globally. Strong positive connections between depression and obesity have been identified by epidemiological research [(1)]. The World Health Organization (WHO) define obesity as" abnormal and excessive fat accumulation in the body that presents a risk to health [(2)]. obesity is commonly measured in terms of body mass index (BMI) more than30 kg/m^2 [(2)]. Obesity is a multifaceted illness on the biological basis which includes genetic and biological components that are involved in normal body growth, eating habits, energy expenditure, and adipose tissue function [(3)]. According to (WHO) In 2022, 1 in 8 people in the world were living with obesity, about 16% of adults aged 18 years and

older worldwide were obese in 2022[2]. An increased risk of metabolic, anxiety, cardiovascular, chronic inflammatory, and some cancers is linked to obesity [4]. Depression, often known as major depressive disorder or clinical depression, is a mood illness that causes people to be unhappy, hopeless, and uninterested in activities [5]. There are about 280 million depressed people in the world, which is about 3.8% of the population. This includes 5% of adults (4% of men and 6% of women) and 5.7% of adults over 60 years old [6]. Depression is about 50% more common among women than among men. Worldwide, more than 10% of pregnant women and women who have just given birth experience depression more than 700 000 people die due to suicide every year [7]. The positive links between depressive disorders and obesity has been argued; several research have been shown that depressive symptoms is also a contribute as a risk factor for obesity [8]. The researcher Faith in (2011) detected obesity and depression associations in 25 studies, of which 15 tested studies "depression a risk factor for obesity" paths and 10 tested "obesity a risk factor for depression" paths [9]. Although about 80% of the studies reported significant obesity-to depression associations (odds ratios 1.0-2.0), while 53% of analyzed studies reported a positive significant depression-to-obesity association [9]. While other studies found no significant association between obesity and depression [10 12].

PATIENTS AND METHODS

A case-control study was conducted in Al- Najaf governorate (Diabetes and endocrinology center) at Al-Sadr Medical City. The study had been conducted from October 2023 until the end of January 2024. The size of the sample comprises 300 subjects (two controls for every case), 100 obese subjects with Body Mass Index (BMI ≥30 $kg/m2$) as a cases group and 200 normal weight participants with BMI around (18.5−24.9$kg/m2$) as a control group selected after being matched with (sex and age). All participant from the same geographical area. Sample selected randomly from subjects attending Nutrition and Endocrinology Unit.

Data Collection: Study data has been obtaining by using a questionnaire form that includes three domains.

Socio-demographic characteristics: This includes age, residence, educational level, occupation, economic status, physical activity and family history of obesity.

Anthropometric Measurements: Body Mass Index (BMI): Height and weight were computed to measure the BMI measurement by the equation BMI=weight kg /height m^2[2].

Beck's depression inventory II: The validated Arabic form of Beck's Depression Inventory-II (BDI-II) was used to measure depression symptoms [13]. The BDI-II is a self-administered assessment that typically requires 5-10 minutes to complete. The assessment comprises 21 items, each rated on a 4-point scale that ranges from 0 (indicating the absence of symptoms) to 3 (indicating the presence of severe symptoms). The scoring is determined by summing the highest ratings for each of the twenty-one items. The minimum score is zero and the maximum score is 63. A score of zero to thirteen indicates the absence of depression, whereas a score of fourteen to nineteen indicates mild depression. Depression is indicated by scores above 20, with scores ranging from 20-28 indicating moderate depression and scores ranging from 29-63 indicating severe depression. Several studies have investigated the validity and reliability of the BDI-II in various groups and nations. These investigations have consistently found that the BDI-II has good internal consistency (Cronbach's alpha = 0.91) and high test-retest reliability (Pearson r = 0.93) [14].

STATISTICAL ANALYSIS

Version 28 of the Statistical Package for the Social Sciences (SPSS) was used to analyze data. To find the mean, standard deviation, rates, and percentages, descriptive statistics were used. Chi-square, t-test, odds ratio used for analysis.

RESULTS

A total of 300 subjects had been included in this study matched for age and gender the mean age of cases was and controls (37.1 ± 10.6 years vs. 34.6 ± 11.4 years). Table (1) compares obese subjects and normal weight subjects according to sociodemographic characteristics. An about (71%) of cases and control lives in urban areas. Regarding education level intermediate school and bachelor degree are a highest percentage (28 %) of cases, while the high school or vocational is highest percentage (28 %) of control group (27.5%).

Regarding occupational level, the highest percentage (31.0%) of cases were housewives compared to control individuals (27.5%) who were self-employed. Most cases and controls groups have a moderate- economic level. Regarding exercise (73%) of controls practice physical activity compare (37%) of cases group. Table (2) represents depression indicators for obese and control groups. The results of this study indicates that the indicators "Permission," " Loss of Pleasure," " Guilty feeling," " Punishment feeling," " Self-dislike," "Criterion-self," " Changes in sleeping pattern," " Change in appetite " and " Body image " show significant differences between cases and controls ($p < 0.05$). While other indicators of depression have no significant association between cases and controls (P. value >0.05). In Table 3, the results of this study indicate that those with moderate/severe depression scores are more likely to be obese than those with mild depression or no depression (odds ratio = 2.306 times, 95% CI: 1.360-3.910). With p. Value = 0.002.

Table (1): The distribution of obese subjects and normal weight subjects according to socio-demographic characteristics.

		Groups				P. value
		Obese		Normal weight		
		No.	%	No.	%	
Age	Mean ± SD (Range)	37.1±10.6 (18-57)		34.6±11.4 (18-56)		0.078
Residence	Urban	71	71.0	146	73.0	0.716
	Rural	29	29.0	54	27.0	
Education	Primary school	15	15.0	42	21.0	0.153
	Intermediate school	28	28.0	33	16.5	
	High school or vocational	21	21.0	52	26.0	
	Diploma (institute)	17	17.0	23	11.5	
	Bachelor degree (college)	28	28.0	41	20.5	
Occupation	Government employee	26	26.0	46	23.0	0.331
	Private sector employee	6	6.0	20	10.0	
	Self-employed	21	21.0	55	27.5	

	Housewife	31	31.0	43	21.5	
	Student	7	7.0	21	10.5	
	Retired	1	1.0	4	2.0	
	Unemployed	8	8.0	11	5.5	
Economic	Low	30	30.0	49	24.5	0.491
	Moderate	56	56.0	126	63.0	
	High	14	14.0	25	12.5	
Exercise	Yes	37	37.0	148	74.0	0.001
	No	63	63.0	52	26.0	
Family history of obesity	Yes	48	48.0	71	35.5	0.037
	No	52	52.0	129	64.5	

Table (2): The distribution of patients with obesity and control subjects according to depression in indicator.

		Groups				P. value
		Case		Control		
		No.	%	No.	%	
Sadness	No depression	42	42.0	95	47.5	0.472
	Mild depression	47	47.0	76	38.0	
	Moderate depression	9	9.0	22	11.0	
	Severe depression	2	2.0	7	3.5	
Permission	No depression	44	44.0	113	56.5	<0.001
	Mild depression	48	48.0	70	35.0	
	Moderate depression	2	2.0	16	8.0	
	Severe depression	6	6.0	1	0.5	
Past Failure	No depression	60	60.0	139	69.5	0.198
	Mild depression	28	28.0	36	18.0	
	Moderate depression	10	10.0	23	11.5	
	Severe depression	2	2.0	2	1.0	
Loss of Pleasure	No depression	39	39.0	111	55.5	0.002
	Mild depression	41	41.0	59	29.5	
	Moderate depression	14	14.0	29	14.5	
	Severe depression	6	6.0	1	.5	
Guilty feeling	No depression	42	42.0	90	45.0	0.025
	Mild depression	38	38.0	94	47.0	
	Moderate depression	13	13.0	10	5.0	
	Severe depression	7	7.0	6	3.0	

Table (3): Association between depression and obesity.

Overall Depression Score	Groups				P. value	OR	95% C. I for OR
	Case		Control				
	No.	%	No.	%			
Mild or no depression	62	62.0	158	79.0	R		
Moderate/ Severe depression	38	38.0	42	21.0	0.002	2.306	1.360-3.910

DISCUSSION

The current study found through the results in table (1) of this study of the sample that there was no significant difference between cases and controls in terms of age, residence, education, occupation, and economic status (P. value>0.05). This result similar to study conducted in Finland 2017 that found no association between cases and controls in above terms [15]. While exercise the current study results indicates a significant association between exercise and obesity ($p < 0.001$), this agreed with a study was conducted in Erbil, Iraq in 2019 that was found there is a significant difference between obesity and practice regular exercise ($p < 0.001$) [16]. Furthermore, new research shows that practice moderate physical activities per day prevents the progress of obesity and fat mass without restricting calories [17].

This study finding also show a significant association between obesity and family history of obesity, this explained that those have family history of obesity more likely for developing obesity and this corresponds with study conducted in Germany that showed people with a family history of obesity have a high prevalence of severe obesity compared with those without a family history of obesity [18]. The results of this study indicates that the indicators "Permission," " Loss of Pleasure," " Guilty feeling," " Punishment feeling," " Self-dislike," "Criterion-self," " Changes in sleeping pattern," " Change in appetite " and " Body image " show significant differences between cases and controls. These differences explained that the cases have high score of depression compared to the control subjects. While other indicators of depression have no significant association between cases and controls. Also, the results of current study found a significant association between depression and obesity. It indicates that moderate or severe depression increases the odds of being obese by more than twofold with an odds ratio of (2.306). This agreed with numerous studies have reported that obesity was linked with depression symptoms with an odds ratio (1.18 to 5.25), depending on the studies and evaluation ways [19 21]. Another recent study conducted by Almarhoon in Saudi Arabia in 2021 that show a significant association between moderate or severe depression and obesity [22]. In contrast, our results disagreed with a study conducted in Iran that reported no association between obesity and depression [12].

Increase of severity of depression has been shown to be associated with decreased physical activity and increased calorie intake, with a consequent increase in the risk of obesity [23]. Depression may be a strong predictor of obesity for a number of reasons. First, hypersomnia and hyperplasia are caused by depression [24]. which consequently increased energy consumption and decreased energy expenditure, respectively [25]. Secondly, people who are depressed tend to eat worse than healthy people, and this can lead to more calories being consumed [26]. Third, people with atypical major depressive disorder had more disability days and days with reduced activities, which may have made them use less

energy expenditure [27]. The raised levels of systemic inflammation and metabolic dysregulation observed in obese individuals are identified as the fourth factor contributing to the association between obesity and depression [28].

CONCLUSIONS

The current study concluded that those who had moderate or severe depression symptoms were more likely for developing obesity than those who had a mild or no depression symptoms.

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