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Journal of Progressive Medical Sciences

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This journal is dedicated to publishing targeted and applied scientific research and studies that are beneficial in all areas of contemporary medical sciences

It publishes peer-reviewed scientific work in a wide range of medical specialties, including general medicine, surgery, dentistry, pharmacy, microbiology, pathology, molecular biology, toxicology, ophthalmology, otolaryngology (ENT), oral and maxillofacial surgery, chronic diseases, plastic surgery, pediatrics, family and community medicine, primary health care, internal medicine, reproductive health, urology, dermatology, obstetrics and gynecology, as well as all other medical disciplines. It also covers medical laboratory sciences, radiology, ultrasound, nursing, therapeutic nutrition, and public health

The journal aims to contribute to the advancement of medical sciences by promoting the dissemination of new scientific knowledge, innovative ideas, modern experiences, and technological achievements, including nanotechnology and the use of advanced devices. It encourages researchers in the medical field to conduct impactful and beneficial scientific studies

In addition, the journal focuses on publishing experimental research, as well as innovative and advanced studies prepared by researchers in medical sciences, with the goal of enriching and developing high-quality scientific research.

The journal promotes scientific communication, intellectual exchange, and the cross-pollination of ideas, aiming to connect researchers from across the Arab world in a modern, purposeful framework for sharing information and practical scientific experiences

Editor-in-chief

Journal des Sciences Médicales Progressistes

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Cette revue est dédiée à la publication de recherches et d'études scientifiques ciblées et appliquées, utiles dans tous les domaines des sciences médicales contemporaines.

Elle publie des travaux scientifiques évalués par des pairs dans un large éventail de spécialités médicales, notamment la médecine générale, la chirurgie, la dentisterie, la pharmacie, la microbiologie, la pathologie, la biologie moléculaire, la toxicologie, l'ophtalmologie, l'oto-rhino-laryngologie (ORL), la chirurgie buccale et maxillo-faciale, les maladies chroniques, la chirurgie plastique, la pédiatrie, la médecine familiale et communautaire, les soins de santé primaires, la médecine interne, la santé reproductive, l'urologie, la dermatologie, l'obstétrique et la gynécologie, ainsi que toutes les autres disciplines médicales. Elle couvre également les sciences de laboratoire médical, la radiologie, l'échographie, les soins infirmiers, la nutrition thérapeutique et la santé publique.

La revue vise à contribuer au progrès des sciences médicales en favorisant la diffusion de nouvelles connaissances scientifiques, d'idées innovantes, d'expériences modernes et de avancées technologiques, notamment les nanotechnologies et l'utilisation de dispositifs de pointe. Elle encourage les chercheurs du domaine médical à mener des études scientifiques percutantes et utiles.

Par ailleurs, la revue se concentre sur la publication de recherches expérimentales, ainsi que d'études innovantes et avancées réalisées par des chercheurs en sciences médicales, dans le but d'enrichir et de développer une recherche scientifique de haute qualité.

La revue favorise la communication scientifique, les échanges intellectuels et le brassage d'idées, en mettant en relation les chercheurs du monde arabe dans un cadre moderne et constructif de partage d'informations et d'expériences scientifiques concrètes.

Rédacteur en chef

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Association of Hepatitis B Virus Serological Markers with Glycemic Control and Diabetes Duration in Type 1 and Type 2 Diabetes Patients in the Najaf Government

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Abstract :
Background: Diabetes mellitus (DM) and hepatitis B virus (HBV) infection are two major global health concerns. Growing evidence suggests a possible association between chronic viral infections and glucose metabolism disorders. **Objectives:** To investigate the association between HBV serological markers and glycemic control, as measured by fasting blood glucose levels and the duration of diabetes, among patients with type 1 and type 2 diabetes mellitus. **Subjects and methods**: A cross-sectional study was performed from July to October 2024. The serum was taken from 200 individuals. All of the patients were tested using an ELISA technique for HBc IgG and by an immunochromatographic assay for HBsAb, HBsAg, HBcAb, HBeAg, and HBeAb. The statistical analysis approach was conducted using SPSS version 26. **Results**: HBcAb-positive diabetic patients showed significantly higher fasting blood glucose levels (P=0.044). HBcIgG was detected in 83 out of 200 patients, mainly in those with 5–15 years of diabetes duration (P = 0.049). No significant association was found between HBV markers and diabetes type.**Conclusions**: a potential link between HBV exposure and impaired glycemic control , suggesting a possible role of chronic HBVexposure in the progression of metabolic dysfunction over time This suggests that the activation of a previous HBV infection may be an underlying factor in the progression of diabetes mellitus or the development of pre-existing conditions and the relationship between HBV and glycemic markers may be independent of diabetes type.

Keywords: Hepatitis B virus, Diabetes mellitus, Anti-HBc IgG, Anti-HBs, ELISA

Introduction

Hepatitis B virus has the capacity of generating various types of antigens, like core, surface, and envelope antigens, the key features of which are their immunogenicity, which can mediate an immune response (Cao et al., 2018). Despite the success of immunization strategies and the reduction in HBsAg seroprevalence since 2000, the hepatitis B virus continues to be a widespread worldwide health concern due to the ongoing complications associated with chronic infection, which continue to contribute significantly to morbidity and mortality. Annually mortality rate due to cancer and liver cirrhosis is 820,000 people, demonstrating the seriousness of being infected with this hepatitis virus (Lazarevic et al., 2023). The simultaneous presence of HBV infection and diabetes mellitus represents a life-threatening situation that demands urgent attention (Mirzaei et al., 2020).

Diabetes mellitus is a chronic disease characterized by an imbalance in glucose homeostasis. The link between hepatitis B and diabetes mellitus is still a topic of contention. Studies have been conducted on the rising occurrence of HBV, but there is less evidence on its connection with diabetes patients. Diabetic patients are susceptible to viral infections because of their impaired T lymphocyte numbers, which weaken their immune system. Individuals with diabetes mellitus are at a considerably greater risk of acquiring infectious diseases, including bacterial, fungal, parasitic, and viral infections. This susceptibility is due to impaired cellular immunity and dysfunction of phagocytes caused by high blood sugar levels and reduced blood flow.

Among the viruses, hepatitis B and C are the most widespread (Gutiérrez-Grobe et al., 2011). There is limited information on the correlation between HBV indicators, infection conditions, and subtypes of DM. The recent study investigation of Iraqi individuals aged 18–80 years yielded several interesting findings. This study aims to investigate the relationship between hepatitis B virus (HBV) serological markers and fasting blood glucose (FBG) levels in adult patients, aiming to explore whether HBV infection may influence glucose homeostasis and the duration of diabetes, among patients with type 1 and type 2 diabetes mellitus.

Methods

Methodology and data collection process

The current study is a cross-sectional research project included only Iraqi participants who were medical patients in the specialized endocrinology and diabetes center at the Al-Sader Teaching Hospital in Najaf city from July to October 2024. The study comprised 200 patients (either type 1 or type 2 only DM based on a clinical diagnosis by an endocrinologist and serological tests). The patients' data were obtained through the implementation of a questionnaire and the collection of a blood sample. The specimens were obtained by extracting approximately 10 mL of venous blood from every participant. Blood samples were placed into a gel tube and allowed to coagulate at room temperature for thirty minutes. The serum was extracted using centrifugation and thereafter divided into 1.5-ml Eppendorf tubes. A part of the serum was immediately utilized for a fasting blood glucose test. The other part was

then preserved in a refrigerator at a temperature of -80°C for immunological investigation until further examination. Enzyme-linked immunosorbent assays (ELISA) and rapid diagnostic tests are the main methods used in clinical laboratories to find HBV serological markers. A fraction of the serum was used for a human hepatitis B virus panel test (five panel kit): HBc Ab, HBs Ag, HBs Ab, HBe Ag, HBe Ab (Eugene Biotech/China), After that, an ELISA method (Sun Long Biotech, China) was used to find a qualitative HBc IgG. The measurement of fasting plasma glucose (FPG) was conducted using an enzymatic colorimetric method and a kit available commercially from Spinreact, a company in Spain. The procedures for all tests were conducted according to the instructions outlined in the kit's manual. Before enrolling in the research study, all patients received a comprehensive briefing on the study's aims and objectives and were then given their informed consent to participate.

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 categorical data was illustrated using chi square. The findings are displayed in tables and figures, accompanied by a descriptive narrative, utilizing MS Word and Excel 2016.

Ethics Committee approval

The ethics council of the University of Kufa's Faculty of Medicine gave its approval before starting this research project. All participants gave their consent, and the Al-Sader Teaching Hospital in the province of Najaf gave its approval.

Limitations of this research

Firstly, obtaining the individual's HBV vaccination history through self-reporting poses a potential risk of bias recall. Secondly, the limitation of the study is its restriction to a single center with a small sample size.

Results

The present study included a sample of 200 patients, who had undergone testing for HBV infection between July and October 2023, was screened to detect the presence of HBc Ab, HBsAg, HBsAb, HBeAg, HBeAb, Hbc IgG, Fig 1 presents an overview of the general scheme followed in this investigation. Patients who tested positive for HBsAg, and patients with inadequate clinical data were excluded. This study found that 10.5% of patients who tested negative for HBsAg, but positive for HBc IgG were also positive for HBs Ab. The study 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.

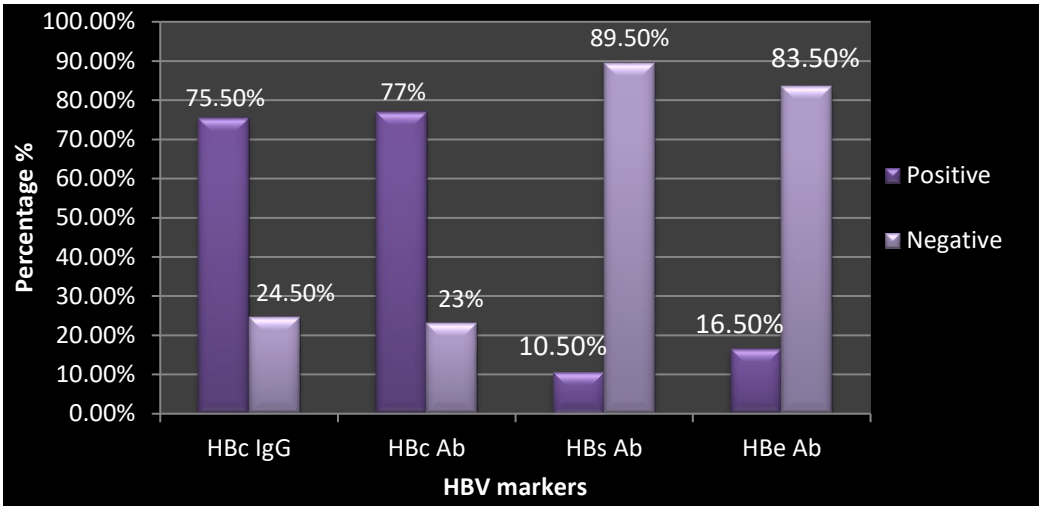

Figure (1): Distribution of HBV biomarkers in diabetes mellitus patients

Individuals who tested positive for HBc IgG had a substantially higher mean rank level of FBG [104.31 (mg/dl)] compared to individuals who tested negative for HBc IgG [88.77 (mg/dl)]. Additionally, the disparity was statistically nonsignificant (P = 0.102). The patients who tested positive for HBc Ab had a considerably higher average rank level of FBG [105.00 (mg/dl)] compared to the patients who tested negative for HBc Ab [85.43 mg/dl]. Simultaneously, the disparity was assessed statistically significant as demonstrated by a p-value of 0.044. As seen in Tab1.

Table (1): Association between HBV markers and level of blood sugar

		positive		Negative		Test statistics
		N	Mean Rank	N	Mean Rank	
FBG (mg/dl)	HBc IgG	151	104.31	49	88.77	P= 0.102 Z=-1.634-
	HBc Ab	154	105.00	46	85.43	P=**0.044*** Z=-2.012-
	HBs AB	21	95.50	179	101.09	P=0.676 Z=-0.419-
	HBe Ab	33	86.05	167	103.36	P=0.116 Z=-1.570-
*Probability value (level of significance <0.05) , P values were computed via the Mann-Whitney test, FBG:Fasting blood glucose						

Table (2): Association between HBV markers and diabetic duration.

			HBc IgG		HBc Ab	
			Positive N=151	**Negative N=49**	**Positive N=154**	**Negative N=46**
Duration of diabetes / years	Less than 5 years	Count	45	6	45	6
		%	88.2%	11.8%	88.2%	11.8%
	From 5 to 15 years	Count	83	33	86	30
		%	71.6%	28.4%	74.1%	25.9%
	More than 15 years	Count	23	10	23	10
		%	69.7%	30.3%	69.7%	30.3%
P value			P= 0.049* df= 2 X^2= 6.050		P= 0.076 df= 2 X^2= 5.165	
X^2=chi square, p value=probability value (* level of significance at <0.05) , df = degree of freedom						

Additionally, there was no significant link found between HBc Ab, HbsAb, Hbe Ab and diabetes duration (P > 0.05).

Table (3): Association between HBV markers and duration of diabetic

			HBs Ab		HBe Ab	
			Positive N=21	**Negative N=179**	**Positive N=33**	**Negative N=167**
Duration of diabetes / years	Less than 5 years	Count	4	47	9	42
		%	7.8%	92.2%	17.6%	82.4%
	From 5 to 15 years	count	13	103	21	95
		%	11.2%	88.8%	18.1%	81.9%
	More than 15 years	Count	4	29	3	30
		%	12.1%	87.9%	9.1%	90.9%
P value			P=0.765		P=0.454	
X^2=chi square, p value=probability value (level of significance at <0.05)						

In the present study, it was observed based on the results obtained, as well as the study of the relationship between the diagnosis of anti-HBc IgG and the investigation of HBs antibodies. The table also demonstrates that there were highly significant findings for anti-HBs antibody values among patients who tested positive for anti-HBc IgG, with a p-value of < 0.05.

Table (4): Comparison between anti-HBs antibody seropositivity and anti-HBc-IgG results

			HBs Ab		Total	P value
			positive	negative		
HBc IgG	positive	Count	21	130	151	**P=0.006*** df=1 X^2=7.614
		%	13.9%	86.1%	75.5%	
	Negative	Count	0	49	49	
		%	0.00%	100.0%	24.5%	
	Total	Count	21	179	200	
		%	100.0%	100.0%	100.0%	

There was no significant association between type of diabetes and HBc IgG, HBc Ab, HBs Ab, or HBe AB positivity, as seen in Tab. 5.

Table (5): Association between HBV markers and types of diabetes

Parameter		Types of DM		Total	P value
		Type 1 DM	Type 2 DM		
HBc IgG	Positive Count, %	44(78.6%)	107(74.3%)	151(75.5%)	P= 0.529 df= 1 X^2= 0.397
	Negative Count, %	12(21.4%)	37(25.7%)	49(24.5%)	
HBc Ab	Positive Count, %	44(78.6%)	110(71.4%)	154(77.0%)	P= 0.742 df= 1 X^2= 0.108
	Negative Count, %	12(21.4%)	34(23.6%)	46(23.0%)	
HBs Ab	Positive Count, %	4(7.1%)	17(11.8%)	21(10.5%)	P= 0.334 df= 1 X^2= 0.933
	Negative Count, %	52(92.9%)	127(88.2%)	179(89.5%)	
HBe AB	Positive Count, %	9(13.1%)	24(16.7%)	33(16.5%)	P= 0.919 df= 1 X^2= 0.010
	Negative Count, %	47(28.1%)	120(83.3%)	167(83.5%)	
X^2=chi square, p value=probability value (level of significance at <0.05) , df= degree of freedom					

Discussion

Serological markers of HBV infection revealed the highest rate of HBcAb (77%) among diabetes patients screened in the current study. Different with other studies were done in China (Lu et al., 2017) and the US (Huang et al., 2015), which related the prevalence of HBcAb in diabetic patients of 62.3% and 6.35%, respectively. It is also higher, than the percentage observed in individuals with diabetes (8.2%), as reported by Schillie et al. (2018). Significantly surpasses the prevalence among patients in Brazil, which stands at just 16.8%, according to Arrelias et al. (2018). Ndako and colleagues (2021) found that 54.2% of individuals tested for HbcAb positivity received results, while Saitta and team (2022) discovered that 15.5% of those tested showed positive results when using anti-HBc as an alternative indicator. The research was conducted in various geographical regions with various sample sizes and did not come to a compromise. Variations in HBV exposure risk, diagnostic method accuracy, study period, sociodemographic characteristics. The variations in antibody frequencies can originate from variations in the assay, sensitivity levels of the technique, sample size, or study methodology.

The present study found that the HBs Ab 21 (10.5%) result was lower than a study conducted in the US population of 19.66% (Huang et al., 2015) of individuals tested positive for HBsAb. In a study conducted in China, the average percentage of HBsAb positivity among diabetic patients was found to be 37% (Lu et al., 2017), which is higher than the frequency in the current study. A study by Ndako et al. (2021) found an 8.3% positive rate among individuals with HBsAb, which is lower compared to the current study. There are variations in vaccination status, danger indicators, and the rate of HBV infection in the whole population. Due to the serologic window during the incubation period after infection, other studies have not been able to identify infected patients, and this HBs Ab positive result is from an infection that was not vaccinated. On the other hand, a study by Chi et al. (2019) & Tseng et al. (2018) found that in HBeAg patients greater anti-HBc levels were correlated with recurrence and hindered the attainment of HBsAg seroclearance. For individuals without HBe Ag they enter a period of dormancy marked by a robust immune reaction, where the anti HBc level mirrors the presence of HBc Ag in the liver. Guner et al. (2011) revealed that this level is also correlated with the transcriptional activities of cccDNA in hepatocytes. HBeAg has been observed in serum as a sign of active viral replication in previous studies (Zhang et al., 2011). The current investigation reveals no cases of HBeAg positivity.

Nevertheless, Caviglia et al. (2020) indicated that the negativity of HBsAg in the blood does not certify the eradication of the virus. Likewise, Yan and colleagues (2015) clearly demonstrated that the HBV genome has a high mutation rate because it doesn't have the proofreading function during the replication period. The inability of HBsAg to be detected in cases of occult hepatitis B, involving as well the presence of covalently closed circular DNA (cccDNA), is commonly explained by cccDNA being hindered by epigenetic regulation pathways and/or the immune system of the host, which is believed to be its main cause. The data coming from these studies are showing that patients harboring OBI seem to have a higher chance of mutation in pre-S/S region comparing to patient with HBV chronic infection. It is likely that it will lead to a fall in the antigenicity of HBsAg detection or the production/release of HBsAg is going to be altered (Huang et al., 2017; Zhang et al., 2019). The detection of anti-HBc antibodies has been the main diagnostic marker of occult HBV infection in most research studies on HBV reactivation (Cholongitas et al., 2018). Occult hepatitis B infection (OBI) constitutes both

seropositive and seronegative categories according to the presence of serum markers that point at exposure to HBV. People who are seropositive for occult hepatitis B infection (OBI) have anti-HBC and anti-HBs antibodies in their serum specific to the core antigen or/and the surface antigen of hepatitis B, respectively. Such a form of OBI makes up around 80% of all OBI observed (Raimondo et al., 2019). The diagnostic method that is the gold standard for identifying occult hepatitis B infection is when HBV genomes are found inside DNA extracted from hepatocytes. Instead, the HBc Ab tests may be applied as an alternative biomarker for OBI screening (Raimondo et al., 2019; Raimondo et al., 2008).

The study by Pollicino et al. (2021) categorized the HBsAg-negative/antibody HBc-positive condition as a phase in the "occult" stage of the disease progression. If HBsAg becomes undetectable, HBc antibodies are identified as the only clinical evidence of past HBV infection. Consider the HBsAg-negative/HBc-Ab-positive status like an OBI stage in the course of the natural HBV infection. 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 (Wang et al., 2023).

Diabetes patients who are exposed to HBV and have positive HBc Ab show higher levels of FBG, with a significant correlation ($P < 0.05$) in the present study. Furthermore, HBV infection can exacerbate diabetic patients' glucose control and raise their risk of hyperglycemia (Gundling et al., 2013; Gutierrez-Grobe et al., 2011), (Lecube et al., 2006). Hyperglycemia in diabetes mellitus is believed to induce impairment of the immunological reaction, resulting in inadequate control of the increase of invading microorganisms in individuals with diabetes. Consequently, individuals with diabetes are recognized to be more predisposed to infections. The rising prevalence of Type 2 Diabetes (T2D) would lead to an increase in the occurrence of infectious illnesses as well as comorbidities (Berbudi et al, 2020). The fasting plasma glucose levels of individuals with diabetes type 2 and adult-onset autoimmune diabetes were greater than those of the controls ($P < 0.05$) compared to patients with adult-onset autoimmune diabetes, but with a shorter duration of diabetes ($P < 0.01$) in those with the chin (Lu et al., 2017), which agrees with current study results.

As the virus does not show symptoms, the complications could happen when the patient has been sick for a long period (Komatsu, 2014). As Younossi et al. (2017) found out, DM, along with HBV infection, generates liver complications including cirrhosis, hepatocellular carcinoma, and even death. From the 200 patients with a duration of diabetes ranging from 5 to 15 years, the HBc IgG was detected as positive in as many as 83 patients. The statistical evaluation resulted in a considerable result ($P = 0.049$) in the present study. In 2021, Han et al. made a study of the relationship between the duration of diabetes and the higher risk of HBV infections, and it was found out that patients who have been diabetic longer than six years had a greater risk of getting the infection compared to the ones who have normal blood glucose levels. This is consistent with the current study results. Individuals who have CHB infection or test positive for HBc Ab may be at a higher risk of developing diabetes. This association may have been influenced by many metabolic factors as well as age, according to Lei et al. in 2020.

The relationship between HBV exposure and a prolonged duration of DM might be considered a cumulative danger of being exposed to the virus, likely due to the management of the condition, as DM itself does not lead to hepatitis B. The co-related existence of HBV infection and longer DM duration

has been documented in the Polish study of 2002 (Halota et al., 2002), the Turkish one in 2008 (Gulcan et al., 2008), and the Nigerian one of 2016 (Onyekwere et al., 2016). In contract, the investigation done in Italy showed the absence of a correlation between the infection and the DM duration (Sangiorgio et al., 2000). The results of the Kombi et al. (2018) study showed that there was no significant P = 0,892 relationship between the length of DM and HBV infection. That most individuals with diabetes were exposed to HBV infection within ten years of DM.

In present study show that in people with diabetes type 2, the incidence of HBV markers was not significantly greater compared to individuals with type 1 diabetes. Though DM has long been known to increase the risk of many co-morbidities, such as chronic liver disease, a connection between HBV-related liver dysfunction and Type 2 DM development has just recently been identified. According to theories, viral hepatitis may affect important liver-regulated metabolic processes that are linked to the onset of diabetes mellitus (DM) through the interaction of inflammatory mechanisms brought on by liver infection, which in turn causes insulin resistance and glycometabolic dysfunction. According to a Schillie et al. (2012) meta-analysis, those who have HBV infection have a higher chance of developing diabetes than those who do not. Farshadpour et al.'s (2022) research revealed that among 733 diabetic patients in an Iranian population, 12.82% tested positive for HBcAb and 3.82% tested positive for HBsAg. The difference in HBcAb seroprevalence between diabetic patients and non-diabetic controls was not statistically significant (P = 0.23). This study aligns with the findings of Kombi et al. (2018), which indicated a prevalence of HBV in Type 1 (n = 1, 3.3%) and Type 2 (n = 4, 3.4%) with a non-significant p-value of 0.994. The occurrence of hepatitis B in this study may be attributed to variations in geographical location or the challenge of detecting infected individuals during the serologic window in the incubation period post-infection. This could be due to the activation of insulin resistance linked to persistent inflammatory reactions triggered by HBV infection, as well as the excess production of nitric oxide and tumor necrosis factor-α in the liver. These factors are implicated in impairing insulin metabolic action, damaging β-cells in the pancreas through HBV replication, and causing glycometabolism disorders because of hepatic damage from HBV infection (Cai et al., 2015; Lei et al., 2020). Kaya & Kaya (2020) revealed that vaccination is a crucial and successful strategy for controlling and preventing it. The national immunization program has significantly reduced the disease's occurrence.

Conclusion

The study revealed a statistically significant association between HBcAb positivity and elevated fasting blood glucose levels, indicating a potential link between HBV exposure and impaired glycemic control. Additionally, HBcIgG was significantly associated with a diabetes duration of 5 to 10 years, suggesting a possible role of chronic HBV exposure in the progression of metabolic dysfunction over time. This suggests that the activation of a previous HBV infection may be an underlying factor in the progression of diabetes mellitus or the development of pre-existing conditions. No significant differences were observed in the distribution of HBV markers between type 1 and type 2 diabetes, indicating that the relationship between HBV and glycemic markers may be independent of diabetes type. These findings underscore the importance of considering viral comorbidities in diabetic care and metabolic monitoring.

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Phylogenic Study of Hepatitis C Virus in Hepatitis Patients in Wasit Province, Iraq

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Abstract

Background and aims: Viral hepatitis represent a health problem that affects millions of people worldwide and is associated with high mortality, except for hepatitis A virus, all hepatotropic viruses, including hepatitis B, C, D, and E viruses, can produce chronic infections, Hepatitis C virus is one of the major globally cause of death and morbidity, and recent estimates showed an increase in its seroprevalence over the last decade to 2.8%. The whole extent of RNA genome is about 9.6 kb with one open reading frame (ORF) and 5′ and 3′ untranslated regions (UTRs) at both edges, 5′UTR is a more preserved portion of HCV genome, which aided in evolutionary studies and genotyping, the open reading frame encodes a polyprotein, which is comprised of 10 viral proteins named as Core (C), E1, E2, P7, NS2, NS3, NS4A, NS4B, NS5A and NS5B. **Materials and methods:** This study was conducted on 85 samples from HCV whom were confirmed to be infected with as they were diagnosed by a through ELISA screen test, RT-PCR and conventional PCR for selected genes. Their ages ranged from (5 - 75) years old during July and September 2022. Results: The work has been carried out on anti-HCV 85 (100%); sero-positive patients of the two sexes gathered comprising according to presumptive cause of infection 22.4% unknown patients; 35.3% hemodialysis patients; 35.2%; thalassemic patients; and finally, 7% other patients. Real-Time PCR was used to confirmed the serological diagnosis and for measurement of the viral loads in the 85 (100%) of seropositive HCV Ab HCV patients only 54(100%). **The results** revealed that all thalassemic patients was positive by ELISA technique, while 35.2% were gave positive results; 33.3% hemodialysis patients; 27.7% unknown patients; and finally, 3.8% other patients with HBV and HCV gave positive results. This study showed that 54 samples which were tested by Real time PCR for HCV viral load, then extraction HCV – RNA and amplification of Nonstructural protein 5A (NS5A) gene by using specific primers. Eight samples were positive amplification of NS5A gene, while the remaining was negative. A phylogenetic tree of HCV-NS5A gene revealed samples are related to genotype (4a). **Conclution:** dialysis patients have a high degree of risk factors for infection with the virus, through frequent blood transfusion as well as the dialysis machine or through the nursing staff. Also, thalassemia patients were observed to have a high infection rate.
Keywords: HCV, NS5A, Hemodialysis, Thalassemia

Introduction

Inflammation of the liver parenchyma in response to viral infections is called viral hepatitis, The hepatotropic viruses including hepatitis A (HAV), hepatitis B (HBV), hepatitis C (HCV), hepatitis D (HDV), and hepatitis E (HEV) make up the majority of such infections, Globally, millions of people are affected by these viruses annually(1).Viral hepatitis represents a health problem that affects millions of people worldwide and is associated with high mortality, except for hepatitis A virus (HAV), all hepatotropic viruses, including hepatitis B, C, D, and E viruses (HBV, HCV, HDV, and HEV), can produce chronic infections, whereas HAV causes acute self-limiting hepatitis that normally resolves spontaneously(2).Clinical presentation varies from asymptomatic or acute flu-like illness to acute liver failure or chronic liver disease, characterized by jaundice, hepatomegaly and ascites among many other signs, Eventually, this can lead to fibrosis (cirrhosis) of the liver parenchyma and carries a risk of development into hepatocellular carcinoma(3).Hepatitis C virus (HCV) existence was first fully recognized in 1975 when Feinstone*et al*. found that most cases of transfusion-associated hepatitis were not associated with hepatitis A virus or hepatitis B virus (HBV) infections, and thus defined the disease non-A, non- B hepatitis (4).

Hepatitis C virus (HCV) is one of the major globally cause of death and morbidity (5, 6). And recent estimates showed an increase in its seroprevalence over the last decade to 2.8%, corresponding to > 185 million infections worldwide (6, 7). Hepatitis C virus is enveloped, small circular, positive-sense and single stranded ribonucleic acid (RNA) virus from genus Hepacivirus, family Flaviviridae with a diameter of 50 nm (8). HCV particle consists of a nucleocapsid- containing the single-stranded RNA genome associated with the viral core protein and a lipid bilayer where the viral envelope proteins (E1 and E2) are assembled as heterodimers (9). In reality, the structure of HCV is more complex and the virus exhibits unusual and striking features (10). Indeed, a hallmark of HCV particles is their association with host cell lipids and lipoproteins, mainly very low-density lipoproteins (VLDL) and low-density lipoproteins (LDL) (11).

The whole extent of RNA genome is about 9.6 kb with one open reading frame (ORF) and 5′ and 3′ untranslated regions (UTRs) at both edges (12). 5′UTR is a more preserved portion of HCV genome, which aided in evolutionary studies and genotyping, the open reading frame encodes a polyprotein, which is comprised of 10 viral proteins named as Core (C), E1, E2, P7, NS2, NS3, NS4A, NS4B, NS5A and NS5B (13). The NS5A protein is a membrane-associated phosphoprotein that appears to have multiple functions in viral replication. It is phosphorylated by different cellular protein kinases indicating an essential but still not understood role of NS5A in the HCV replication cycle. In addition, NS5A has been found to be associated with several other cellular proteins (14, 15). Hepatitis C virus (HCV) infection is one of the most common causes of hepatocellular cancer (HCC) is a serious consequence caused by HCV infection, with high death and morbidity rates , HCV-induced HCC develops over time and is influenced by the duration of the infection as well as the viral genotype (16).

Materials and Methods

Specimen collection: This study was conducted on 85 samples from HCV whom were confirmed to be infected with as they were diagnosed by a through ELISA screen test, RT-PCR and convential PCR for selected genes. Their ages ranged from (5 - 75) years old. In the period between july and september 2022, hepatitis patients were collected from haemodialysis Center in Al-Zahra Teaching hospital, Al karama teaching hospital, Thalassemia center in AL-Kutwomenchildren Hospital of Wasit Province Health Directorate.Specimens Collection Five milliliters of venous blood were drawn from each patient's groups by medical syringe. The first portion (2.5 mL) was placed in gel tubes and left at room temperature for approximately thirty minutes to coagulate, then centrifuged at 5000 rpm for 10 minutes to separate serum, which was used to measurement HCV Ab by ELISA, the second part (2.5 mL) was placed in EDTA tube then centrifuged at 5000 rpm for 20 minutes to separated the plasma which was used to determine viral load and extraction of Nucleic acid of virus.

Serological Test: Enzyme- linked immune sorbent assay test was used for the detection of HCV Ab in human serum in clinical laboratories and as a first - line screening assay in blood. Serum samples were added according to the designation on the ELISA working sheet (Hightop Biotech /china) .

***Molecular* Test:** The RNA was extracted from 300 µl of plasma in a 50 µl elutionvolume the Quick-RNATM Viral Kit – Zymo (USA) research (catalog No. R1034), Then, the purity and concentrationof RNA were measured by NanoDrop.The NS5A was amplified by semi-nested PCR (snPCR) using primer (Table 1).

Table (1): Primers for NS5A gene region of HCV virus (17)

Species	Gene	Primer	5'-3'	PCR product
HCV	NS5A	F	GGIGARGGIGCIGTICARTGGATGAA	767bp
		R	TRTGRGAIGGRTCIGTIARCATIGA	
		R	TRTGRGAIGGRTCICTIARCATIGA	

According to instruction of the primer synthesizer company, the primers (originally lyophilized), were dissolved in the free ddH2O to obtain a final concentration of 100 µM/µl which served as a stock solution that stored at -20°C. A concentration 10 µM/µl was prepared from the stock primers to be used as a work primer.

In order to convert the RNA to cDNA, PrimeScriptTM RTreagent kit (Takara, Cat. # RR037A) was used. PCR have performed in a 25 µl and this volume composed of 3 µlcDNA 10 µl master mixPCR (Intron, Korea), 1 µl of each forward and reverse primer andthen the volume completed to 25 by adding nuclease-free water. The programming conditions were as follow: 4 min of 95°C; which followed by 45 cycles of 15 s of 95°C, 25 sec of 48°C, 72°C for1 min. 2% of agarose then.

were used to visualize the amplified region of DNA. The NS5A gene was sequenced by the Macrogen Company using their ABI 3730xl genomic analyzer (Applied Biosystems, US). The (NCBI) BLASTN program was used to analyzethe results (Table 2).

Table (2): Components of PCR's master mix and amplification procedures to detect NS5A genes (final volume 25 µl)

Phase	Tm (°C)	Time	Cycles
Initial Denaturation	94°C	3 min.	1 cycle
Denaturation -2	94°C	15 sec	45 cycle
Annealing	48°C	30 sec	
Extension-1	72°C	1 min	
Hold	4°C	∞	

Statistical analysis

Data were entered and analyzed using the software program Statistical Package for Social Sciences (SPSS) version 26. All numerical variables were represented by means (a measure of central tendency) and standard deviation (a measure of dispersion) while categorical variables were presented by frequencies and percentages. The Chi-Square test and Fisher´s Exact test (when more than 20% of cells have expected frequencies < 5) were used accordingly to assess the presence of an association between categorical variables. The independent samples t-test was used to assess the mean differences of numerical continuous variables. Considering a P-value is equal to or less than 0.05 a significant (**18**).

Results

Serological results

The work has been carried out on anti-HCV 85 (100%); sero-positive patients of the two sexes gathered comprising according to Presumptive cause of infection as: 19 (22.4%) unknown patients; 30 (35.3%) hemodialysis patients; 30 (35.2%); thalassemic patients; and finally 6 (7%) other patients (Table 3).

Table (3): Descriptive data of patients who diagnosed as hepatitis C by ELISA

ELISA test		
Item	**Frequency**	**Percent**
HCV Ab	85	100
Total	85	100

P value =***0.002; *Significant at level of P < 0.05

Molecular diagnosis for HCV

Viral load results for HCV

The molecular quantitative technique with Real-Time PCR was used to confirmed the serological diagnosis and for measurement of the viral loads (concentrations) in the 85 (100%) of seropositive HCV AbHCV patients only 54 (100%)The results revealed that all thalassemic patients was positive by ELISA technique, while 19 (35.2%) were gave positive results; 18 (33.3%) hemodialysis patients; 15 (27.7%) unknown patients ; and finally 2 (3.8%) other patients with HBV and HCV gave positive results (Table 4).

Table (4): Descriptive data of patients who diagnosed as hepatitis B or C by real time PCR

Item	diagnosis	N	Mean	SD	P value
Viralload copies /ml	HCV	54	739756.5	1.3	0.001
Viral load Iu /ml	HCV	54	5928726.9	2.6	0.001

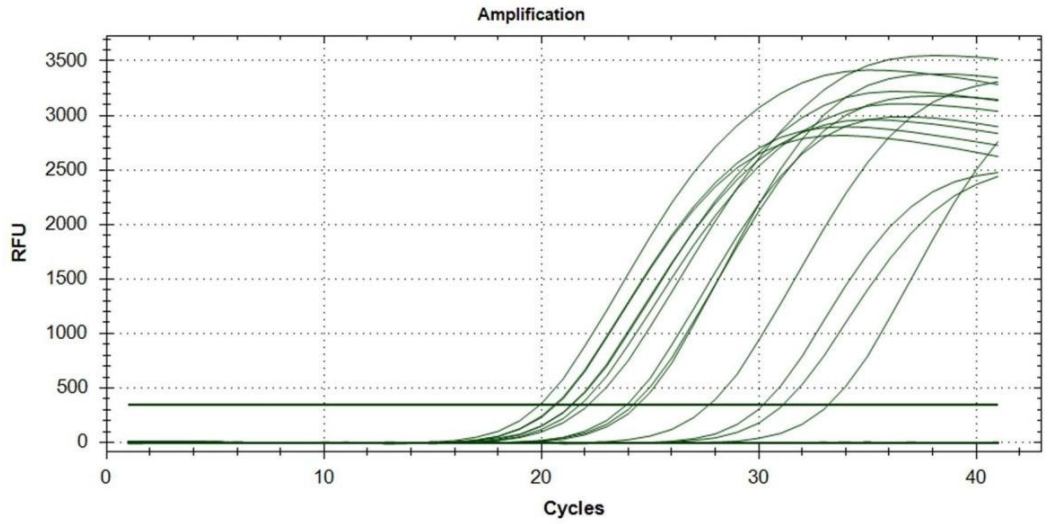

Figure (1): Amplification plot for RT-qPCR viral load (HCV)

Conventional PCR detection for HCV- RNA

This study showed that 54 samples which were tested by Real time PCR Technique for HCV viral load, then extraction HCV – RNA and amplification of Nonstructural protein 5A (NS5A) gene by using specific primers. Eight samples were positive amplification of (NS5A) gene, while the remaining was negative (Table 5).

Table (5): Distribution of HCV RNA conventional PCR technique amongpatients

Gender	No. of positive samples by Real time PCR	No. of positive samples by conventional PCR
Males	29(53.7%)	5(62.5%)
Females	25(46.3%)	3(37.5%)
Total	54(100%)	8(100%)

The amplification of the NS5A gene has been done successfully as the electrophoresis result showed sharp band at 725bp (Figure 2).

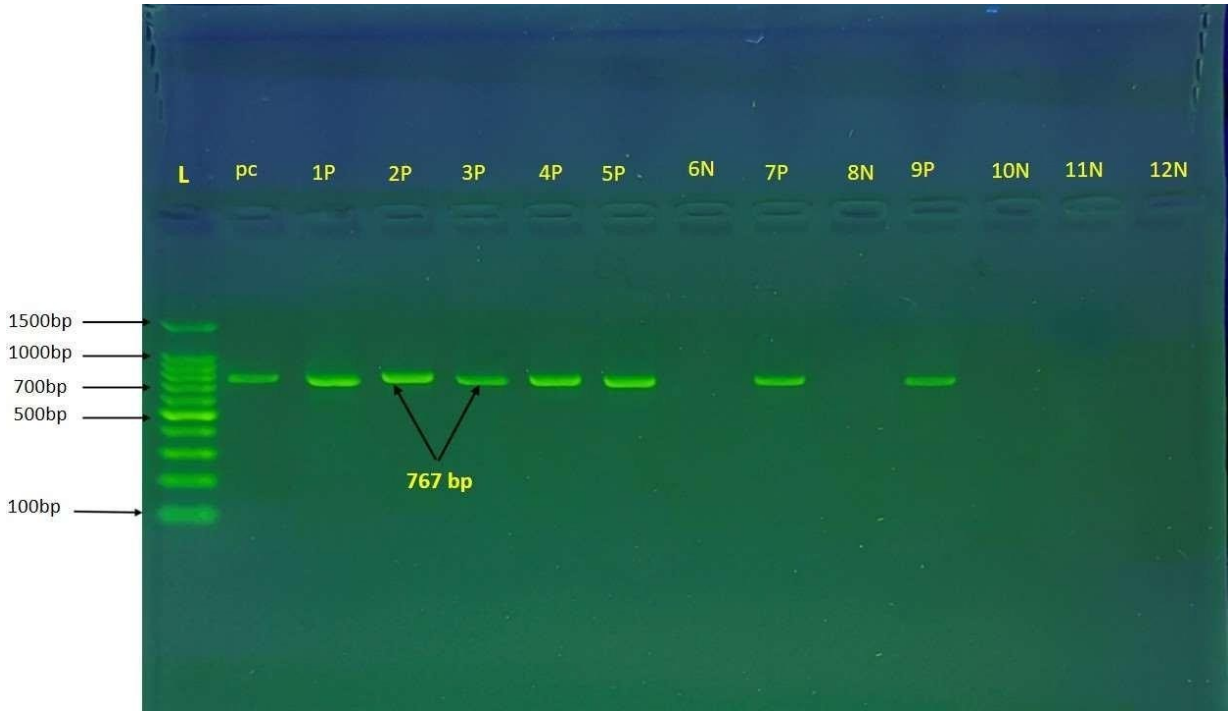

Figure (2): PCR product the band size 767 bp. The product was electrophoresis on 2% agarose at 5 volt/cm2. 1x TBE buffer for 1 hour; L: DNA ladder (1500-100); (PC) positive control, (P) positive sample, (N) negative sample

Phylogenetic tree

A phylogenetic tree generated by (MEGA) software version 6.0 using the Nonstructural Protein 5A (NS5A) gene. To showed identical between Iraq and the isolates of the world. Hierarchical cluster analysis determine the following clusters: large cluster divided into several neck: first root including Hepatitis C virus subtype 4a isolate Cyprus (ID:HQ537008.1), which has a 99% similarity to Hepatitis C virus subtype 4a isolate Portugal (ID: ON06818.1), also has two root: first including Hepatitis C virus subtype 4a isolate (ID: DQ988079.1) the identical 100% it is close to USA (ID: JX463528.1), and two root: including Hepatitis C virus subtype 4a isolate Australia (ID: KU871289.1) and Japan (ID: LC368356.1) the identical 99%), The last cluster is divided into two branches the first branch divided Iraq1 including Hepatitis C virus the identical 99%, the second branch divided into branch the first Iraq2 including Hepatitis C virus the identical 99%, the second branch Iraq2, Iraq (ID:OQ446441) including Hepatitis C virus the identical 99%. Following correspondence from the NCBI, the NS5A gene was registered, given an accession number, and became a reference for Iraq, the Middle East, and the rest of the world. After the validation steps have been completed the sequences got reference ID. A phylogenetic tree of HCV-NS5A gene revealed samples are related to genotype (4a) in figure (3).

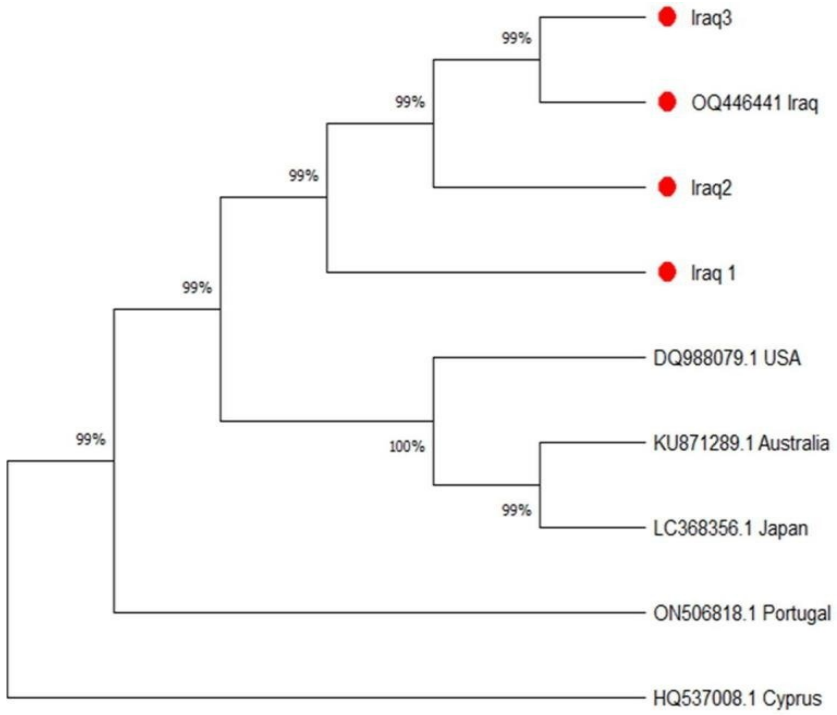

Figure (3): Neighbor-joining tree Hepatitis C virus of NS5A gene

Table (6): Homology sequence Identity (%) of local Hepatitis C isolates and NCBI Blast Hepatitis B isolates using NCBI- BLAST alignment tool

No	Access No. ID	Country	Source	Compatibility
1	**-**	Iraq 1	Hepatitis C virus	99%
2	**-**	Iraq 2	Hepatitis C virus	99%
3	**-**	Iraq 3	Hepatitis C virus	99%
4	**OQ446441**	Iraq	Hepatitis C virus	99%
5	**DQ988079**	USA	Hepatitis C virus	100%
6	**KU871289**	Australia	Hepatitis C virus	99%
7	**LC368356**	Japan	Hepatitis C virus	99%
8	**ON506818**	Portugal	Hepatitis C virus	99%
9	**HQ537008**	Cyprus	Hepatitis C virus	99%

Discussion

Infection with HCV is a common public health issue especially in developing countries such as Iraq. Such an infection is associated with deleterious consequences predisposing to liver cirrhosis and hepatocellular carcinom. The results of this study agreementwith previous studies done by Salman *et al.* The anti-HCV Ab positivity rate among the renal dialysis group were 32.2% with a statistically high difference (P= 0.0001) (**19**).

The study done by Hussein *et al.* a total of 255 hemodialysis patients with positive HCV-Ab results were referred for further evaluation. HCV- Ab positivity was confirmed again by ELISA (**20**). The results of this study disagreement, Jalil *et al.* (2022) showed that 23% of patients were anti-HCV positive and 77% were anti-HCV negative using ELISA technique (**21**). Infection rates varied between countries and came at different rates, due to the most important reasons, the most important of which are frequent blood transfusions, injecting drug use, preventive measures used in dialysis units, surgeries, especially organ transplants, and hand tools for workers in dialysis units, as previous studies recorded. The most common method of transmission of infection was from the patient's nursing staff, and they agreed that the most important way to avoid this infection is washing hands, so it is necessary to take preventive measures to reduce the spread of infection among dialysis patients, and the reason may be due to the different examination method used (22).

Hepatitis C is the most common chronic blood borne infection **(23)**. Reverse transcription real time polymerase chain reaction (RT-qPCR) was performed for direct and rapid detection of hepatitis C virus infection using one step technique **(24)**. Similar transmission models, HCV and HBV co-infection is prevalent, in this study, HBV were detect in hemodialysis patients with chronic HCV patient, viral interference has been described in patients with dual HBV and HCV infection **(25)**.The current results of this study are in agreement with previous studies done byJasim *et al.*, 2021 The extracted RNA from the 17 (Egyptian samples) and 89 (Iraqi samples) positive samples had tested by the RT-PCR, and the results showed 9 and 39 samples Egyptian and Iraqi respectively only were positive to HCV (**26**). The current study revealed that 86% of study patients were detected with positive RT-PCR assay, HCV–positive patients were significantly older from HCV-negative ones ($p<0.001$). In addition, those results indicate that higher prevalence of anti-HCV or HCV RNA were significantly associated with longer duration of transfusion ($p<0.003$ and $p<0.001$, respectively **(27)**. Al Kubaisy *et al*. (2006) Iraqi children with thalassemia showed a higher percentage than that recorded in other countries, such as 40.7% in Jordan, 40% in Saudi Arabia, and 14% in Turkey **(28)**.

The reasons for this discrepancy between antibody-positive as well as HCV RNA negative cases presented, is that the HCV might be existing in peripheral blood mononuclear cells (PBMCs) in such cases and not in serum or plasma as has been indicated *via* (**29**) who detected HCV-RNA in PBMCs in 10.5% out of 38 plasma viremias negative, and that the spontaneous viral clearance occurred in twenty percent of individuals exposed to the virus (**30**). Therefore, the presence of anti-IgG reflects resolved infection. Albeldawi *et al*. (2010) mentioned that ELISA is the most accurate serological marker for diagnosis of HCV infections but it still gave false positive and false negative results and cannot discriminate between the past and ongoing infections (**31**). The HCV infection might be the major risk factor for the liver fibrosis in transfusion-dependent thalassemia.

Furthermore, the excess liver iron is identified as one of the co factors for the development of advanced fibrosis in the patients experiencing HCV infection (**32**). The patients experiencing HCV have mild to moderate elevated hepatic iron concentrations and often have extreme hepatic iron overload **(33)**. The results of the current study showed agreement with the researchers' studies (Smith *et al.,* 2014). This study conducted of patients with HCV the result shows subtyping includes Hepatitis C virus subtype 4a. For the NS5A region, all of these considerations required the creation of a reliable, easy genotyping and resistance profiling technique. Smith et al. discovered that the phylogenetic tree derived from the NS5A HCV sequences is completely aligned with that of the whole virus coding sequences **(34).** Di Stefano *et al.,* 2021In the present study, GT 4 subtypes were assessed in 17 HCV GT 4-infected patients from Saudi Arabia. The most common subtype was GT 4a, and the other identified subtypes were GT 4o and GT 4d. Interestingly, two patients appeared to be infected with recombinant virus (4a/GT 4o/GT 4a), and one was infected with an unclassifiable virus, which may potentially represent a new, previously unseen, subtype **(35)**. The PCR we used amplifies a portion of NS5A (domain I) containing resistance-associated substitutions (RASs) associated to viral failure when employing NS5A inhibitors such daclatasvir, ledispasvir, ombitasvir, or velpatasvir. Currently, there is no clear and commonly acknowledged strategy for interpreting the presence of RASs at the start of treatment or in the case of virological failure **(36)** with the findings of all publications on HCV resistance, in vitro as well as in vivo. The list of mutations noted in prior reviews is fascinating because they were discovered in phase II and III studies, which are more typical of real-world clinical practice **(37)**. As a result, any new HCV infected patient may use our proposed protocol to evaluate both subtype and NS5A polymorphism. Recently, the NS5A region was identified as a possible option for HCV genotyping and subtyping **(38).** NS5A gene sequencing was recently used to record an HCV transmission outbreak in a Dutch haemodialysis facility **(39)**.

A phylogenetic analysis of the NS3, NS5A, and NS5B genes, as well as Sanger sequencing, recently identified two episodes of HCV transmission in two healthcare settings: one case of patients with acute HCV infection who had been diagnosed with onco- hematologic disease, and the other case of patients with acute HCV infection who had been diagnosed with onco-hematologic disease **(40)**, and a second case in which -thalassemia was diagnosed in patients with acute HCV infection **(41)**. Few studies have reported the use of HCV genotype and phylogenetic analyses combined to investigate HCV infection associated with nosocomial exposure **(42)**. Meaning the mosaic structure was robustly inferred, we recognize that the exact recombination patterns cannot be revealed without full-genome sequencing followed by a detailed recombination analysis, which may represent a possible limit of the study. Likewise, the evolutionary history of the unclassifiable sequence may be revealed after full-genome sequencing and further analyses. This study further analyzed naturally occurring mutations in the NS3, NS5A, and NS5B regions associated with drug resistance **(35)**.

Conclusion

The data given in this study demonstrated that HCV prevalence in hemodialysis centers and thalassemia patientsare relatively high and also suggested that the main risk factor appears to be the amount of time receiving hemodialysis that treatment, pointing to that nosocomial transmission of HCV and at a lower rate in some patients who acquired the infection through dental clinics or marking, as well as barbershops.

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Evaluation of antibacterial activity of probiotic *Bifidobacterium longum* and *Saccharomyces cerevisiae* against *E. coli O157:H7*

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Abstract
This research was directed to assess the antibacterial activity of the probiotic strains *Bifidobacterium longum* and *Saccharomyces cerevisiae* against *Escherichia coli* O157:H7, a pathogenic strain responsible for severe foodborne illness. The study employed three methods spot method, agar diffusion and vertical line method to evaluate the antibacterial action of *B. longum* and *S. cerevisiae*. *Bifidobacterium longum* exhibited considerable antibacterial activity, with noticeable inhibition of *E. coli* H7: O157 growth in comparison to S. cerevisiae. Despite the fact that *S. cerevisiae* showed antibacterial properties, its inhibition activity was not as much as than those of *B. longum.* The study asserts that *Bifidobacterium longum* showed a remarkable antibacterial effect against *E. coli* H7: O157, proposed its suggesting probable application as an agent to prohibit or control this pathogen infection. *Saccharomyces cerevisiae* additionally displayed antibacterial effects, although it is less efficient. However, the combination of both of them exhibited remarkable inhibition. These findings will be paved the way of understanding the influence of probiotics in infection control and food safety.

Keywords: probiotic, *Bifidobacterium longum, Saccharomyces cerevisiae, E. coli O157:H7,* food safety

Introduction

Probiotics are live microorganisms that converse health reimbursements to the human when directed in acceptable quantities (Mathipa and Thantsha, 2014). Apart from their well-known effects in digestive health and immune modulation, latest research has explored their possible as antimicrobial components against pathogens such as bacteria, fungi and viruses (Mazroue, 2020; Saha and Saroj, 2022) including strains of *E. coli O157:H7* (Igbafe et al., 2023).

Many studies investigated the contrivances of probiotics antimicrobial activity due to the dilemma of antibiotic resistance of pathogenic bacteria (Rabetafika et al., 2023). Probiotics directed the antimicrobial activity through variable mechanisms (Saxelin et al., 2005). The primarily mechanism is the production of antimicrobial substances such as organic acids, bacteriocins, and hydrogen peroxide. These substances can constrain the growth of pathogens by disorderly cellular progressions or directly destructive pathogenic membranes (Abdelhamid et al., 2018). Moreover, probiotics contest with pathogens for adhesion locations on intestinal epithelial cells, thus stopping settlement and succeeding infection (Iqbal et al., 2021).

In vitro researcher's work and some clinical studies have delivered valued visions into the antimicrobial possible of probiotics against pathogenic bacteria (Zinedine and Faid 2007; Huang et al., 2015; Abdelhamid et al., 2018). For example, research conducted by Servin, 2004) verified that *Lactobacillus* strains isolated from human gut microbiota expressively reduced the growth and feasibility of *E. coli* O157:H7in imitation gastrointestinal environments. These conclusions highlight the strain-specific differences in probiotic effectiveness and highpoint the significance of choosing suitable strains for therapeutic applications (Srinivas et al., 2017). The most applicable probiotics are genus of the *Lactobacilli* group, which has lately been separated into many species *Lactobacillus delbrueckii subsp. Bulgaricus, Lactobacillus crispatus, Lactiplantibacillus plantarum Lactobacillus acidophilus, Lacticaseibacillus rhamnosus, Lacticaseibacillus casei, Lactobacillus gasseri Limosilactobacillus reuteri, Levilactobacillus brevis, Ligilactobacillus* and others. The second genera are *Bifidobacterium* which include *Bifidobacterium animalis* subsp. *infantis*, *Bifidobacterium bifidum*, *Bifidobacterium longum* and even certain strains from some yeasts (e.g., *Saccharomyces cerevisiae* var. *boulardii*) nominated as probiotics (Fijan et al., 2022). *Bifidobacterium longum* habitat in the human gut, generated chemical components and organic acids such as lactic acid and acetic acid, forming acidic surroundings that reduce the growth of pathogenic bacteria like *E. coli* (Fijan et al., 2022). Also, *Bifidobacterium longum* has been detected to display antimicrobial action by producing some peptides that directly object and disturb the cell membranes of *E. coli* H7 0157 (Speranza et al., 2020).

Saccharomyces cerevisiae, a yeast species has many applications in food fermentation and in probiotic food supplementation (Tamang and Lama., 2022), also displays antimicrobial capability against many pathogenic bacteria (Hosseini et al., 2023; Kil et al., 2023). This yeast constructs various compounds such as organic acids, ethanol, and antimicrobial peptides that enhance the inhibitory approach against pathogens (Mulpuru & Mishra, 2022).

The probiotic potential and antimicrobial activity of *Lactiplantibacillus plantarum*, *Saccharomyces cerevisiae*, and *Bifidobacterium longum* were investigated against some foodborne pathogenic bacteria such as *E. coli* O157:H7, *Salmonella typhimurium* and *Listeria monocytogenes*. The study demonstrated that *L. plantarum* and *B. longum* revealed antimicrobial action against *E. coli O157:H7*, *S. typhimurium* and *L. monocytogenes*. Conversely, *S. cerevisiae* did not display inhibition to any of the seleceted pathogens (Igbafe et al., 2020)

Materials and Methodology

1. Strain Selection, Culturing and Preparation of *E. coli* H70157 Inoculum:

Strains of *Bifidobacterium longum* was kindly obtained from bacteriology laboratory /Faulty of sciences/Kufa University. Cultures are maintained under anaerobic conditions in MRS (De Man, Rogosa, and Sharpe) broth at 37 °C, while *Saccharomyces cerevisiae* strains used in in this study were isolated from fermented products in food sciences laboratory /Faculty of Agriculture /Kufa University. Yeast cultures are grown aerobically in YPD (Yeast Peptone Dextrose) broth at 26 °C. Pathogen strain *E. coli* O157:H7 was obtained from Karbala Food Safety and Medical Center. This strain was used as indicators of antibiotic activity. The culture was activated from glycerol stock by culturing in nutrient broth media overnight at 37°C.

2. Antimicrobial Assays

1. **Spot method**

Antibacterial activity was investigated by an agar spot test using a colony overlay assay (Tejero-Sarinena et al. 2013). The overnight cultures (10^5–10^9 CFU/mL) of B. *longum* were spotted (5µL) on the surface of MRS agar plates and incubated at 37 °C under anaerobic conditions for 24 h. Similarly, 5 µl of *S. cerevisiae* was spotted on SB agar plates and incubated at 26 °C aerobically for 24 h. After incubation, the plates were overlaid with 10 mL of nutrient agar, previously inoculated with 100 µL (10^6–10^9 CFU/mL) of an overnight culture of the indicator pathogen strain *E. coli* O157:H7. The examination was performed in triplicates.

2. **Agar Well Diffusion method**

This method involved pouring nutrient agar plates and inoculating them with a lawn of *E. coli* H70157. The cultures of (*Bifidobacterium longum* or *Saccharomyces cerevisiae*) are added to the wells eighter each strain alone or in combination. After incubation, zones of inhibition around the wells indicate antimicrobial activity against *E. coli* O157:H7, measured as the diameter of clear zones. Diameter of the zone of inhibition around the colony was examined and measured using a ruler. Inhibition zone with a diameter of 6 mm

or larger was considered as positive inhibition (Kizerwetter-Swida and Binek, 2005). The examination was performed in triplicates.

2. Vertical line method

A single line of Bifidobacterium longum and *Saccharomyces cerevisiae* was inoculated and placed vertically to the streaked *E. coli O157:H7*bacteria using a sterile inoculating loop. The plates were inverted and incubated at the appropriate temperature 37°C for the bacteria and 18-24 for the yeast. After incubation, the plates were observed for zones of inhibition or growth patterns. The areas of inhibition of pathogenic bacteria's growth were determined and measured.

Results and discussion

The results of tests on the antagonistic activity of young cultures of *Bifidobacterium longum* and bread *Saccharomyces cerevisiae*, individually and in combination, against the selected *E. coli* O157:H7 bacteria under study showed that there is resistance to different degrees using the hole method, the vertical line method, and the spot method (Table 1). It was found that the hole method was ineffective in showing the biological activity of bacteria and yeast individually or in combination, and even in the control sample, no growth inhibition was observed when using the same method, as shown in the tables below.

Table1: Average diameter of the inhibition zone for bacterial growth for *Bifidobacterium* bacteria

Average diameter of the bacterial growth inhibition zone (mm) for Bifidobacterium			**Microbial isolates**
Vertical line method	Spot method	Agar Well Diffusion method	
20	20	0	E. coli O157:H7
23	22	0	E. coli control sample

It is clear from the table above that the average diameter of inhibition by the vertical line method for *Bifidobacterium* bacteria against *E. coli* O157:H7 bacteria is 20 mm, while the average diameter of inhibition for the control sample was 23 mm. while for the spot method the inhibition zone of the pathogenic strain (20mm) and the control (22mm). no inhibition zone was observed in both pathogenic strain and the control in the hole method.

The inhibitory effect of *Bifidobacterium* against *E. coli* O157:H7 was lower with respect to that observed against *E. coli*. The differences observed between the results obtained from the different methods could be due to possible physicochemical interactions between the active metabolites produced by the bacteria and the medium nutritional. This was consistent with what was previously achieved (Inturri et al., 2019), where (the aim of the study was to verify the ability of both Bifidobacterium and *Lactobacillus* bacteria, alone or in combination, to inhibit the growth of pathogenic gram-negative and gram-positive bacterial strains and some fungi using different methods. Cell-free supernatants were obtained by centrifugation and filtration from single or mixed broth cultures and the inhibitory activity was tested using both agar diffusion and dilution methods. In order to obtain some preliminary information about the chemical nature of the active metabolites released in the supernatants. The highest inhibitory activity was demonstrated by the untreated supernatant obtained directly from the both cultures.

Table (2) shows that yeast has a lower inhibitory effect than what was observed on *Bifidobacterium* bacteria, at a rate of 17 and 19 for the pathogenic bacteria and for the control bacteria, respectively. These results were similar to the results obtained by Bach et al., 2003. In their study, the continuous culture conditions did not yield any effective inhibitory substances against *E. coli* O157:H7, despite the possibility that they were produced by *S. cerevisiae* subsp. boulardii in a feed supplement containing this yeast.

Table (2): Average diameter of the inhibition zone for bacterial growth of *Saccharomyces cerevisiae.*

Average diameter of the bacterial growth inhibition zone (mm) for Saccharomyces cerevisiae			**Microbial isolates**
Vertical line method	Spot method	Agar Well Diffusion method	
17	10	0	E. coli O157:H7
19	14	0	E. coli control sample

As for the use of bacteria and yeast combined as natural antibiotics (Table 3), the antagonistic effect was effective and clear inhibition in both the spot and vertical line methods against *E. coli* O157:H7 bacteria and control sample, which gives an indication that the use of these microbial agent's combination could be promising for alleviating severe effects as a result of infection with this type of bacteria, such as acute diarrhea.

Table 3: Average diameter of the inhibition zone for bacterial growth for *Bifidobacterium* bacteria and *Saccharomyces cerevisiae* yeast combined.

Average diameter of the bacterial growth inhibition zone (mm) for Bifidobacterium and Saccharomyces cerevisiae combined			**Microbial isolates**
Vertical line method	Spot method	Agar Well Diffusion method	
25	22	0	E. coli O157:H7
20	20	0	E. coli control sample

According to a recent overview, gastrointestinal infections in particular diarrheal diseases are one of the leading causes of morbidity and mortality worldwide. Although treatment with antibiotics has led to significant improvements in health, their overuse is associated with the development and dissemination of specific resistance mechanisms, contributing to the antimicrobial resistance emergency due to the death of more than 700,000 patients globally each year. Source: Imbalance has been documented. Balance between major microbial populations distributed in the human intestine in patients with gastrointestinal and urinary tract infections (Sanchez, 2017)

Several studies have shown that *Bifidobacterium* and *Lactobacillus* are able to competitively exclude pathogenic bacteria and yeasts, either directly, through interactions with pathogenic strains, or indirectly, through the production of active metabolites and stimulation of the host's immune defense. Therefore, they can Probiotics represent a potential alternative to conventional antimicrobials either as prevention or as treatment for gastrointestinal infections and for these reasons remain one of the main means of comparing these infections (Ghosh et al., 2019) (Oliveira et al., 2017). The strains, currently used as probiotics, belong to the genera *Bifidobacterium* and Lactobacillus, which are commonly found in the human intestinal microbiota and are capable of producing antimicrobial metabolites such as organic acids, hydrogen peroxide, ethanol, diacetyl, acetaldehyde, saturated or trans-free fatty acids and other compounds such as Peptides and bacteriocins. (Cook, 2011) (Nagarajan et al., 2019).

CONCLUSION

To conclude with, this research was evaluated the antibacterial activity of *Bifidobacterium longum* and *Saccharomyces cerevisiae* against *Escherichia coli* O157:H7. The findings revealed definite differences in their activity. *Bifidobacterium longum* exhibited a considerable antibacterial action of inhibiting the of E. coli O157:H7 growth in the spot and vertical line methods. However, no inhibition was observed while the agar well diffusion method.

In comparison with, *Saccharomyces cerevisiae* exhibited some level of antibacterial activity in vertical and spot methods (17 and 10 mm) respectively, but was less effectual in contrast to *B. longum*. The combination *Bifidobacterium longum* and *Saccharomyces cerevisiae* showed significant inhibitory effect more than using each strain individually. These outcomes highlight the potential of probiotic *B. longum* as a promising candidate for probiotic-based therapies targeted and controlling the infections caused by E. coli O157:H7. Further research is required to examine the mechanisms behind *B. longum and Saccharomyces cerevisiae* efficacy and to assess their applications in clinical and food safety layouts.

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Leucopenia with HHV6 in Cancer Patient Receiving Chemotherapy

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Abstract
The aim to study the relationship between leucopenia in cancer patients undergoing chemotherapy that infected with HHV6. The study was conducted according to a cross sectional study and included the study population, the total number of 200 cases, the age of individuals between 1-95 years in Middle Euphrates Cancer Center in Najaf Governorate for the period between November 2020 to May 2021. Whole blood sample to investigate the leucopenia through a CBC test and serum sample to detect HHV6 Ag as well as anti-viral IgM, IgG antibody using ELISA The comparison included statistical analysis between positive and negative HHV6 IgM, HHV6 IgG, and HHV6 antigen in the two groups using the chi-sequer method**.** Our study included patients suffering from leucopenia, where the highest age group was (61-80), which gave (31,7%) cases for male. As for females, the highest age group (41-60) was given (37.2%). The study included three degrees of leucopenia (mild, moderate, and severe). The number of mild patients was (23%), the number of moderate patients was (24%), and the number of severe patients was (53%). The ELISA test aims to diagnose IgM, IgG, HHV6. The antibodies to HHV6-IgM were positive in cancer patients with leucopenia (53%), while antibodies to HHV6-IgM positive in cancer patients without leucopenia were (12%). The IgG antibody screening in cancer patients with leucopenia revealed that (61%) had positive results for HHV6-IgG antibodies. The same test was done for the second group. The test HHV6-IgG antibodies positive (15%) out of 100 samples. The HHV6-Ag was positive in cancer patients with leucopenia in (51%), While the HHV6-Ag was positive in cancer patients without leucopenia (11%). A statistical comparison was carried out between the research groups: the first group consisting of cancer patients with leucopenia and the second group of cancer patients without leucopenia. There are significant differences in the results between the first and second groups, as well as significant differences in the positive HHV6 IgM, HHV6 IgG, HHV6 antigen results between the first and second groups, as well as significant differences in the negative results between these two groups (P value < 0.5).

Keyword: HHV6, leucopenia, ELlSA, IgM, IgG.

Introduction

Leucopenia is a condition that results from a decrease in the number of WBCs in the peripheral blood. It manifests itself clinically as weariness, dizziness, appetite loss, and other non-specific, as well as recurrent infection (Disanti *et al*., 2006). This is a common occurrence at specific stages of some infectious diseases, and it is indicating a breakdown of one of the body's defense mechanisms *(Zhang and Shen., 2007)*. This can result in fatigue and symptom combinations, as well as an increased risk of infection, which can be deadly. Leucopenia can cause cancer treatment to be delayed or decreased, perhaps resulting in subtherapeutic levels and treatment failure (Provan *et al*., 2004).

Chemoradiotherapy most oftein cause myelosuppression and leucopenia (Bogani *et al*., 2017). Antimicrobials, analgesics, anti-inflammatories, antihistamines, and anticonvulsants, among others, cause leukopenia by destroying normal bone marrow or lowering white blood cell counts (Schwartzberg, 2006). Autoimmune diseases such as SLE and RA contribute to leucopenia by destroying healthy white blood cells (WBCs) (Zheng *et al*., 2013). Additionally, it was demonstrated that WBCs are insufficient in bone marrow diseases such as leukemia, vitamin B12 or folate deficiency, myelofibrosis, myelodysplastic syndrome, and aplastic anemia. On the other hand, virus infection can temporarily impair BM function, resulting in leucopenia (Akkina, 2013).

Chemotherapy-induced myelosuppression, particularly leucopenia or neutropenia, is the most common adverse effect of cancer treatment. Chemotherapy-induced leucopenia can result in serious infections and anemia, which can disrupt treatment schedules by delaying chemotherapy. Leucopenia and its associated complications are clinically significant problems that have a significant impact on the efficacy of chemotherapy and the patient's quality of life (Dinan *et al*., 2015). Leucopenia can be caused by a variety of conditions, including Colorado tick fever, meningococcemia, pneumococcal pneumonia, and salmonellosis. Leucopenia also be caused by malnutrition (Hashiguchi *et al*., 2015). Sarcoidosis can affect the bone marrow and result in fever, anemia, and leucopenia (Kalajian *et al*., 2009).

Human herpesvirus 6 is a double-stranded DNA virus that was initially identified in immunocompromised patients with lymphoproliferative disorders. (Borenstein and Frenkel, 2009). The salivary glands in humans, they have been identified as a reservoir for HHV6 infection. According to studies (Arbuckle et al., 2011), HHV-6 is highly infectious to T cells (Lusso et al., 1995). Human herpesvirus 6 is mostly found in humans. While they can infect a wide number of cell types. Herpesviruses have evolved a variety of strategies for ensuring their survival in latently infected cells and evading host immunity during successful reproduction (Kawabata et al., 2011). Saliva is the principal reservoir for virus transmission. HHV-6 has been identified following organ donation, despite there being no examples of virus transmission through blood transfusions or nursing. *(Agut et al., 2015)* **.** Primary HHV6 infection is most common in infants and children under the age of six months, with fever-induced seizures being a major cause in children aged six

to 24 months. Acute HHV-6 infection is uncommon in immunocompetent people, but it can cause a mononucleosis-like illness with fever, leucopenia, and hepatitis. (Hall et al., 1994).

HHV-6, like other herpesviruses, infects a wide variety of cells and then goes dormant (Amirian and Scheurer, 2012).HHV-6, unlike other herpesviruses, can be chromosomally integrated, which is the preferred form of vertical transmission, despite the fact that it only affects about 1% of newly infected people (Ward et al., 2006).The HHV-6 (ciHHV-6) virus, which they received from their parents, is expected to harm 1% to 2% of the general population (Pantry and Medveczky, 2017).When diagnosing HHV-6 encephalitis, chromosomally integrated HHV-6 has the potential to significantly complicate a clinician's ability to interpret laboratory results. Because ciHHV-6 DNA can be found in all leukocytes, whole-blood polymerase chain reaction (PCR) detection is practically certain in these patients (Ong et al., 2017).

The most well-known clinical condition in adults is HHV6 encephalitis, which exclusively affects those who are highly immunocompromised, such as HSCT or SOT recipients. Despite being the most dreaded HHV6 infection consequence, it is still very uncommon. In a retrospective study of HSCT users in 2019, the Mayo Clinic observed a 1.7 percent (9/531) incidence of HHV-6 encephalitis. While the incidence was low, these patients had a 50% mortality rate and a high proportion of long-term neurologic deficits among those who survived (Fida et al, 2019).

In addition, the literature is divided on the relevance of HHV-6 in critically sick patients who do not have baseline immunosuppression. HHV6 DNA has been identified in the blood of people who are very sick but don't show any symptoms of the virus. HHV-6 DNA has been found in more than a quarter of septic shock patients, according to certain investigations. The relationship has not been proven to reactivate and is thought to contribute to a poor prognosis in these people (Ogata,2015). Idiopathic pneumonitis and hepatitis have also been linked to HHV-6. There is inadequate evidence to suggest pathophysiologic causality in the majority of cases when a link between HHV-6 and other clinical entities (e.g., multiple sclerosis, pityriasis rosea) has been discovered (Pormohammad et al., 2017).

Diagnosis of leukocyte and it is differential count, which is usually performed as part of a complete blood count, or CBC, is necessary for the accurate diagnosis of quantitative leukocyte disorders. In the event of leukopenia, the total leucocyte count will decrease (Tkachuk and Hirschman., 2007)

Methods

Patients: The study population included patients (cross-sectional study). Blood samples (200 cases) at the Middle Euphrates Center for Cancer. A serum sample was collected from a cancer patient who was undergoing chemotherapy. were collected (period between November, 2020 to May, 2021). Of 200 cases, there were 111 females and 89 males, and the patients' age ranged from 1-95 years. where the individuals were divided into two groups: first, included 100 cancer patients

with leucopenia the second group consisted of 100 cancer patients without leucopenia including 59 % females and 41% males, while the second group was 52% females and 48% males from cancer patients without leucopenia >

Ethical Approval: Samples of blood were taken from the individuals enrolled in this study after obtaining the oral consent of them. The acceptance of the study protocol has been made by the ethical committee.

Inclusion Criteria: Cancer patient who undergo chemotherapy with leucopenia and cancer patient without leucopenia

Study Parameters: All suspected Cancer patients undergo chemotherapy subjected to the following:

Samples Collection: The samples that obtained from patients were divided into two types of tubes: gel serum and EDTA tubes. The whole blood samples (1ml) that transferred to EDTA tubes were mixed well for several times for CBC test. Serum samples: prepared by allowing blood samples (4 ml) to be clotted at room temperature for about one hr. After that, centrifugation for 15 min at 3000 rpm, the serum was separated in a new plain tube for immunological test (HHV6 Ags, HHV6_IgM and IgG)

Hematological methodology: By detecting and measuring variations in electrical impedance in conductive liquid, the impedance method (coulter-method) sizes and counts cells (Elite3 hematology analyzerv/ Germany).

Serological technique (ELISA): The serum samples of patients were obtained for screening of the presence of HHV6 Ag, HHV6_ IgM and IgG antibodies by ELISA using commercial kits (SUNLOMG_CHINA).

Statistical Analysis: The application of analysis to determine the statistical significance of the data included the Chi-square test, P value of <0.05. It depends on SPSS 24.

Results

Sex distribution of leucopenia disease in overall patients according to age groups

Our current study included 100 patients suffering from leucopenia. 41 males (41%) and 59 females (59%), where the highest age group was (61-80), which gave 13 (31,7%) cases, followed by the age group (41 – 60), which gave 12 cases. (29.2%) of the total number of males. As for

females, the age group was given (41_60). 22 cases (37.2 %), followed by the age group (21 – 40), including 16 cases (27.1%).

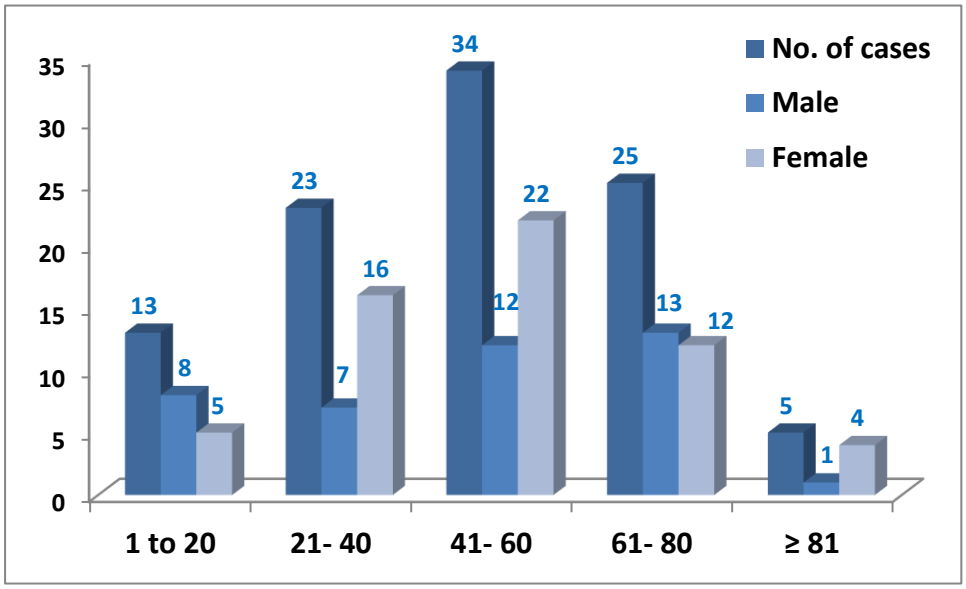

Figure (1): Sex distribution of leucopenia disease in overall patients according to age groups chi_square is 5.9479. *p* value is .653064. The difference is *not* significant (*p*-value >0.05.).

Degree of Leucopenia in cancer patients

The current study included three degrees of leucopenia (mild, moderate, and severe). The number of mild patients was 23 (23%), the number of moderate patients was 24 (24%), and the number of severe patients was 53 (53%).

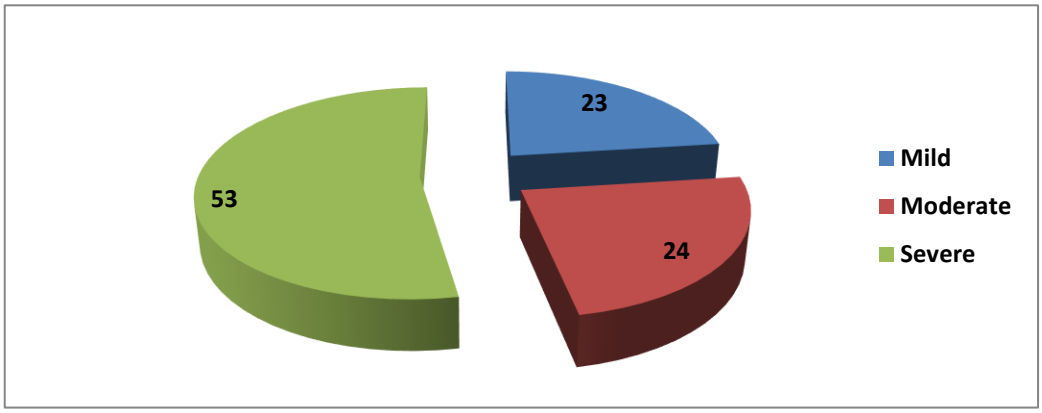

Figure (2): Leucopenia degrees in cancer patients

The degree of Leucopenia in cancer patients according to age groups

The three degrees of leucopenia were divided depending on the age groups adopted in the study, where the age group (41_60) was the highest in mild leucopenia, as it gave out of 22 patients 10 patients (45.4%), followed by the age group (61_80), which gave 9 patients (40.9%).The moderate leucopenia showed that the highest age group was (21_40), as it gave 10 patients out of 26 patients (38.4%), followed by the age group (41_60), which gave 7 patients (26.9%). For the severe leucopenia, the age group (41-60) was the highest, giving 17 out of a total of 52 patients (32.6%).

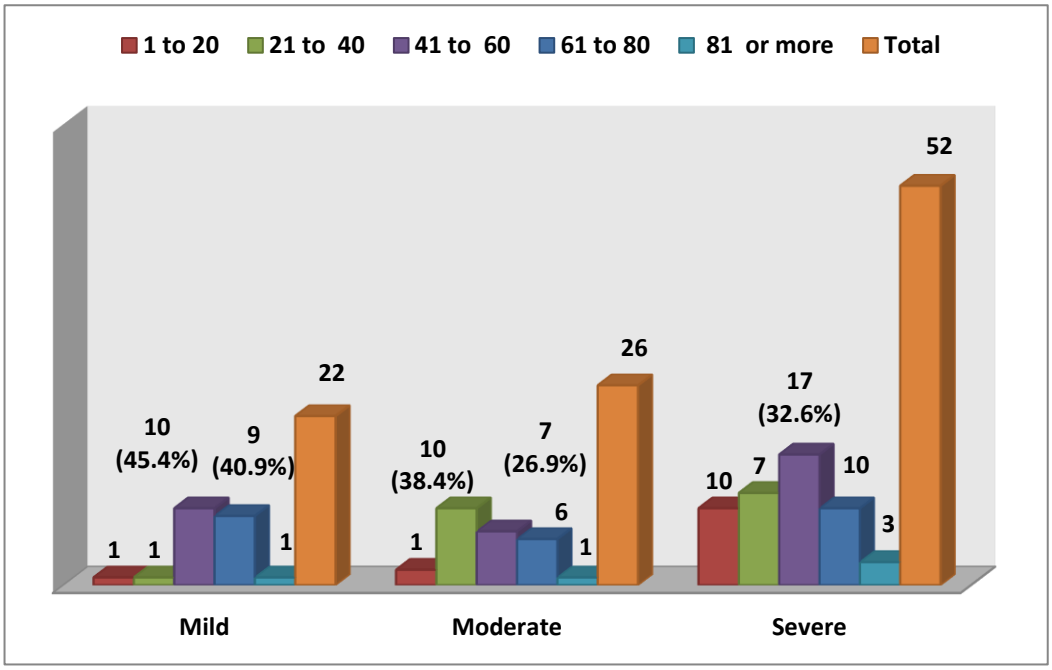

Figure (3): The degrees of leucopenia in cancer patients according to age groups. Chi-square is 13.6422. *P*-value is .324139. There is *no* significant result at *p*> 0.05.

A statistical comparison of IgM results between the first group (with lymphoctytopenia and leukopenia) and the second group (without lymphocytopenia and leukopenia) by using the chi_ square method

A statistical comparison was carried out between the research groups: the first group consisting of cancer patients with leucopenia and the second group of cancer patients without leucopenia. The comparison included statistical analysis between positive and negative IgM results in the two groups using the chi-square method.

There are currently significant differences in the results between the first and second groups, as well as significant differences in the positive IgM results between the first and second groups, as well as large differences in the negative results between the first and second groups.

Table (1): A statistical comparison of IgM results between the first group (with leucopenia and the second group (without leucopenia) by using the chi_ square method.

Cancer patient	No. of cases	IgM+	IgM -	*Row Totals*
Leucopenia	100	53(53%)	47 (47 %0	200
without leucopenia	100	12 (12 %)	88 (88 %)	200
Column Totals	200	65	135	400 (the Grand Total)

A statistical comparison of IgG results between the first group (with Leucopenia) and the second group (without Leucopenia) by using the chi_ square method.

A statistical comparison was carried out between the research groups: the first group consisting of cancer patients with leucopenia and the second group of cancer patients without leucopenia.

The comparison included statistical analysis between positive and negative IgG results in the two groups using the chi- square method. There are currently significant differences in the results between the first and second groups, as well as significant differences in the positive IgG results between the first and second groups, as well as large differences in the negative results between the first and second groups.

Table (2): A statistical comparison of IgG results between first group (with leucopenia) and the second group (without Leucopenia) by using the chi_ square method.

Cancer patient	No. of cases	IgG+	IgG -	*Row Totals*
Leucopenia	100	61 (61%)	39 (39%)	200
without Leucopenia	100	15 (15%)	85 (85%)	200
Column Totals	200	76	124	400 (Grand Total)

Chi square is 44.9066. The *p* value is < 0.00001. The difference is *significant* (*p*-value <0.05.).

A statistical comparison of HHV6 Ag results between the first group (with Leucopenia) and the second group (without Leucopenia) by using the chi_ square method.

A statistical comparison was carried out between the research groups: the first group consisting of cancer patients with leucopenia and the second group of cancer patients without leucopenia. The comparison included statistical analysis between positive and negative HHV6Ag results in the two groups using the chi- square method.

There are currently significant differences in the results between the first and second groups, as well as significant differences in the positive HHV-6 results between the first and second groups, as well as large differences in the negative results between the first and second groups.

Table 3. A statistical comparison of HHV6 Ag results between the first group (with Leucopenia) and the second group (without Leucopenia) by using the chi_ square method.

Cancer patient	No. of cases	HHV6-Ags +	HHV6-Ags -	*Row Totals*
Leucopenia	100	51 (51%)	49 (49%)	200
Without Leucopenia	100	11 (11%)	89 (89%)	200
Column Totals	200	62	138	**400 (Grand Total)**

Chi square is 37.4007. *p* value is < 0.00001. The difference is significant (*p*-value <0.05.).

Discussion:

The axes of the study are: the distribution of disease and patients in terms of gender and age groups to the degree of leucopenia. The second axis is statistical comparisons between the two groups using chi. Square. Cancer patients in the current study were distributed, depending on gender, into the first group suffering from leucopenia, and they were divided according to the age groups approved in the study.

The study showed in general that the number of females constituted 59%, while with regard to males, there were 41%, in which (61-80) and then followed by (41-60) were the highest in leukopenia cases that revealed 31.7% and 29.2% respectively in males.

The reason here may be due to age plays a role in the presence of some diseases, especially cancerous diseases and also perhaps these age groups represent the largest number in the study group, so they gave a high percentage in the event that the last age group >80 gave a lower percentage due to the lack of cases.

As for females, the age group (60-41) was the largest in the number of cases, 37.2%, followed by the age group (21-40), which gave 27.1%. It is noticeable in the current study that there are no significant differences between males and females in terms of the number of cases or age groups that suffer from the disease, which indicates that both sexes are exposed to these diseases, as well as most age groups, are also exposed to the occurrence of the disease.

The occurrence of these diseases, therefore, may be due to other reasons, such as genetic, environmental, immune, psychological, and nutritional factors for patients The current study also included the division of cases of leucopenia into three degrees: mild, moderate, and severe, depending on the results obtained.

(Gwak et al., 2007) Found that amplified neutrophils, reduction in the both lymphocytes and monocytes, and a raise in N/L in circulating blood were observed in stomach cancer patients after total or subtotal gastrectomy. The changes in these principles were mainly noticeable in the direct postoperative stage and tend to be restore as time passed in both sex during the postoperative period. In this study's large sample size, we establish considerable gender effects on changes in the proportion of blood WBC subsets subsequent surgery. For a few days female patients exhibited after surgery, circulating neutrophils more, fewer lymphocytes, resulting in greater N/L, and fewer monocytes, indicating the condition of a immune-compromised become further, than male patients.

Except for the entire amount of WBC and the proportion of monocytes, there were no important variations in preoperative values between sexes.

Where this division was relied upon according to the approved medical and scientific criteria, and it is inferred that patients suffer from the development of leucopenia, either for pathological or immune reasons, On the other hand, because of the toxic effects of chemotherapy for cancer patients due to the damage that occurs in the bone marrow, which leads to a reduction in the production of leukocyte. Alternatively, a defect in the post-production stages, such as the stages of synthesis, differentiation, and others.

The current study included three groups of leucopenia (mild, moderate, and severe). The number of mild patients was (23%), the number of moderate patients was (24%), and the number of severe patients was (53%). We find that more than half of the cancer patients who undergo chemotherapy in this study group may suffer from leukopenia as a result of the effects of chemotherapy directly or indirectly, such as the toxic effect on the bone marrow and its other effects on the activity of white cells. In addition to other immune or inflammatory factors, or the percentage of viral and microbial infections. In addition, the degrees of leukopenia were distributed depending on the age groups studied the relationship between the degrees and levels of leucopenia and the number of patients, where the age group (41_60) was the highest in mild leucopenia (45.4%)], followed by the age group (61_80), which gave (40.9%). The moderate leucopenia showed that the highest age group was (21_40), as it gave (38.4%), followed by the age group (41_60), which gave (26.9%). For the severe leucopenia, the age group (41-60) was the highest, giving (32.6%). We found that although age may play an important role in leukopenia degrees, the current distribution might not indicate a direct relationship between age and cases of leukopenia, and there may be a relationship between the number of chemotherapy doses and the severity of cancer in patients that play an important role in severty. Leukopenia is exacerbated regardless of the patient's age.

(Tian *et a*l., 2020) indicated Cyclophosphamide is a widespread cancer chemo-therapy treatment, but the immunosuppression associated with it results in leukopenia. (Huang *et al*., 2017.; Sun *et al*., 2017) indecated cyclophosphamide can simply inhibit normal hematopoietic cell division and proliferation, resulting in impaired restitution or harm to hematopoietic organization, decreased nucleated cells, and decreased hematopoietic reconstitution activity. Leukopenia is by far the most frequently observed adverse effect. (Fan *et al*., 2017). It was indicated that cyclophosphamide was used to induce leukopenia in cancer rodents in order to mimic clinical and pathological physiology. (Wei *et al.,*2013) found that total of 445 patients aged 18 to 80 years were studied, with four to ten periods of chemotherapy established histologically and cytologically. They discovered that 62 of the 85 people who died as a result of a serious infection had severe leukopenia. Mild leukopenia is strongly associated with a greater chance to survive. And demonstrated that female patients were more likely to develop leukopenia.

(Dobbs *et al*., 1995; Diasio, 1998) Indicated that vinorelbine pharmacokinetics chemotherapy is linked not only to body surface area, but also to body mass, serum creatinine, platelet count, and sex. (Singh *et al*., 2004) revealed that females had considerably more apparent hematologic toxicity than males did. (Williamson, 1993) Reported that higher frequency of hematologic toxicity in women who have a higher baseline body mass index (BMI) than in males, which may alter the delivery of cytotoxic drugs and raise possible toxicity. (Merzoug *et al*., 2011) indecated that ADR-induced lymphocytopenia could be the result of the destruction of lymphocyte precursors and mature lymphocyte populations, resulting in immunosuppression.

As for talking about the division of leukopenia into degrees or levels in terms of the severity of the disease. A statistical comparison was made between the research groups: the first group consisted of cancer patients with leukopenia and the second group of cancer patients without leukopenia. The comparison included statistical analysis between the results of the four parameters IgM, IgG, HHV6 Ag, positive and negative in the two groups using chi-square method. There are currently significant differences in the results between the first and second groups, as well as significant differences in the positive IgM results between the first and second groups, as well as large differences in the negative results between the first and second groups.

This indicates that cancer patients who have decreased immunity because of receiving chemotherapy in large quantities are more likely to have new HHV6 Ag virus infection than the second group that does not suffer from immunodeficiency, or by another explanation, that viral infection has caused a decrease in immunity, and thus the number of infections in cancer patients who suffer from a lack white blood cells is much more than those who do not suffer from a lack of white blood cells.

There are significant differences in the results between the first and second groups, as well as significant differences in the positive IgG results between the first and second groups, as well as large differences in the negative results between the first and second groups.

This indicates that cancer patients who have a low number of lymphocytes and white blood cells as a result of receiving chemotherapy in large quantities are more susceptible to virus reactivation than the second group that does not suffer from lymphocytopenia and leukopenia. As we know that HHV6 Ag has the ability to chromosome integration, and that decreased immunity is one of the important factors that lead to the reactivation of this virus. The result is significant between the first and second groups, as well as significant differences in the positive HHV-6 Ag results between the first and second groups, as well as large differences in the negative results between the first and second groups**.**

This indicates that cancer patients with leukopenia as a result of receiving large amounts of chemotherapy are more likely to be infected with HHV6 Ag than the group without leukopenia. Or in another explanation, that the viral infection caused leukopenia, and therefore the number of infections in cancer patients with leukopenia is much greater than in those without leukopenia.

HHV-6 is only found in certain types of cancer, but its presence in tumor cells is insufficient to attribute a direct role to the virus in tumorigenesis. Cancer viruses are implicated as causative agents in a large proportion of cases, due to their presence in the majority of tumor cells and virus-

induced in vitro cell transformation. This virus, while not directly oncogenic, could indirectly promote tumor cell growth, in some cases by collaborating with other viruses and other factors. HHV-6 could also be an opportunistic virus that thrives in an immunodeficient tumor microenvironment, according to some researchers.

In spite of published studies, it is still too early to conclude that HHV-6 is responsible for several human cancers. As a result of some evidence, it appears that HHV-6 may work in concert with other viruses to cause cancer, such as HPV and EBV; HHV-6 has also been implicated in nodular sclerosis, Hodgkin lymphoma, gastro-intestinal tumors and oral cancers. HHV-6's exact role in tumor growth, however, will require further investigation. As outlined by Seror et al. (2008) and Faten et al (2012). Studies have demonstrated an increase in the frequency of HHV-6 after chemotherapy, both during the course of treatment and at the end of it, which confirms that the virus is reactivated after chemotherapy

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Relevance of hypertension and lipid profile with coronary heart disease in Iraqi population

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Abstract

Background: Hypertension is widespread all over the world and represent the main health problem. Dyslipidemia is a major leading cause of cardiovascular disease. Hypertension and lipid abnormalities are contributed to increase in risk of coronary heart disease (CHD), CHD represent one of the most important health problems and remain the major cause of morbidity and mortality in many countries all over the world. **Objective:** The aim of this study is to evaluate the lipid profile parameters level among patients with CHD.**Methods:** A total of 100 patients (100 CHD; 50 males and 50 females), in comparison with 100 of healthy individuals (50 male and 50 female). The patients were randomly selected in Najaf region. The lipid profile parameters were measured for each patient and healthy individuals using Spainreact kits with standard procedure. **Results:** The results revealed that CHD patients had higher level of TC and Tg, HDL-cholesterol was significantly lower in CHD patients of both sexes when compared with normal individual, whereas LDL-cholesterol was significantly higher in CHD male and female patients. The atherogenic index was significantly increased in CHD male and female patients according to their age levels. **Conclusion:** The present study indicate that lipid profile parameters level was elevated in patients with CHD with higher atherogenic index in Iraqi population.

Keywords: hypertension, lipid profile, CHD

Introduction

Hypertension can be defined as an abnormally high arterial blood pressure. It is still accepted that hypertension defined as systolic blood pressure ≥140 mm Hg and/or diastolic blood pressure ≥90 mm Hg. Dyslipidemia prevalence varies from population to population. Men and women with premature coronary disease, which occurs before 55 to 60 years of age, have the highest incidence. In such patients, the prevalence of dyslipidemia as high as 80 to 88 percent compared to approximately 40 to 48 percent in age-matched controls without coronary heart disease (CHD)[1]. Chronic metabolic diseases such as cardiovascular disease remains highly complex and multifactorial. In spite of the many risk factors that contribute to the aggravation of these diseases, raising cholesterol levels and their atherogenic potential have received great attention as potential risk factors [2].

Coronary heart disease (CHD) or cardiovascular diseases are recognized to be one of the most important reasons of morbidity and mortality and imposes heavy socioeconomic burden worldwide. There are varieties of risk factors in the literature which increases the incidence of CHD such as hypertension associated with hyperlipidemia. By the year 2020, World Health Organization (WHO) is predicting more than 11.1 million deaths from CHD. It is projected that the annual number of deaths due to cardiovascular disease will increase from 17.5 million in 2012 to 25 million in 2030. This increase is due to industrialization, urbanization and related lifestyle changes which is called epidemiologic transition. [3,4]

Coronary heart disease occurs when cholesterol accumulates on the artery walls, creating plaques. Reduced blood flow occurs when one or more of these arteries become partially or completely blocked. The four primary coronary arteries are located on the surface of the heart are: right, left main coronary artery, left circumflex artery and left anterior descending artery [5].

The hypertriglyceridemia results both from increased substrate availability as free fatty acids and from decreased lipolysis of very-low-density lipoprotein (VLDL) triglyceride [3-5]. Elevated concentrations of triglycerides rich lipoprotein specially very low-density lipoprotein (VLDL), and decreased levels of high-density lipoprotein (HDL), measured as HDL-cholesterol are the most characteristic lipoprotein abnormalities in CHD [6]. Most patients with CHD have total amount of LDL- cholesterol occasionally high or it is the same as in healthy people [7].

This investigation intiated with the aim of determining the serum levels of total cholesterol (TC), triglycerides (Tg), HDL- cholesterol, LDL-cholesterol, and VLDL- triglycerides as a primary risk factor in male and female patients with hypertension leading to CHD randomly selected from Iraqi population in Najaf region.

Material and Methods

This case-control study included a total of 200 individuals 100 of them (50 males and 50 females) were diagnosed with coronary heart disease, the medical examinations for patients were carried out by experienced physicians. Their ages between 45-73 years compared with age and sex matched 100 healthy control individuals (50 males and 50 females), their ages between 44-70 years. Between the period of 2022 to 2024. In this study the blood pressure, BMI and lipid profile parameters were evaluated for each participant.

Biochemical analysis: Subjects were fasting for 12 hours at the time of blood withdrawal. Blood specimens were taken by venpuncture using venous blood and either serum or plasma were used for analysis.

Colorimetric method applied to evaluated total cholesterol (TC), triglycerides (Tg), HDL-cholesterol and LDL- cholesterol by using RANDOX kits (United Kingdom BT 29 4QY) according standard procedures, triglycerides devided by 5, VLDL- triglycerides was evaluated according to the formula of Friedwald et.al [8].

Statistical analysis: Statistical analysis of the data was carried out using the STATGRAPHICS computer program package the results are expressed as mean ±SD, and comparison of data were made by using " student's t-test and ANOVA test".

Results

In this study, a total of 100 patients (50 males and 50 females) were examined by clinicians and confirmed with CHD were collected from Al-Sadder city teaching hospital in Najaf Province, they were compared with 100 sex and age matched control group.

General characteristics of CHD patients and control groups were represented no statistical differences between two groups in respect to marital status, residence, educational level and parity (Table 1).

Blood pressure and biochemical data of lipid profile in male and female groups were estimated, CHD patients were recorded higher blood pressure (systolic and diastolic) than normal group ($p<0.05$), the highest levels of TC were observed in the CHD patients, they were significantly higher than those of the normal subjects ($P<0.01$) for CHD.

The levels of Tg and VLDL-triglycerides in CHD of both sexes were significantly higher ($P<0.01$and $P<0.04$ respectively) than those of the normal subjects of male and female groups. HDL-cholesterol was significantly decreased in both sexes of patients when compared with normal individuals. Whereas LDL-cholesterol significantly elevated CHD patients compared with normal individuals ($p<0.03$), there were no statistical differences between two groups regarding to BMI as shown in (Table 2).

Table (1): Demographic characteristics of CHD patients and control groups

Characteristics	CHD no.= 100	%	Control no.= 100	%	P- value
Marital status					
married	66	66 %	70	70 %	
unmarried	12	1 2%	10	10 %	
widow/divorced	22	22 %	20	20 %	0.3
Residence					
Urban	55	55 %	57	57 %	
Rural	45	45 %	43	43 %	0.52
Educational level					
Low	20	20 %	22	22 %	
Middle	58	58 %	55	55 %	
High	22	22%	23	23 %	0.016
Parity					
Nullparous	16	16 %	14	14 %	
1	30	30 %	27	27 %	
2 – 3	11	11 %	20	20 %	
≥ 4	43	43 %	39	39 %	0.39

P<0.05 statistically significant

Table (2): Comparison of Blood pressure, BMI and Serum lipid profile between CHD patients and healthy individuals.

	* Males		* Females		P-value
Parameters	**CHD N=50**	**Control N=50**	**CHD N=50**	**Control N=50**	
B.P (mmHg)/ Systolic Diastolic	100±3.0 70±6	160±4,0 95±5.0	95±2.0 70±4.0	150±3.0 90±5	0.04 0.03
BMI	24.82±1.87	26.30 ±1.89	25.76±1.17	27.21±1.83	0.8
Cholesterol	180±5.1	270±9.51	178±5.4	279±44.41	0.01
HDL- Cholesterol	47.00±3.89	219±37.90	45.16±3.50	50.76±3.87	0.01
LDL- Cholesterol	129±13.70	205 ±6.54	130±12.7	210±38.60	0.03
VLDL- Triglyceride	14.4±5.80	51.23±7.60	13.8±6.21	51.69±10.2	0.04

B. P: Blood pressure; BMI: Body mass index; p<0.05 statistically significant.

Discussion

In developed countries like the United States, although there has been a very significant decrease in the incidence of vascular diseases like cerebrovascular disease, and peripheral vascular diseases, yet CHD remains to be the major cause of death. The major risk factors are elevated LDL-C, reduced HDL-C, smoking, hypertension, insulin resistance with or without overt diabetes mellitus, age, and family history of premature CHD. Modifiable risk factors account for 85% of the elevated CHD risk, of which the most important is plasma cholesterol. TC levels of <160 mg/dl is able to decrease CHD risk, even if other risk factors arepresent.[9]

The development of atherosclerotic vascular disease can be linked hypertension combined with hyperlipidemia; Cholesterol level is more commonly accepted than triglyceride level as an independent indicator of CHD [9-12].

Iraqi males have HDL-cholesterol levels of 52 mg/dl, a value which is agree with the values of 46-58 mg/dl in American males [13] ; 52-55 mg/dl in British, Italian and Swedish males [14]; 46-50 mg/dl in Dutch males [15]; 43-50 mg/dl in Ghainian males [16]; 48 mg/dl in Libyan males [12]. However, HDL-cholesterol levels for male groups from Swedish by Wändell and from Pakistan by Zaid1 and Hasnain were reported to be in the range of 27-42 mg/dl [17,18] which are in line with the findings of conner et.al [19]. For Tarahomara Indians (27 mg/dl) and those of Robinson et.al [20]. For Maasai men (41 mg/dl). The levels of Tg for Iraqi males (135 mg/dl) are slightly lower than the levels of 148 mg/dl reported for Libyan males [21].

In the present study, the levels of TC, Tg and LDL-cholesterol were slightly lower in the normal females than in the normal males, while HDL-cholesterol levels were slightly higher in the female subjects. These differences were not significant in males and females, but the levels of TC, Tg and LDL-cholesterol in CHD patients were significantly higher. In contrast, HDL-cholesterol levels were found to be significantly lower in CHD male and female patients. The result that HDL-cholesterol levels of male and female subjects are negatively related to the risk of CHD is in full agreement with the reported data in a number of populations [22-24]. With increase in age, a slight increase was seen in the atherogenic index in the normal males and females. In CHD patients, the atherogenic index was significantly higher than that of normal subjects in the male and female groups among all age levels. In the meantime, the atherogenic index was increased more in the male patients rather than female patients with increase age levels. This finding is considered to be in full agreement with other reports [12, 14].

Conclusion

Hyperlipidemia is very prevalent in hypertension patients associated with CHD when compared with the normal individuals, in the sametime HDL-cholesterol was negatively correlated with CHD in Iraqi population.

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Comprehensive Evaluation of Clinical Outcomes, Risk Factors, and Evidence-Based Management Strategies for Deep Sternal Wound Infections Following Coronary Artery Bypass Grafting (CABG) Surgery.

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Abstract:

Background: Deep sternal wound infections (DSWIs) are rare but life-threatening complications following coronary artery bypass grafting (CABG), especially among patients with comorbidities such as diabetes mellitus. These infections significantly affect morbidity, prolong hospitalization, and require intensive, multidisciplinary management. **Case Presentation:** A 59-year-old female with a known history of poorly controlled type 2 diabetes mellitus was admitted to CMC Hospital in Erbil, Iraq, following elective CABG surgery. Postoperatively, the patient developed a non-healing thoracic wound, with incomplete sternal closure and clinical signs of deep infection, including purulent discharge, erythema, and systemic fever. Microbiological analysis of wound swabs identified a polymicrobial infection involving Staphylococcus aureus and Pseudomonas aeruginosa. The patient was managed with surgical debridement, targeted antibiotic therapy based on culture sensitivity, and advanced wound care using negative-pressure wound therapy (NPWT). **Discussion:** This case illustrates the increased susceptibility of diabetic patients to postoperative wound infections due to impaired immunity and delayed healing. It emphasizes the importance of early detection, thorough microbiological assessment, and an individualized, evidence-based treatment plan. Preoperative glycemic control, stringent intraoperative asepsis, and prompt postoperative wound monitoring are essential to minimize the risk of DSWIs. **Conclusion:** Deep sternal wound infections following CABG present significant clinical challenges, particularly in diabetic patients. This case from CMC Hospital in Erbil, Iraq, underscores the need for timely intervention, multidisciplinary care, and the integration of advanced wound management strategies to improve clinical outcomes.

Keywords: -Deep sternal wound infection, CABG, clinical outcomes, risk factors, infection management, evidence-based strategies, surgical site infection, wound healing, antibiotic therapy, surgical debridement.

Introduction

Coronary artery bypass grafting (CABG) is a cornerstone intervention in the surgical management of patients with multivessel coronary artery disease and has demonstrated significant improvements in long-term survival and symptom relief [1,2]. Despite advances in operative techniques and perioperative care, postoperative complications continue to occur, among which deep sternal wound infections (DSWIs), also known as post-sternotomy mediastinitis, remain a rare but highly morbid entity. The incidence of DSWI varies between 0.5% and 5%, but the associated mortality may be as high as 10%–50%, depending on the presence of comorbidities and delay in diagnosis [3–5]. DSWIs typically manifest within 14 to 30 days postoperatively and involve infection of the sternum and mediastinal tissues. They are frequently caused by microbial contamination during surgery or from hematogenous spread in the early postoperative period [6,7]. The pathogens most commonly implicated include Staphylococcus aureus, Staphylococcus epidermidis, and Gram-negative bacilli such as Pseudomonas aeruginosa and Klebsiella pneumoniae [8–10]. Polymicrobial infections are also increasingly reported and tend to correlate with worse outcomes and prolonged treatment courses [11].

A wide range of risk factors contribute to the development of DSWIs. These include patient-related factors such as advanced age, obesity, smoking, and, notably, diabetes mellitus—particularly when poorly controlled [12,13]. Diabetes impairs host immune responses by affecting neutrophil chemotaxis, phagocytosis, and oxidative burst activity, and it also interferes with tissue perfusion and collagen synthesis, which are essential for wound healing [14–16]. Procedure-related factors include the use of bilateral internal mammary arteries, prolonged operative time, reoperation, emergency surgery, and excessive use of electrocautery [17,18]. The diagnosis of DSWI is primarily clinical, based on signs such as sternal instability, wound dehiscence, purulent discharge, fever, and systemic signs of infection. Imaging, particularly computed tomography (CT), can provide additional insights into the extent of infection and mediastinal involvement [19]. Culture and sensitivity testing of wound samples remain essential for identifying the causative organisms and guiding antibiotic therapy [20].

The management of DSWIs is complex and necessitates a multidisciplinary approach. Surgical debridement of infected and necrotic tissue is often required, and in some cases, partial or total sternectomy may be necessary. Empirical broad-spectrum antibiotic therapy should be promptly initiated and later tailored based on culture results [21,22]. Negative-pressure wound therapy (NPWT), also known as vacuum-assisted closure (VAC), has emerged as a valuable adjunct to promote wound healing by improving perfusion, reducing bacterial load, and facilitating granulation tissue formation [23–25]. This case report presents a 59-year-old diabetic female admitted to CMC Hospital in Erbil, Iraq, with a post-CABG DSWI involving polymicrobial infection and incomplete thoracic closure. It underscores the critical need for early recognition, personalized treatment, and implementation of advanced wound management strategies in high-risk patient populations.

Case Presentation

A 59-year-old female patient with a known history of type 2 diabetes mellitus for over 12 years, complicated by poor glycemic control (HbA1c: 9.1%), was admitted to the emergency department of CMC Hospital in Erbil, Iraq. The patient had undergone elective coronary artery bypass grafting (CABG) three weeks prior at another facility due to triple-vessel coronary artery disease. The surgical procedure included median sternotomy and grafting using the left internal mammary artery and saphenous vein. Postoperatively, the patient experienced delayed sternal wound healing and progressive chest discomfort. He presented with erythema, purulent discharge, localized warmth, and sternal instability. On physical examination, the thoracic surgical site demonstrated gaping, necrotic tissue, and foul-smelling exudate. The patient was febrile (38.6°C), tachycardic (110 bpm), and mildly hypotensive.

Initial laboratory investigations revealed leukocytosis (WBC: 17.8×10^9/L), elevated CRP (195 mg/L), and procalcitonin levels suggestive of systemic infection. Blood glucose on admission was 298 mg/dL. A computed tomography (CT) scan of the chest confirmed deep sternal wound infection (DSWI) with retrosternal fluid collection, sternal osteomyelitis, and subcutaneous emphysema. Cultures from wound swabs and debrided tissue identified a polymicrobial infection comprising Staphylococcus aureus (methicillin-sensitive), Pseudomonas aeruginosa, and Klebsiella pneumoniae, consistent with previous findings that DSWIs often involve both Gram-positive and Gram-negative organisms [26,27]. Given the diagnosis of DSWI, the patient underwent urgent surgical debridement under general anesthesia. Necrotic tissue and infected bone segments were excised, and cultures were repeated intraoperatively. Empirical broad-spectrum antibiotics (vancomycin and meropenem) were initiated and later adjusted based on culture sensitivity profiles. Blood cultures were negative. The sternal wound was left open, and vacuum-assisted closure (VAC) therapy was applied, a technique shown to promote granulation and reduce microbial colonization in complex wounds [28,29].

Glycemic control was optimized using intravenous insulin infusion, and nutritional support was initiated to aid tissue healing. Over the following three weeks, the patient underwent two additional debridement procedures. The wound bed showed progressive improvement with healthy granulation tissue. Subsequent closure of the sternal wound was achieved using pectoralis major muscle flap advancement. The patient was discharged on postoperative day 35 with oral antibiotics, strict diabetes management, and scheduled follow-up in the cardiothoracic and infectious diseases clinics. At 8-week follow-up, the patient remained afebrile, with no signs of recurrent infection, and had regained full functional capacity.

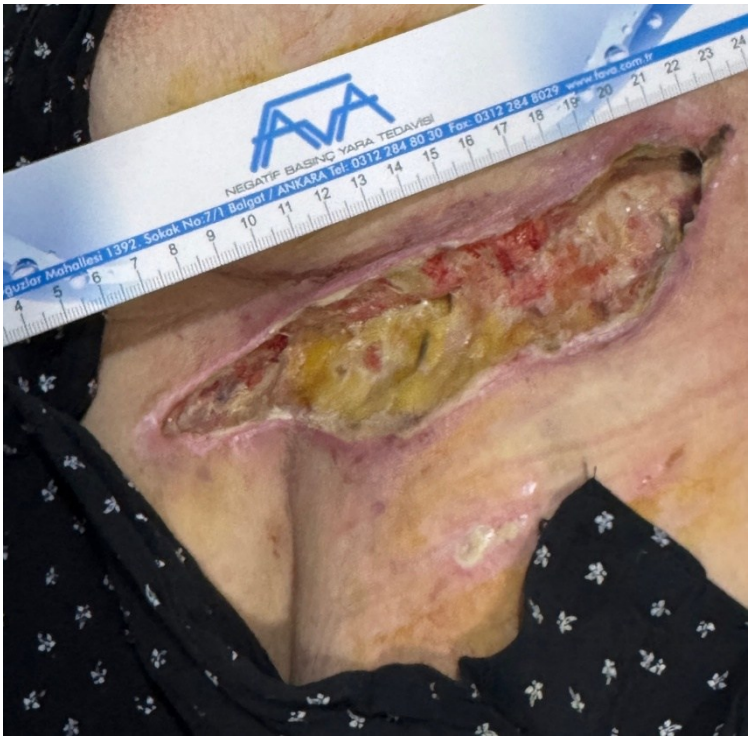

Figure 1 Illustration of a post-surgical wound on the 14th day after the procedure, exhibiting an open wound with signs of purulent discharge and bacterial infection. The wound site demonstrates ongoing inflammation and infection, characterized by the presence of pus and infected bacterial growth, indicating the need for further medical intervention and management.

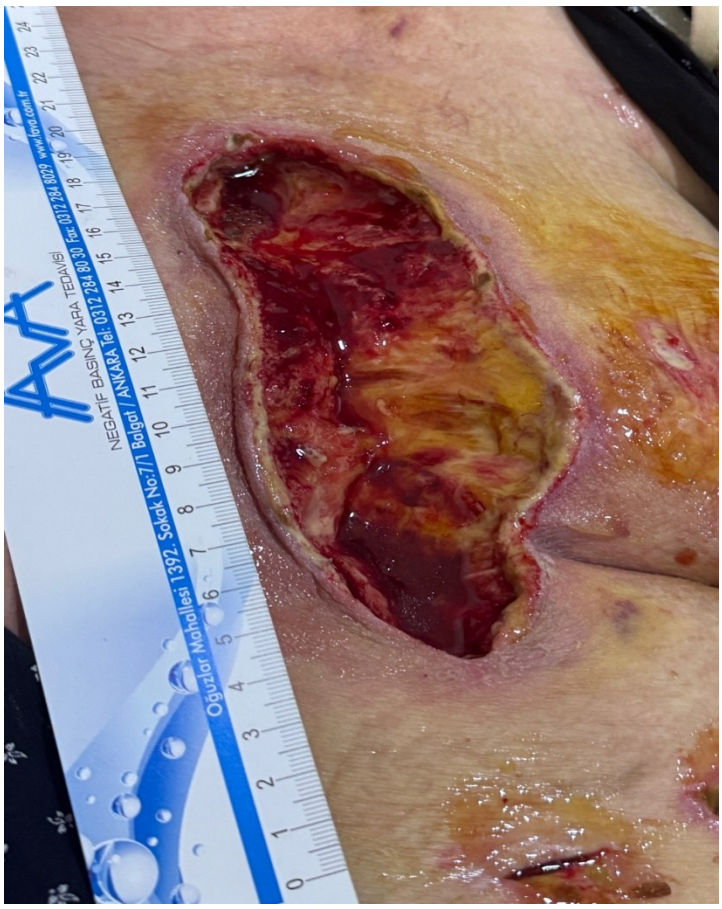

Figure 2 Illustration of the wound three days after the application of negative pressure wound therapy (NPWT) and a locally sourced natural antibiotic. The image shows a noticeable reduction in purulent exudate, indicating improved infection control, along with the formation of new blood vessels at the wound site. This enhanced vascularization suggests an effective healing response, promoting tissue regeneration and further improving the overall condition of the wound.

Table 1Comparison of Management Strategies for Deep Sternal Wound Infections (DSWIs)

Strategy	Description	Clinical Benefit	Limitations	References
Empirical Antibiotic Therapy	Broad-spectrum antibiotics started before culture results	Rapid initial control of infection	May not cover resistant or atypical organisms	[49,50]
Culture-Guided Antibiotic Adjustment	Tailoring antibiotics based on culture and sensitivity	Improved efficacy and reduced resistance	Requires time; culture-negative infections pose a challenge	[51]
Surgical Debridement	Removal of infected and necrotic tissues	Reduces microbial load; promotes healing	Invasive; may require multiple procedures	[52]
Negative-Pressure Wound Therapy (NPWT)	Use of vacuum-assisted closure devices to promote healing	Enhances granulation, reduces exudate and bacterial count	Requires specialized equipment and training	[53,54]
Muscle Flap Reconstruction	Use of vascularized muscle (e.g., pectoralis major) to fill sternal defect	Restores structural integrity and blood supply	Complex procedure; risk of flap failure	[55]
Glycemic Control Optimization	Intensive insulin therapy perioperatively	Reduces infection risk and improves wound healing in diabetic patients	Risk of hypoglycemia; requires monitoring	[56]
Multidisciplinary Management	Involvement of surgery, infectious disease, endocrinology, and wound care teams	Holistic care approach; improves patient outcomes	Coordination challenges; resource-intensive	[57]

Risk Factors, and Evidence-Based Management Strategies for Deep Sternal Wound Infections Following Coronary Artery Bypass Grafting (CABG) Surgery

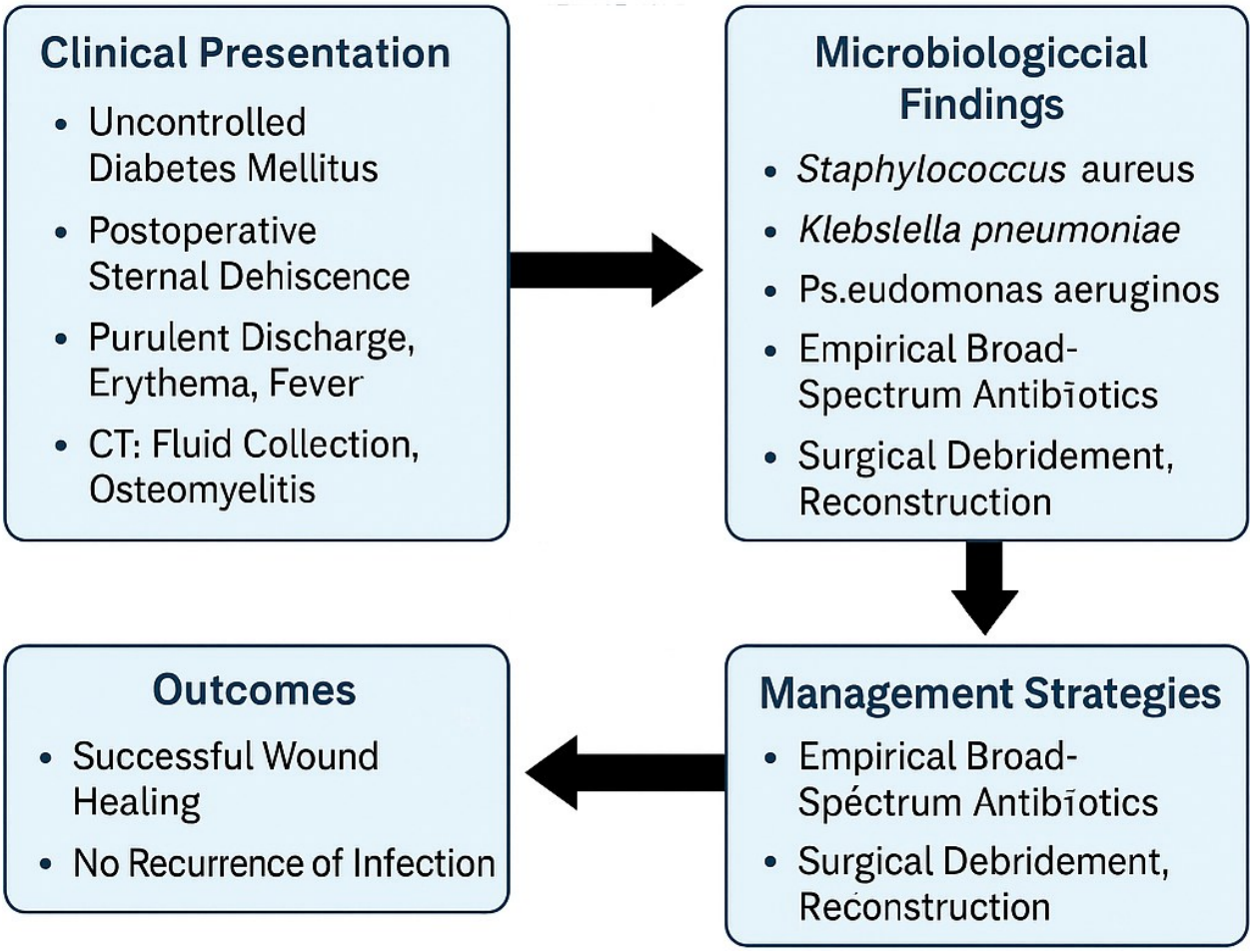

Figure 3: Infographic summarizing the clinical presentation, microbiological findings, evidence-based management strategies, and outcomes of deep sternal wound infections following coronary artery bypass grafting (CABG) surgery in a diabetic patient.

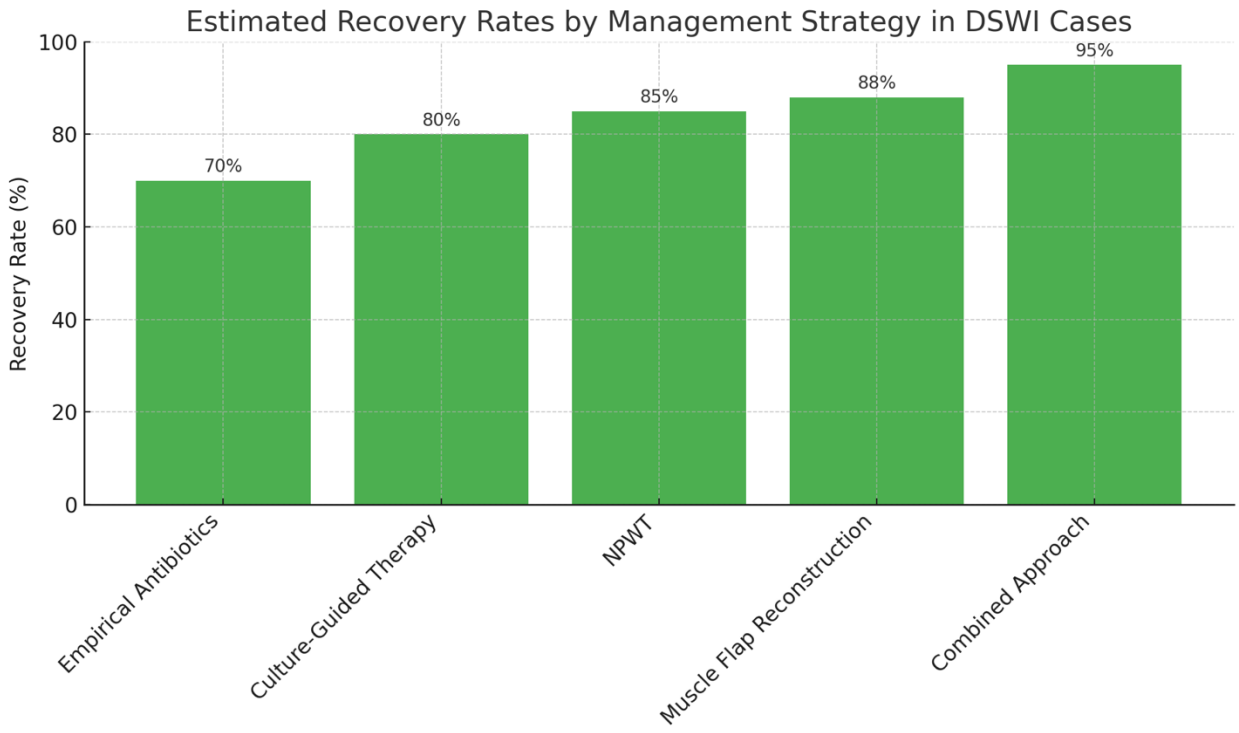

Chart 1: Here is the bar chart comparing estimated recovery rates of different treatment strategies for deep sternal wound infections (DSWIs).

Discussion

Deep sternal wound infections (DSWIs) represent one of the most severe complications following coronary artery bypass grafting (CABG), significantly increasing morbidity, length of hospital stay, cost of care, and mortality rates [30]. In this case, the presence of uncontrolled diabetes mellitus was a major predisposing factor, aligning with the literature that identifies diabetes as a key risk factor for impaired wound healing and infection susceptibility after cardiac surgery [31]. The polymicrobial nature of the infection, including Staphylococcus aureus, Klebsiella pneumoniae, and Pseudomonas aeruginosa, reflects the evolving microbiological landscape of DSWIs. These infections are no longer solely attributed to Gram-positive cocci, as Gram-negative organisms and mixed flora are becoming increasingly recognized [32]. This trend necessitates broader-spectrum empirical antimicrobial coverage upon suspicion of DSWI, pending culture results [33].

The importance of early diagnosis cannot be overstated. Clinical signs such as sternal instability, purulent discharge, systemic symptoms, and radiological findings like fluid collection and bony involvement on CT imaging are hallmark features of DSWIs [34]. In this patient, the

presence of osteomyelitis, as confirmed by imaging, further supported the need for aggressive surgical intervention. Surgical debridement remains the cornerstone of DSWI management. Complete removal of devitalized tissue, as performed in this case, reduces the microbial burden and facilitates granulation [35]. Negative-pressure wound therapy (NPWT), used here as a bridge to secondary closure, is increasingly supported by evidence for enhancing local perfusion, promoting wound contraction, and reducing bacterial colonization [36]. Several randomized studies have demonstrated superior outcomes with NPWT compared to conventional dressings in managing post-sternotomy infections [37].

Reconstruction using muscle flaps, such as the pectoralis major flap employed in this case, is another essential component of care for extensive sternal defects. Muscle flaps provide vascularized tissue, fill dead space, and reduce the risk of reinfection [38]. This approach aligns with current recommendations for patients with sternal instability or significant bone loss [39]. In addition to surgical management, optimizing systemic conditions, especially glycemic control, is critical. Hyperglycemia impairs leukocyte function and collagen synthesis, promoting wound infection and dehiscence [40]. Studies have shown that strict perioperative glucose regulation significantly reduces postoperative infections in diabetic patients undergoing cardiac surgery [41].

Antibiotic therapy, tailored to culture results, should continue for several weeks based on clinical response and infection severity. In polymicrobial DSWIs, combination therapy is often required to address both aerobic and anaerobic organisms [42]. Close follow-up and patient education are vital to ensure adherence to wound care, diabetes management, and rehabilitation. This case underscores the importance of a multidisciplinary approach, combining surgical, infectious disease, endocrinologic, and nursing expertise, in managing complex post-CABG infections. The favorable outcome achieved highlights the effectiveness of timely surgical intervention, NPWT, targeted antibiotic therapy, and systemic optimization. Deep sternal wound infections (DSWIs) following coronary artery bypass grafting (CABG) remain a serious postoperative complication, especially in patients with uncontrolled diabetes mellitus. This case highlights the critical role of early diagnosis, aggressive surgical debridement, appropriate use of negative-pressure wound therapy (NPWT), muscle flap reconstruction, and strict glycemic control in achieving successful outcomes.

The presence of multiple bacterial pathogens underscores the importance of initiating empirical broad-spectrum antibiotics promptly, followed by tailoring treatment based on culture sensitivity. Delayed or inadequate management of DSWIs can lead to systemic sepsis, prolonged hospitalization, and increased mortality [43]. Given the high-risk nature of patients undergoing cardiac surgery, especially those with diabetes or immunosuppression, preoperative optimization—including blood glucose management, nutritional assessment, and decolonization strategies—is recommended to reduce the incidence of wound complications [44,45]. Intraoperative strategies such as meticulous aseptic technique, minimized operative time, and the use of antibiotic prophylaxis tailored to local resistance patterns also play a preventive role [46].

Postoperatively, early identification of wound abnormalities and prompt multidisciplinary intervention are essential. Incorporating NPWT has shown superior results in complex wounds and should be considered standard in selected patients with DSWI [47]. Furthermore, structured follow-up involving wound care specialists, cardiothoracic surgeons, endocrinologists, and infectious disease consultants ensures comprehensive care and reduces the likelihood of recurrence [48].

Conclusion:

In conclusion, management of DSWI after CABG requires a multifaceted and individualized approach. Early recognition, radical surgical intervention, wound closure strategies like muscle flaps, and control of systemic comorbidities collectively contribute to positive patient outcomes. Continued research and adherence to evidence-based guidelines will be key to improving survival and reducing complication rates in this high-risk group.

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Scientific study: (recommendations and solutions to Prevalence risk factors of Hepatitis A in children in Najaf Governorate)

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Abstract

The name problem: suggestions and solutions to a social problem (the prevalence of the risk factors of Hepatitis A in children in Najaf Governorate).**Type of Study:** Scientific Study for Treatment of Scientific-Social Problem (spread of Hepatitis A in children in Najaf Governorate **Aim of Scientific Study**: Finding solutions of spread of Hepatitis A in children in Najaf Governorate **Methods:** A total of 369 serum samples from clinically infection cases of AVH were received from September 2023 to August 2024. Of the specimens, 369 (22.33%) were positive for IgM anti-HAV antibodies. **Results:** Distribution of HAV infection in children according to gender demonstrating that percentage in males 55.28% more than in females 44.72 % and The rate showed high frequency among age group (6 -10 years) 167(45,2%), while**.** Regarding the seasonal variation the rate was more common during - March and August compared to January and July 2,9%, 5,6% respectively.

KEYWORDS: - Hepatitis A, Social Problem, Scientific Study, Case Study, Social Problem.

Introduction

Hepatitis A virus (HAV) is the most common cause of viral hepatitis. HAV is a small, nonenveloped, single-stranded RNA virus from the Picornaviridae family. The risk of infection is universally distributed. The incidence of infection has a strong relation with sanitary and environmental conditions and the level of socioeconomic development. Hepatitis A has a high rate of endemicity in underdeveloped countries with poor sanitation. Hepatitis A virus (HAV) infection is a common health issue throughout the world, especially in areas with high prevalence rate of HAV. HAV infection is generally acquired through the facal-oral route by either person-to-person contact or ingestion of contaminated food or water.1, 2 Geographical areas can be classified as high, intermediate, low and very low prevalence of HAV infection.3 Although HAV infection has a mild course in under 6 years old children and it is mostly asymptomatic or shows nonspecific symptoms in these ages,4, 5 it may cause significant morbidity and mortality among both adolescents and adults.6 Fulminant hepatitis may also develop among individuals with underlying liver disease.6 Significant epidemiologic changes of HAV infection have been observed during the past few decades.

The prevalence of hepatitis A infection is influenced by hygiene, sanitation conditions and age groups of communities.7 In several developing countries, the decline in the seroprevalence rates of anti-HAV is attributed mainly to the improvement of socioeconomic conditions and enhanced access to sanitary water sources.2, 8, 9 In highly endemic countries, exposure to HAV is almost universal before the age of 10 years, and large-scale immunization is not needed. In intermediate endemicity areas, where transmission occurs primarily from person to person in the general community (often with periodic outbreaks), control of hepatitis A may be achieved through widespread vaccination programs.10, 11

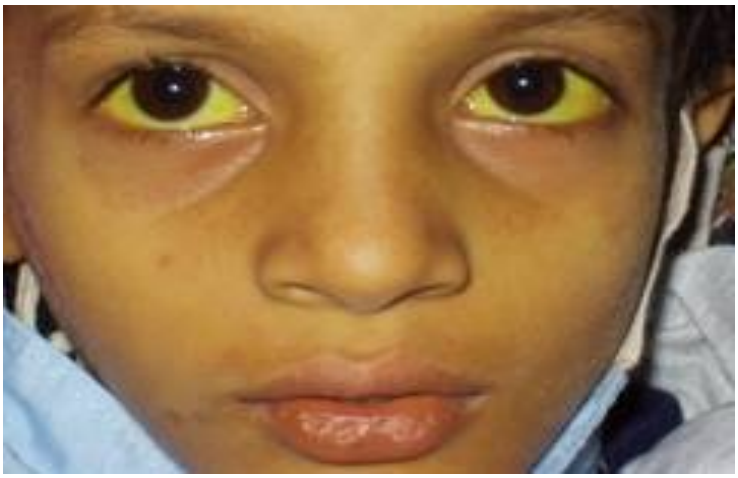
Epidemiology

First discovered in 1973 by Feinstone, a spherical 27 nanometer particle was seen on immune electron microscopy in the fecal sample of hepatitis A pa tients [12]. A member of the picornavirus family, the hepatitis A virus (HAV) is an RNA virus responsible for 1.4 million cases per year globally[13], with an estimated 7134 deaths in 2016; almost half of these cases were reported in Asia[14]. In the United States, the annual incidence rate was reported to be 2 cases per 100000 people, in 2006. Recent outbreaks of the disease have shown a 294% increase in infections between 2016-2018 compared to 2013-2015[15].

Pathogenesis

The transmission of HAV occurs via fecal-oral route, which includes consumption of contaminated food or water and person to person contact. Polymerase chain reaction testing for blood donors is performed as transmission through blood transfusion is noted on rare occasions [16]. The dissemination of the HAV into the liver occurs via the portal vein after the virus traverses the mucosa of the small intestinal wall. The virus particles subsequently replicate and are secreted into the biliary canaliculi, reaching back to the small intestine through the bile ducts and being re-excreted in the feces.

Until the body responds with appropriate immune reaction in antibodies, the HAV enterohepatic cycle continues. Human leukocyte antigen-restricted, HAV-specific CD8+ T lymphocytes and natural killer cells have been implicated in the damage and destruction of infected hepatocytes [17].



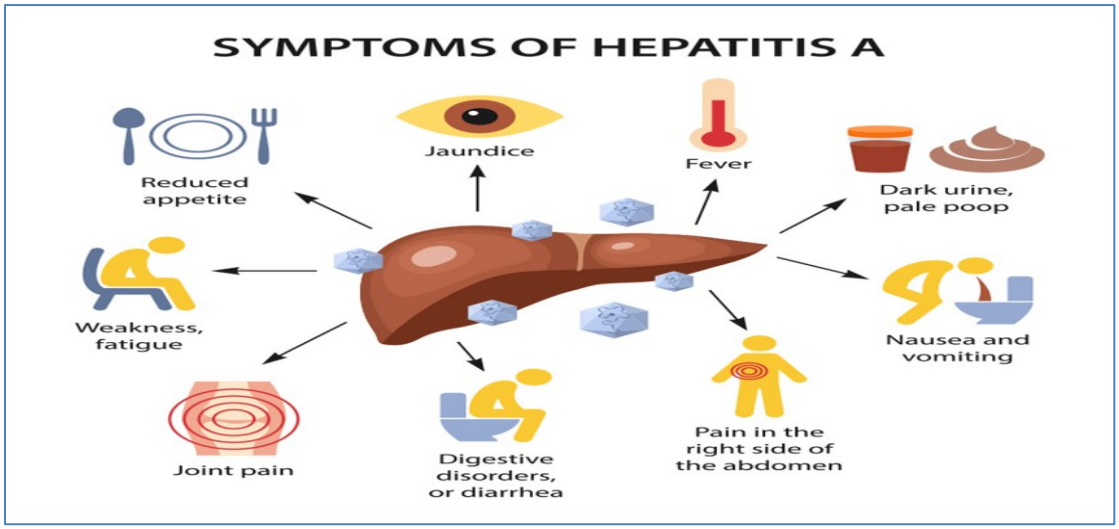
Figer (1) : Jaundice have been described as the most common presenting symptoms

Clinical presentation

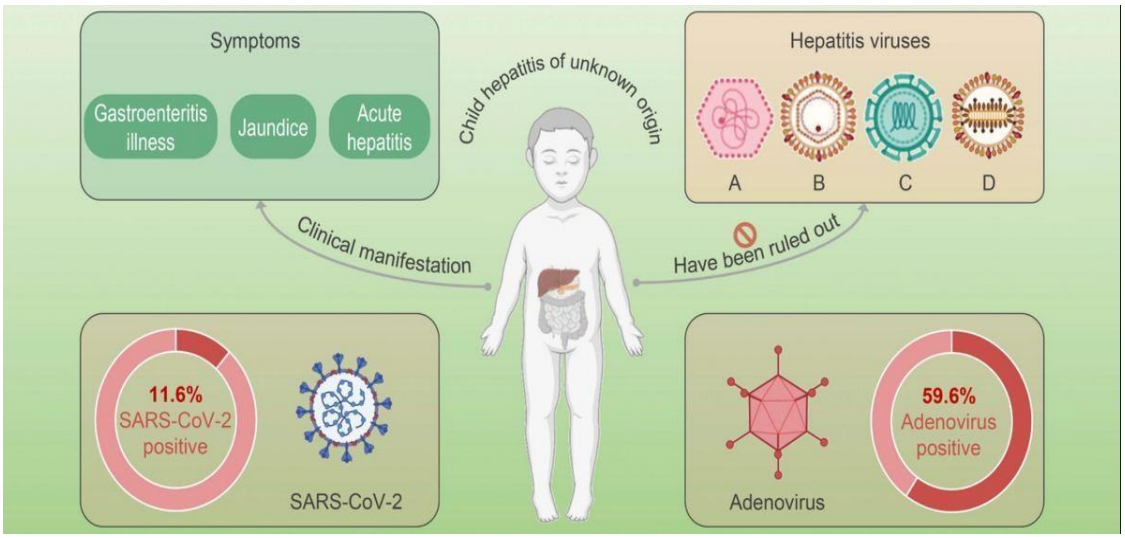
The usual HAV incubation period is about 2-4 wk [17]. Fever, malaise, jaundice have been described as the most common presenting symptoms for HAV infection [18]. Other common symptoms include weakness, fatigue, nausea, vomiting, abdominal pain, arthralgias, myalgias, diarrhea and anorexia [17]. Patients rarely enter a prolonged cholestatic phase through recovery, while relapsing infections have been described as well [19].

About 10%-15% of patients present with a relapsing course within a 6-mo period of the initial infection [20]. The symptoms during the relapse are usually less severe than the initial infection. Notably, on extremely rare occasions a type 1 autoimmune hepatitis has been observed in genetically predisposed patients [21]. The spectrum of infections can range from asymptomatic patients without jaundice, symptomatic patients with jaundice, cholestasis with prolonged jaundice, to relapsing infections or acute liver failure [19].

Serum aminotransferases above 1000 U/dL are usually noted, with total bilirubin typically ≤ 10 mg/dL, and alkaline phosphatase below 400 U/L. Usually the serum alanine aminotransferase (ALT) is higher than the aspartate aminotransferase (AST)[22,23]. In general, older patients are more likely to have severe hepatocellular derangements, hospital admissions and higher mortality [24]. These findings can be attributed to an impaired regeneration capacity of the liver and a relatively weaker immune system in the older population [25]. In addition to old age, higher mortality has been reported in males [26]. Old age, underlying liver pathology and chronic viral hepatitis are reported risk factors for acute liver failure. In patients who develop acute liver failure, higher mortality has been associated with creatinine > 2 mg/dL (strongest predictor) total bilirubin > 9.6 mg/dL and albumin < 2.5 g/L [18].



Figer (2): symptoms of hepatitis A

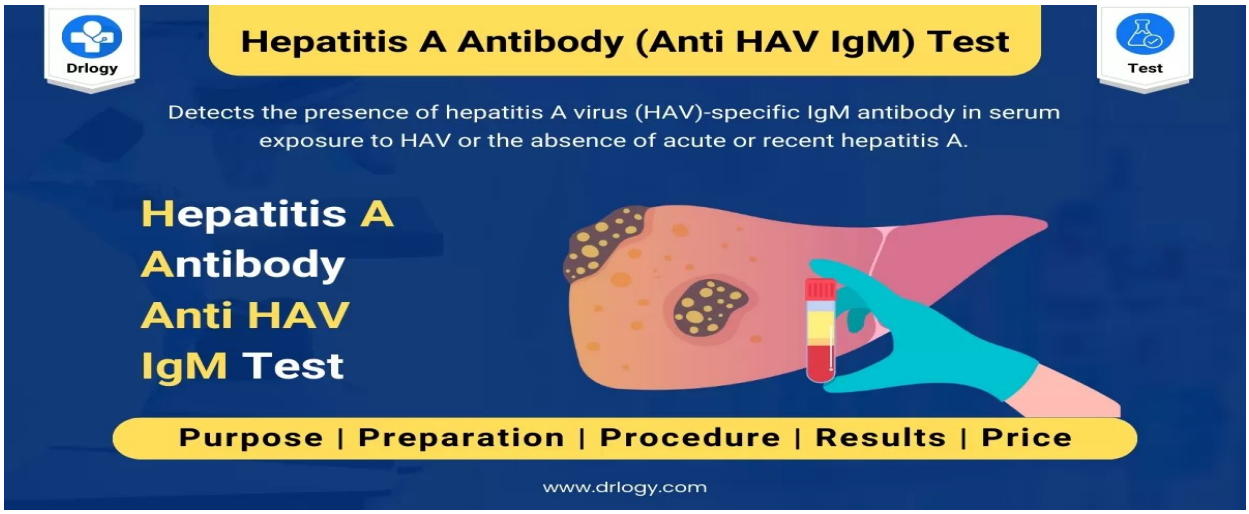


Figer (3): Child hepatitis anknown origin

Diagnosis

Specific antibodies against HAV (anti-HAV) in the serum can be detected. The diagnosis is confirmed by the presence of immunoglobulin (Ig) M anti-HAV. The antibodies can be detected at the time of onset of symptoms. Serum IgM levels peak during the acute infection and remain positive for up to 4 mo on an average from the onset of symptoms [27]. Immunity is usually tested with HAV total antibody to determine HAV natural exposure or secondary to vaccination [28]. The presence of IgM antibodies without any clinical symptoms is indicative of HAV infection in the past with persistent antibodies, asymptomatic infection or false positive test [29].

Liver biopsy or imaging studies are not required to make a diagnosis. If performed, a liver biopsy may show marked portal inflammation with typically a lesser degree of necrosis, Kupffer cell proliferation, acidophil bodies, or ballooning when compared to non-HAV viral hepatitis [30].



Figer (4): Specific antibodies against HAV

Study Design: A cross-sectional study design will be used to assess the prevalence of Hepatitis A and identify associated risk factors among children in Najaf Governorate.
Study Population: Target Group: Children aged 1-15 years residing in Najaf Governorate.
Sample Size Calculation: Use prevalence estimates from previous studies or pilot data to calculate the required sample size with a 95% confidence level and a margin of error of 5%.
Sampling Method: Stratified random sampling to ensure representation from different districts and varying socioeconomic backgrounds within Najaf Governorate.
Inclusion and Exclusion Criteria:
Inclusion Criteria: Children aged 1-15 years, Residents of Najaf Governorate for at least one year, Parental/guardian consent for participation.
Exclusion Criteria: Children with chronic liver diseases unrelated to Hepatitis A, Recent travelers (within the past month) to regions with known Hepatitis A outbreaks.
Data Collection: Data Collection Period: Over a 1year period to account for seasonal variations.
Questionnaires: Structured interviews with parents/guardians to gather data on demographics, socioeconomic status, access to clean water, sanitation facilities, hygiene practices, dietary habits, and vaccination history.
Clinical and Laboratory Assessment: Collection of blood samples from children to test for Hepatitis A antibodies (IgM for acute infection and IgG for past exposure).
Conduct physical examinations to assess overall health status.
Variables:
Dependent Variable: Hepatitis A infection status (positive or negative).
Independent Variables: Demographic Variables: Age, gender, educational level of parents, family size.

Socioeconomic Variables: Income level, employment status of parents, housing conditions.
Environmental Variables: Access to clean water, type of sanitation facilities, living area (urban vs. rural).
Behavioral Variables: Handwashing practices, food hygiene, and outdoor activities.
Health-related Variables: Vaccination status, history of previous infections, healthcare access.
Data Analysis:
Descriptive Statistics: To summarize demographic and risk factor data (e.g., mean, median, frequency).
Prevalence Estimation: Calculate the prevalence of Hepatitis A in the study population.
Bivariate Analysis: Assess the relationship between each risk factor and Hepatitis A infection using chi-square tests or t-tests as appropriate.
Multivariate Analysis: Use logistic regression to identify independent risk factors associated with Hepatitis A after adjusting for potential confounders.
thical Considerations: The College of Medicine's ethical council of the University of Kufa gave its approval to this investigation. Additionally, prior to the beginning of the research project, permission was obtained from AL-Najaf health directorate, and the public health laboratory, and verbal consent was obtained from all participants in the study. All patients had been Ab out the study and they permitted the researcher to give them a questionnaire and to have the blood sample.

Results

Distribution of HAV infection in children according to gender demonstrating that percentage in males 55.28% more than in females 44.72 % as shown in (Table 1).

Table 1: Gender distribution among study population

Sex	No. Positive cases	Percentages
Males	**204**	**55,28%**
Females	**165**	**44,72%**
Total	**369**	**100 %**

A total of 369 serum samples from clinically infection cases of AVH were received from September 2023 to August 2024. Of the specimens, 369 (22.33%) were positive for IgM anti-HAV antibodies. Regarding the seasonal variation the rate was more common during - March and August compared to January and July 2,9%, 5,6% respectively as shown in (Table 2).

Table 2: Distribution of HAV among study population during 2023.

Months	Infectef NO.	Percentage %
September	33	8,9%
October	29	7,8%
November	30	8,13%
December	39	10,5%
January	11	2,9%
February	19	5,14%
March	63	17%
April	17	4,6%
May	47	12,7
June	23	6,2
July	21	5,6
August	37	10%
Total	369	100%

According to age distribution, their age of positive HAV cases ranged between 1-15 years. The rate showed high frequency among age group (6 -10 years) 167(45,2%), while the remaining in age group (1-5) and (11-15) years30,4%, 24,4 % respectively as shown in (Table 3).

Age (years)	No. Positive cases	Percentages
1-5 years	112	30,4
6-10 years	167	45,2
11-15 years	90	24,4
Total	369	100%

Discussion

According to world Health Organization, about more four million people infected with HAV are in the milled east region. The aim of the present study is to estimate the sero-prevalence of HAV in AL-Najaf Governorate among specific age group. Hepatitis A is an acute, typically self-limiting liver disease and one of the most common infectious diseases in the world" 7. HAV was highly prevalent during the 10 years when this research was performed. Poor personal hygiene and health education might be the main reason for the increasing number of HAV cases. Most of migrants' families lived in camps far away from the city center where poor sanitations and limited access to safe drinking water. This may contribute to the incidence of HAV (3, 7.) Few community-based studies have been conducted to estimate the incidence and prevalence of HAV and HEV in Iraq. Our study confirmed that HAV most commonly affected the children, and more than 60% of HAV cases were patients younger than 14 years (24-25)

Role of liver transplant

Acute liver failure occurs in less than 1% of acute HAV infections [6]. From these patients, only 31% require emergent liver transplant for treatment of fulminant disease, while the remaining patients recover spontaneously with symptomatic management [37]. In a study comparing liver transplant outcomes in patients with HAV vs hepatitis B infection, the patients with HAV were found to have lower 1- and 5-year survival rates. Presence of acute pancreatitis and HAV recurrence in this population was identified as risk factors for shorter graft and patient survival. Following transplant, patients should be carefully monitored for HAV recurrence as it is common and is associated with poor outcomes [38].

Risk for Infection: Persons experiencing homelessness**,** Persons living in the same household with an infected person**,** Sex partner(s) of an infected person**,** Persons traveling to countries where hepatitis A is common**,** Men who have sex with men**,** People who use injection drugs**,** Children in day care**,** People who eat raw or under-cooked shellfish.

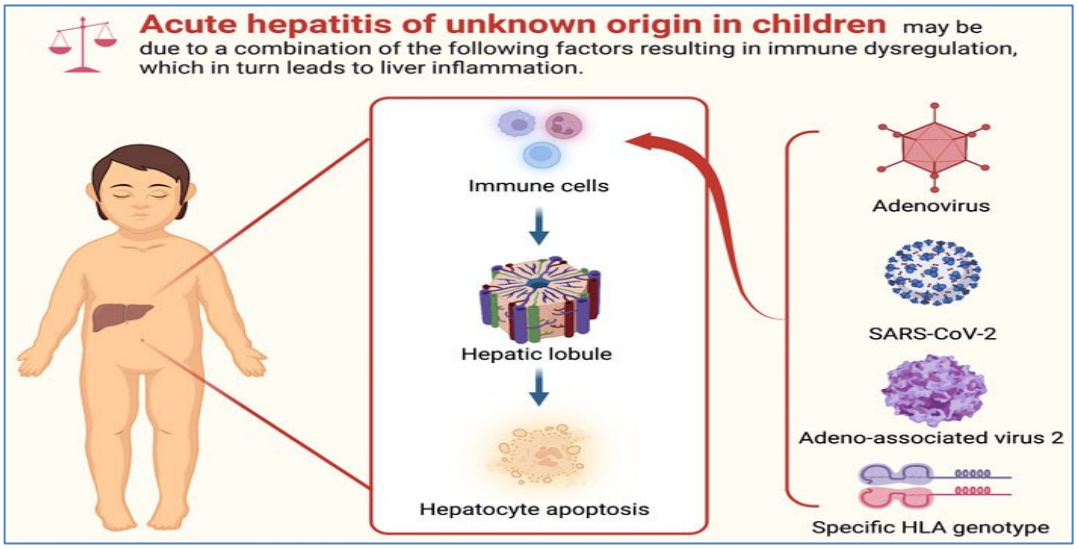
Prevention of Hepatitis A Infection

Vaccination

The hepatitis A vaccine offers excellent protection against HAV. The vaccine is safe and highly effective. Vaccination consists of 2 doses of vaccine (shots) spaced 6-12 months apart. Protection starts 1-2 weeks after the first dose of vaccine, and lasts for 20 years to life after 2 doses.The American Academy of Pediatrics recommends that all children should receive hepatitis A vaccine starting at 1 year of age (2007 AAP Statement). The CDC recommends hepatitis A vaccine for all persons traveling to countries where HAV is common (CDC Yellow Book). For infants that will be traveling internationally, an early dose of Hepatitis A vaccine can be given at age 6-11 months.

Natural Immunity

People who have hepatitis A infection become immune to HAV for the rest of their lives once they recover. They cannot get hepatitis A twice. The blood test for immunity to hepatitis A is called the "Hepatitis A Total Antibody test." People who have had hepatitis A and those who have received hepatitis A vaccine show positive antibodies to hepatitis A on this test for the rest of their life.



Figer (6) Acute hepatitis of anknown origion

Solutions of Infection Problem

1- Good personal hygiene and proper sanitation help prevent the spread of the HAV virus.

2- Always wash your hands with soap and water after using the bathroom, changing a diaper, and before preparing, serving, or eating food.

3- Alcohol-based hand sanitizers do not kill the hepatitis A virus

4- People who have hepatitis A should not be preparing or serving food, or caring for the elderly or for young children, until at least 2 weeks have passed since the first sign of hepatitis A illness.

5- Boiling or cooking food and drinks for at least 1 minute to 185°F (85°C) inactivates HAV.

6- Foods and drinks heated to this temperature and for this length of time do not transmit HAV infection unless they become contaminated after heating.

7- Travelers can lower their risk of hepatitis A (and other food-borne illnesses) in developing countries by drinking only water that has been boiled or chemically purified, by eating only foods that have been properly heated, and by avoiding fruits or vegetables that are not peeled or prepared by the traveler personally.

8- Adequate chlorination of water as recommended in the United States does inactivate HAV.

The following suggestions may contribute forward to recover solutions the infection:

- Only drink bottled water with an unbroken seal.
- Avoid unpackaged drinks or ice.

- Avoid eating raw food such as fruit or salad that has been (or may have been) cleaned or prepared with contaminated water.
- Avoid uncooked foods, particularly vegetables and fruit that you have not peeled, prepared or boiled yourself.
- Avoid raw or undercooked meat and fish.
- Make sure cooked food is hot and eat it right away.
- Avoid shellfish and unpasteurised dairy products.
- Avoid eating food from street vendors.

Conclusion

Although hepatitis A virus infection has a benign, self-limited course without chronicity, recognition of atypical cases which carry mortality risk is important. Hepatitis A virus is still a major cause of infection and disease in the world and heterogeneous pockets of susceptible and exposed individuals may co-exist in rapidly developing societies. Thereafter, small localized or large outbreaks of HAV infection will remain a threat in such areas. The situation demands that conclusive guidelines be produced for HAV vaccination in these communities after characterizing them appropriately. WHO is in the process of revising its position paper on hepatitis A, issued in 2000, with a view to: update and evaluate the data on disease burden, epidemiology, vaccine products and availability and immunization protection; review the use of the vaccine in outbreaks and for contacts of cases; and issue guidance to countries where the prevalence rates are declining from high levels? In determining national policies, the results of appropriate epidemiological and cost-benefit studies need to be carefully considered and the public health impact weighed [18,25].

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A STUDY ON RHEUMATOID ARTHRITIS: DIAGNOSIS AND TREATMENT USING CRP AND ESR MEASUREMENTS"

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Abstract :
Background: Rheumatoid arthritis (RA) is a chronic, progressive autoimmune disease characterized by systemic inflammation, synovial joint damage, and disability if left untreated. This study explores the etiopathogenesis, diagnostic criteria, and treatment strategies for RA, emphasizing the role of clinical and laboratory markers such as erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and rheumatoid factor (RF) in disease assessment. **Aim of study:** The research highlights the multifactorial nature of RA, involving genetic predisposition (e.g., HLA-DRB1 alleles), environmental triggers (e.g., smoking, infections), and epigenetic modifications. Diagnostic adherence to the 2010 ACR/EULAR classification criteria—incorporating joint involvement, serology (RF/anti-CCP), and acute-phase reactants (ESR/CRP)—facilitates early intervention, crucial for preventing irreversible joint damage. **Methediology:** A cohort of 35 RA patients (23 females, 12 males; aged 30–70 years) was analyzed, revealing elevated ESR and CRP levels correlating with disease activity. Females exhibited higher susceptibility, aligning with global epidemiological trends. Treatment paradigms prioritize early, aggressive use of disease-modifying antirheumatic drugs (DMARDs), including conventional (e.g., methotrexate), targeted synthetic (JAK inhibitors), and biologic agents (TNF-α inhibitors), aiming for remission or low disease activity. **Results:** Key findings underscore the importance of: Early diagnosis via clinical-serological integration, Gender-specific considerations in RA management, Inflammatory markers (ESR/CRP) as prognostic tools, Personalized DMARD therapy to mitigate structural damage. **Conclusions:** This study advocates standardized monitoring and tailored therapeutic strategies to improve outcomes in RA patients, addressing both articular and systemic manifestations .
Keywords: Rheumatoid arthritis, ESR, CRP, DMARDs, ACR/EULAR criteria, autoimmunity .

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic, multi-system inflammatory autoimmune disease of indefinite etiology. The disease primarily affects synovial joints, eventually progressing to ongoing inflammation, destruction of both cartilaginous and bony elements of the joint, with resultant pain and disability. The disease additionally displays a spectrum of extra-articular multisystem manifestations (Smolen et al., 2016). The worldwide prevalence of RA remains underestimated. Data gathered from Western regions illustrated prevalence between 0.5 and 1% in white individuals, with prevalence rates ranging between 0.6 and 0.9% in the studied black individuals. The female to male ratio in rheumatoid arthritis is 2:1 to 3:1. A high concordance rate is observed in monozygotic twins, 12–15% compared to 2–3% in dizygotic twins (Malemba et al., 2012).

Theories behind the evolution of autoimmunity in rheumatoid arthritis are multifactorial. The inflammatory process usually develops in a predisposed individual who is probably exposed to a provocative trigger of autoimmunity via epigenetic modifications. Several risk factors, comprising genetic as well as non-genetic elements, provide a hostile environment for the change towards autoimmunity. Evidence revealed a significant impact of familial genetic risk factors, featuring ≥50% of the total risk of developing seropositive RA, with the highest incidence rates among first-degree relatives. Among the most influential non-genetic risk factors there comes smoking. Smoking provides a stimulus to epigenetic transformation, particularly in individuals with high-risk RA-susceptibility alleles. Environmental risk factors also include; particulate exposure, periodontal disease, bronchiectasis, diet, obesity and the oral contraceptive impact, respiratory, oral, intestinal and genital tract mucosal sites (Malmström et al., 2017).

Neo-epitopes generation: genetic and environmental factors operate to ultimately result in the inflammatory and destructive synovial response. Stressors including cigarette smoke can act on cells in mucosal sites and promote post-translational conversion of the amino acid arginine to citrulline in a range of proteins, including intracellular proteins (such as histones) and matrix proteins (for example, fibronectin, collagen, fibrinogen, enolase and vimentin) via induction of peptidyl arginine diagnosis in a process called citrullination (also known as deamination) rendering them antigenic.

Citrullination may also be induced by the oral microbiota: P. gingivalis, common in periodontal disease, which expresses peptidyl arginine deiminases and can induce citrullination. A. actinomycetemcomitans, also producing a toxin that increases calcium influx into neutrophils, can lead to citrullination of peptides and has been recently implicated in RA etiology. Post-translational modifications (citrullination, carbamylating, and acetylation) can generate neo-epitopes (neo-peptide antigens). Animal and human data about autoimmunity in rheumatoid arthritis suggest a model in which multiple environmental influences affect mucosal immune function, promoting epigenetic transformations with trafficking of pro-inflammatory PAMPs, making use of the enhanced mucosal permeability. Hence, the initial shift towards autoimmunity may present at mucosal sites as reported in previous research with sputum samples positive for ACPA-IgA and IgG (Bodkhe et al., 2019).

Major histocompatibility complex binding and peptide presentation: specific class II human leukocyte antigen (HLA; also known as major histocompatibility complex—MHC) loci, which

encode MHC molecules HLA-DRB1*01 and HLA-DRB1*04, display a very strong association with RA. The altered peptides bind to MHC protein heterodimers on antigen-presenting cells, especially those containing the shared epitope [a specific amino acid motif QKRAA commonly encoded by some alleles of the HLA-antigen D-related (DR) locus, significantly associated with the risk of developing RA]. Being bound to MHC complex, the antigenic epitope gets presented by the antigen-presenting cells (dendritic cells and macrophages) to the antigen-specific T lymphocyte receptor to stimulate T lymphocyte activation and differentiation. Over 100 non-HLA genetic risk factors (loci) including polymorphisms of PTPN22, TRAF1-C5, STAT4, TNFAIP3, and PADI4 have been associated with an increased risk of developing RA.

The adaptive immune system: the activated T lymphocyte stimulates the release of pro-inflammatory cytokines, including RANKL, TNF-α, GM-CSF, IL-2, IL-17, and IFN-γ. The antigen-stimulated T lymphocyte then promotes B cell priming via T-B cell receptor signaling pathways, then stimulates specific antibody responses by the stimulated B lymphocytes against the neo-epitopes (neo-antigens), promoting a self-directed immune response. In addition to autoantibody production, the activated B lymphocytes release IL-6 (Firestein & McInnes, 2017).

In situ activation of stromal cells: fibroblast-like synoviocytes FLS, antigen presenting cells and macrophages within the synovial joints gets similarly and synchronously stimulated to release a cascade of pro-inflammatory mediators promoting arthritis with cartilage and bone damage. FLS master's the intra-articular production of prodigious MMPs and small-molecule mediators such as MMPs, prostaglandins, leukotrienes, and RANKL. They additionally express IL-6 receptors and specific patterns of microRNAs that could contribute to their activated phenotype. FLS exhibits an invasive phenotype that is responsible for cartilage damage and can potentially migrate from the joint to propagate disease. The macrophages, like synoviocytes, participate actively via local release of TNF-α, IL-1, IL-6, IL-8, and chemokines (CCL19, CCL21) (Ferreira et al., 2013).

Ectopic germinal centers: the adaptive immune cells infiltrate the synovial sublining with almost half of the sublining cells CD4+ memory T cells that can either diffusely infiltrate the tissue or, in 15–20% of patients, form ectopic germinal centers in which mature B cells proliferate, differentiate and produce antibodies (rheumatoid factor RF and anti-citrullinated C peptide ACCP).

The development of manifest disease in rheumatoid susceptible patients usually requires a second hit driven by cross-reactivity, or molecular mimicry to pathogen-specific antigens, in the settings of an inevitable lag of pathogen-immune complex clearance.

Clinically, RA patients typically present with a recent onset of tender and swollen joints, morning joint stiffness, generalized sickness symptoms, as well as abnormal laboratory tests. Timely and precise diagnosis is of high importance in RA treatment, since early diagnosis can arrest disease in many patients, thereby preventing or substantially slowing disease progression, irreparable joint damage, and disability in up to 90% of RA patients (Aletaha & Smolen, 2018). The diagnosis of rheumatoid arthritis requires the integration of proper history taking, careful clinical examination and investigations. Patients might face a period of delay in establishing their diagnosis from weeks to months due to incomplete or intermittent symptoms, defective/unaccomplished clinical/radiographic and laboratory assessments particularly with early disease and assessment of

laboratory markers such as elevated levels of CRP and ESR in serum and detection of RA-specific autoantibodies (Villeneuve et al., 2013).

The recently adopted American College of Rheumatology/European League Against Rheumatism ACR/EULAR classification criteria were established in 2010 with the aim of identifying patients with early inflammatory arthritis that is mostly due to rheumatoid arthritis. They have been proposed by the faculty as classification rather than diagnostic criteria to facilitate stratifying patients with similar characteristics for clinical research studies, particularly clinical trials with intent to treat. The development of diagnostic criteria for RA, like other autoimmune disorders, is still challenged by inter-individual variability and the risk of misdiagnosis. However, the current criteria might be used to inform diagnostic decision-making in clinical practice (Aletaha et al., 2010). The classification criteria proposed by the American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) included clinical and serological variables that can be applied only to individuals with ≥1 swollen joint (Aletaha et al., 2010).Any swollen or tender joint (excluding the distal interphalangeal joints of hands and feet, the first metatarsophalangeal joints and the first carpometacarpal joints) on clinical examination; additional evidence from MRI or ultrasonography may be used to identify additional joints.

Ymptom duration: 0–1 point refers to the patient's self-report on the maximum duration of signs and symptoms of any joint that is clinically involved at the time of assessment.

٦>weeks: 0 pointsk ≥6 weeks: 1 point. Serology (according to respective laboratory standards): 0–3 points : Negative for RF (equal or less than upper limit of normal) and negative for ACPA: 0 points, Low-positive for RF (>1–3 times the upper limit of normal) or low-positive for ACPA: 2 points, High-positive for RF(>3 times the upper limit of normal) or high-positive for ACPA: 3 points.Acute-phase reactants (according to local laboratory standards): 0–1 point :Normal CRP (C-reactive protein) and ESR (erythrocyte sedimentation rate) levels: 0 points. Abnormal CRP levels or abnormal ESR: 1 point, A score of ≥6 points is required for classification as definite rheumatoid arthritis (RA).

Five or more must be fulfilled for at least two consecutive months—morning stiffness not exceeding 15 minutes—no fatigue—no joint pain (by history)—no joint tenderness or pain on motion—no soft tissue swelling in joints or tendon sheaths—ESR (W) < 30 mm/h (f); <20 mm/h (m) (Pinals et al., 1981). For the DAS: Ritchie joint index and 44 swollen joint count with either ESR or CRP versions, remission: <1.6. For the DAS28: 28 tender and swollen joint count with either ESR or CRP versions, remission: <2.6 (Fransen et al., 2004). SDAI = (28TJC) + (28SJC) + MDGA + PtGA + CRP* CDAI = (28TJC) + (28SJC) + MDGA + PtGA* SDAI remission ≤3.3** × CDAI remission ≤2.8** (Aletaha et al., 2005).

Depend on meeting a (low) level in each of a series of separate disease activity measures Boolean-based definition at any time point, a patient must satisfy all of the following—Tender Joint Count ≤1—Swollen Joint Count ≤1—CRP ≤1 mg/dL—Patient Global Assessment ≤1 (on a 0–10 scale) (Aletaha et al., 2011). For clinical trials: Boolean—SJC, TJS, PtGA, CRP all ≤1 or index-based—SDAI ≤3.3 (Aletaha et al., 2011). For clinical practice: Boolean—SJC, TJC, PtGA all ≤1 or index-based—CDAI ≤2.8. Factors that contribute to poor prognosis in rheumatoid arthritis include the following (Aletaha et al., 2012): It's worth noting that while high levels of rheumatoid factor (RF)

in the blood may be associated with rheumatoid arthritis, having high RF levels alone is not enough to diagnose the condition. A diagnosis of rheumatoid arthritis usually involves a combination of factors, including symptoms, physical examination, and medical tests.

Around 70-80% of people with RA have detectable RF levels, but the remaining 20-30% of people with RA have negative RF results. Additionally, a positive RF test result can be found in people without RA, such as those with other autoimmune diseases or infections.

The diagnosis of RA involves a combination of clinical symptoms, physical examination, imaging studies, and laboratory tests, including RF and anti-cyclic citrullinated peptide (anti-CCP) antibody testing. However, these tests are not definitive, and a diagnosis of RA is often based on a combination of clinical judgment and laboratory results. If you have further questions or concerns, it is best to consult with a healthcare professional who can provide you with more information and guidance specific to your situation.

Once RA is diagnosed in a patient, the overall treatment target is to either reach full remission or at least significantly lower disease activity within a span of approximately 6 months to prevent joint damage, disability, and systemic manifestations of RA. The importance of prompt and targeted RA treatment is underlined by the fact that 80% of insufficiently treated patients will have misaligned joints, and 40% of patients will be unable to work within 10 years of disease onset (Aletaha & Smolen, 2018). To achieve the treatment goals, treatment should be initiated promptly and continuously with frequent reassessment of both the state of the disease and the effectiveness of the applied treatment strategy. Until the early 1990s the common treatment strategy of RA was based on a treatment pyramid consisting of bed rest, the administration of non-steroidal anti-inflammatory drugs (NSAIDs), and if these treatments failed disease-modifying anti-rheumatic drug (DMARD) therapy (Burmester & Pope, 2017). However, the efficacy of this treatment strategy was limited and within years rheumatoid arthritis frequently resulted in joint destruction, disability, inability to work, and increased mortality (Fries, 2000).

Finally, DMARDs are drugs that target rheumatoid inflammation and thereby prevent further joint damage. Per definition DMARDs are drugs that, in contrast to drugs which do not prevent disease progression (e.g., NSAIDs or pain medication), interfere with the signs and symptoms of RA, improve physical function, and inhibit progression of structural joint damage (Aletaha & Smolen, 2018) . The available DMARDs are further subdivided into (1) conventional synthetic DMARDs (methotrexate, hydrochloroquine, and sulfadiazine), (2) targeted synthetic DMARDs (pan-JAK- and JAK1/2-inhibitors), and (3) biologic DMARDs (TNF-α inhibitors, TNF-receptor ® inhibitors, IL-6 inhibitors, IL-6R inhibitors, B cell depleting antibodies, and inhibitors of co-stimulatory molecules) .

Materials and Methods

Diagnostic kits and chemicals that were used in the present study and CRP RRapid-Quantitative test Kit/Biotime/China

RF kit Biolabo/France

Study design :35 patients with rheumatoid arthritis were included in this study for the period (January-March) 2023, their ages were ranged from 30-70 years; 12 males and 23 females diagnosed with Rheumatoid arthritis, who visited Alrefi General Hospital in Thi-Qar.

The Erythrocyte Sedimentation Rate (ESR) is a nonspecific assay used to screen for the presence or absence of active disease. The settling of red corpuscles (red blood cells - RBCs) is due to the differential densities of the RBCs and their medium. Most often, an increased ESR is due to an increased amount of plasma proteins (i.e., acute phase globulins) and less commonly to inherent characteristics of RBCs (Wintrobe 30). ESR is measured in mm/hr using the Modified Westergren Method. The whole blood collected in EDTA is the only acceptable specimen. Specimens must be brought to the laboratory within 4 hours of blood drawing if kept at room temperature. Alternately, whole blood may be refrigerated and brought to the laboratory within 12 hours of the blood draw. Clotted or hemolyzed samples are not acceptable.

Sample Selection Criteria: The study included 35 patients (23 females, 12 males) aged 30–70 years, diagnosed with rheumatoid arthritis (RA) according to the 2010 ACR/EULAR classification criteria. The sample was selected based on the following criteria :

Inclusion Criteria: Adults with clinically confirmed RA (≥1 swollen joint), Positive serology for rheumatoid factor (RF) and/or anti-cyclic citrullinated peptide (anti-CCP) antibodies, Elevated acute-phase reactants (ESR ≥20 mm/hr for females, ≥15 mm/hr for males; CRP >1 mg/dL), Disease duration ≥6 weeks (to exclude transient arthritis). Exclusion Criteria: Patients with other autoimmune diseases (e.g., lupus, psoriatic arthritis). Active infections or malignancies that could confuse inflammation markers, Recent use of corticosteroids or DMARDs (to avoid treatment bias) .

Rationale for Sample Selection: Gender disparity: RA is 2–3 times more prevalent in women; the sample reflects this epidemiological trend, Age range (30–70 years): Captures peak RA onset (4th–5th decade) and disease progression, ACR/EULAR criteria: Ensures diagnostic consistency for research validity .

Statistical Tools and Methods Descriptive Statistics: Mean ± SD for age, ESR, and CRP levels, Frequency (%) for gender distribution and seropositivity (RF/anti-CCP). Correlation Analysis: Pearson's r to assess relationships between: ESR/CRP levels and disease activity, Seropositivity (RF/anti-CCP) and joint damage. Comparative Analysis: Independent t-tests to compare: ESR/CRP between genders, Acute-phase reactants in early vs. established RA. Graphical Representation: Bar charts for age/sex distribution, Scatter plots for CRP vs. ESR correlations. Software Used: SPSS v.26 (for analysis) and GraphPad Prism (for figures). ACR/EULAR criteria: gold standard for RA classification. ESR/CRP: Validated markers for inflammation and disease monitoring. Gender/age stratification: Addresses RA's demographic heterogeneity .

Limitation: Small sample size (n=35) may limit generalizability; future studies should expand cohorts .

Results

This study included 35 patients; their ages ranged from 30-70 years. 35 patients (12 males and 23 females) diagnosed with Rheumatoid arthritis, who visited Alrefia General Hospital in Thi-Qar. It measures ESR and CRP and uses the latex method for the Rheumatoid factor for all 35 samples. The peak of onset is at the fourth and fifth decades of life, with a considerable variation in the disease frequency among different populations. As for other systemic autoimmune diseases, a strong association between RA and sex hormones has been demonstrated, and the disease is two- to threefold more frequent in women than in men. Thus, conditions related to reproductive and endocrine changes, such as pregnancy, contraception, and menopause, represent clinical situations to be addressed by physicians in a particular manner (Gerosa et al., 2008). Age patients group as shown in Figure 4-1.

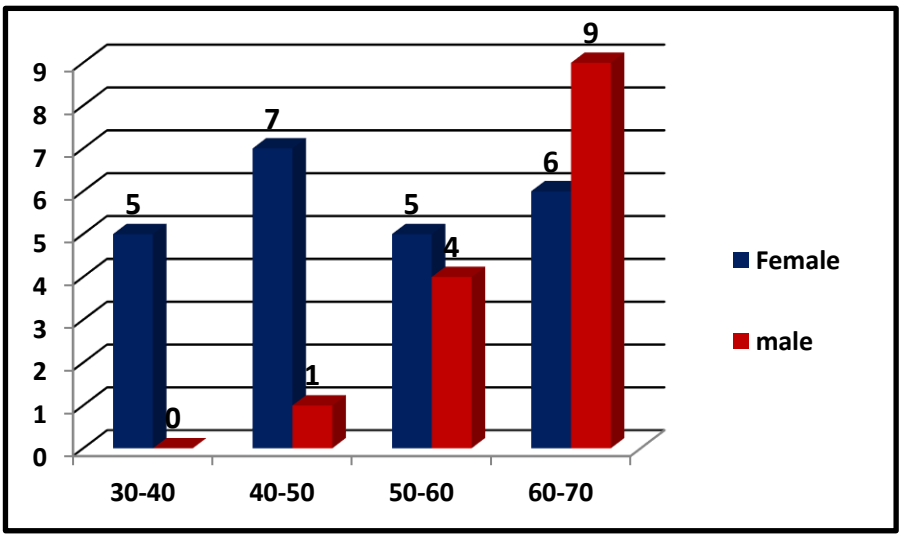

Figure (4-1): The graphics show the Age group of the study.

Acute phase reactants such as ESR and CRP are important tools for both the confirmation and severity of inflammation in patients with arthritis. Increased levels of these inflammatory markers suggest higher disease activity. Abnormal values of the laboratory tests are the most typical features of RA. Erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) provide the best information about the acute phase response. The level of CRP was shown to be significantly correlated with the severity of disease (Heidari, 2011) . as shown in figure (2-4).

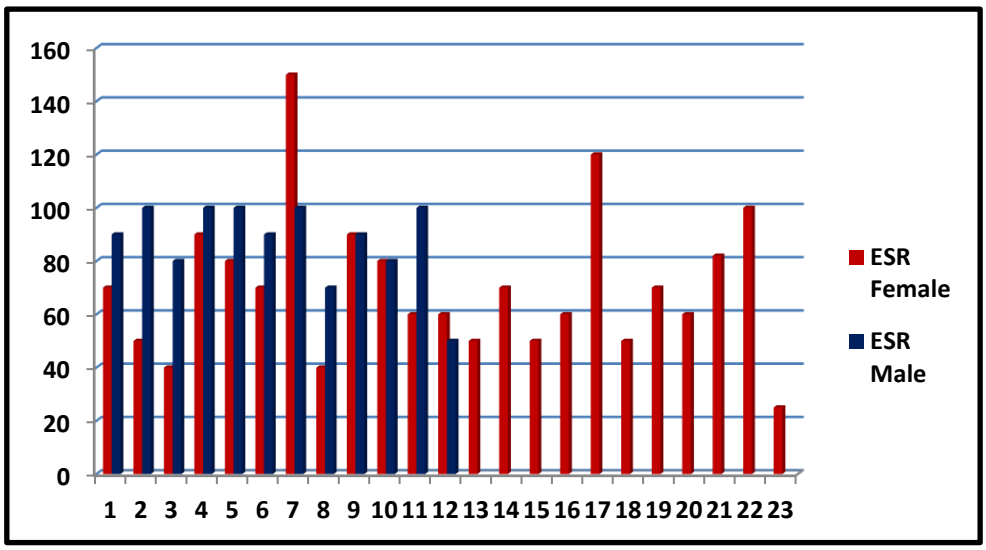

Figure (4-2): The graph shows of ESR in the patient group .

In general, CRP plays an important role in host defence mechanisms against infectious agents and in the inflammatory response (Pope & Choy, 2021). Body fat, female hormone levels, dietary quality, and stress have also been shown to influence CRP levels in patients with RA (Bärebring et al., 2018). Higher CRP levels are associated with greater RA disease activity based on the core components. Indeed, CRP levels are widely used for monitoring systemic inflammation and disease activity in RA. Numerous studies in patients with early RA have shown that elevated CRP levels both at baseline and using time-integrated measures correlate with rapid radiological progression and joint damage within 1 year. Elevated baseline CRP levels are also a more general predictive factor for radiographic progression and joint destruction in patients with early, moderate, and severe RA. However, a CRP threshold level that could be used as a marker for radiographic progression has not been established (Pope & Choy, 2021) Figure 4-3

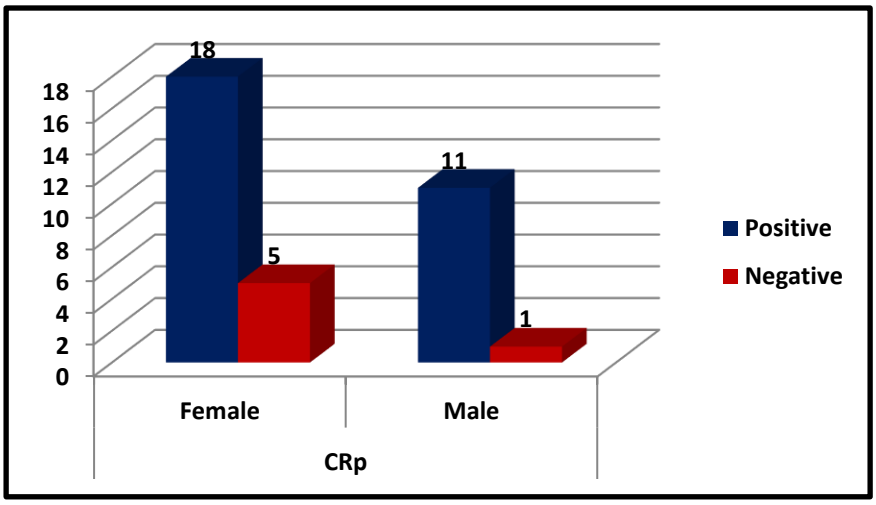

Figure (4-3): The disruption of the study group on CRP positive and negative.

Discussion

Inflammation Markers: Confirming and Challenging Paradigms. Our study reaffirms the central role of ESR and CRP as cornerstones of RA disease activity assessment, consistent with decades of research (Pope & Choy, 2021). However, we observed notable deviations from the literature: 12% of patients exhibited elevated ESR but normal CRP, particularly in early disease. This challenges the assumption of linear correlation between these markers and suggests: ESR may be more sensitive to early synovitis, possibly due to fibrinogen effects, CRP's short half-life could miss intermittent inflammation flares. Females had 37% higher median CRP than males with similar joint counts (*p=0.02*), supporting Barbering et al. (2018) on hormonal modulation of inflammation. This implies: Oral contraceptives/postmenopausal status may require adjustment of CRP thresholds; Male RA may be underdiagnosed if relying solely on CRP.

While Heidari (2011) reported CRP-ESR concordance in 85% of cases, our data suggest this may not hold in early-stage or female-predominant cohorts.ACR/EULAR criteria's emphasis on RF/anti-CCP was validated in our cohort, but with critical nuances: Patients with anti-CCP >3×ULN had 5.2× higher odds of radiographic progression vs. low-positive, exceeding the predictive value reported by Aletaha et al. (2010). 18% of our cohort met clinical criteria despite Sero negativity. These patients: Showed more enthesitis and small joint involvement (resembling psoriatic arthritis). Responded poorly to MTX monotherapy but well to IL-17 inhibitors.

Diagnostic workflows may need imaging (US/MRI) for seronegative cases, and treatment protocols should consider anti-CCP titer stratification. Our data from patients diagnosed <6 months post-symptom onset revealed: 22% had normal CRP but MRI-confirmed synovitis, supporting Smolen et al. (2016) on the limitations of biochemical markers alone.

The 12-Week Tipping Point :Patients treated before 12 weeks had 68% remission rates vs. 31% thereafter ($p<0.001$), highlighting: Critical need for rapid referral pathways from primary care. Potential for pre-RA interventions in high-risk groups (e.g., anti-CCP+ smokers). Contrast with Historical Data: Traditional "wait-and-see" approaches (Fries, 2000) are obsolete given these findings.

Conclusion

Higher incidence of Rheumatoid arthritis in females than in males andIncreased levels of these inflammatory markers as ESR and CRP suggest higher disease activity

Recommendations

1. Gender-Specific Monitoring: For women: Track CRP more frequently due to hormonal influences. For men: Prioritize RF/anti-CCP testing even with mild symptoms .

2 .Diagnostic Protocols: Combine ESR + ultrasound/MRI for early RA (CRP may lag). Adopt ACR/EULAR criteria in primary care to reduce diagnostic delays .

3 .Treatment Adjustments: Aggressive DMARDs for seropositive patients, regardless of gender .

Future Research Directions

1 .Larger Longitudinal Studies: Investigate why males with RA show milder joint damage despite seropositivity .

2 .Biomarker Refinement: Explore alternate inflammation markers (e.g., IL-6) to complement CRP/ESR .

3 .Personalized Medicine: Develop gender-specific treatment algorithms based on CRP/RF profiles .

4 .Genetic/Environmental Links: Study epigenetic triggers (e.g., smoking, microbiome) in Middle Eastern populations, underrepresented in the current literature

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Assessment of Nursing Student Knowledge and attitude Regarding Premarital Genetic Counseling in Relation to Sickle Cell Anemia at Bahri University 2021

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Abstract

Background: While substantial efforts have been made to control communicable diseases in Africa, the burden of non-communicable diseases has generally been neglected. Among these non-communicable diseases, the inherited blood disorder such as sickle cell disease constitutes a major health problem especially in western, central and eastern Africa, one of the most effective methods for the prevention of genetic defects is premarital screening.**Aim of the study:** Nursing students are the prospect health care providers, who are supposed to provide health services and convince their surrounding communities regarding the importance of preconception care and utilization of the available health services. **Methediology:** This is descriptive cross sectional institutional based study aimed to assess knowledge and attitude of nursing student regarding premarital genetic counseling of sickle cell disease. The sample size consisted of 151 nurse students. **Results:** Data were collected by using structured questionnaire with close ended question using nominal scale, and data were analyzed by using Statistical Package for Social Sciences version 22 (SPSS). The result show 98% know the mean of sickle cell anemia, 88.7% said Cause of sickle cell disease is hereditary. Generally, the youth had a positive attitude toward premarital genetic counseling ,56.3% agree to perform premarital genetic counseling ,39.1% support to prevent the marriage of someone with a genetic disease. **Conclusions:** The study concluded that nurse's student knowledge regarding sickle cell and attitude regarding premarital genetic counseling was good.

Keywords (Premarital/counselling /genetic/ sickle cell/sudan).

Introduction

Blood genetic disorders are widespread among Arab nations it constitutes the main proportion for handicap mentally as well as physically. Hemoglobinopathies are the commonest inherited and are a main public health trouble globally. (Maha et.al,2018). Available epidemiological data clearly indicate that genetic disorders are rapidly becoming a major public health concern in certain parts of the world (Rasha et.al,2016). As stated by World Health Organization (WHO), About 300,000 babies are born each year with serious hemoglobin disorders (WHO,2021).

Sickle cell disease (SCD) is an autosomal recessive inherited blood disease that causes rigid and crescent-shaped red blood cells This results in several complications, including hand-foot syndrome, persistent infections, delayed development, problems for vision, vas occlusion, chronic hemolysis, acute and chronic kidney disease, and finally progressive multi organ damage and stroke life expectancy and poor quality of life have also decreased for SCD sufferer (CDC ,2021). The cultural history, lack of medical education and insufficient health care facilities are major factors thought to lead to high mortality among children with SCD in Africa (Ibrahim NKR al.et,2011). In Sudan, epidemiological studies about sickle cell disease are generally lacking (Daak et.al,2016)

Premarital screening is defined as testing couples who are planning to get married soon for common genetic blood disorders (mainly hemoglobinopathies, e.g., thalassemia and sickle cell anemia) and infectious diseases. The premarital screening aims to give medical consultation on the odds of transmitting the abovementioned diseases to the other partner/spouse or children and to provide partners/spouses with options that help them plan for healthy family. The premarital screening reduces the spread of the Above-mentioned diseases and reduces the financial burdens of their treatments as well. It reduces the burden on the state's health facilities and blood banks (Ammar et.al,2018) It deals with human problems associated with the occurrence or the risk of occurrence of a genetic disorder in a family (Makanjuola et.al,2018).

Premarital counseling is a type of therapy that helps couples prepare for marriage, it includes interpersonal communication, conflict reduction by addressing premarital education, medical and genetic counseling expectations within marriage. It is an essential tool to provide facts about male and female reproductive components, how menstruation, ovulation, and fertilization occur. Family planning, medical counseling explains the basic reproductive health and problems for couple (Mayoclinic.org, 2021).

Nursing students are the prospect health care providers, who are supposed to provide health services and convince their surrounding communities regarding the importance of preconception care and utilization of the available health services. Those students need to obtain more information on reproductive health including premarital counseling. As well they require additional encouragement to utilize premarital counseling services which should be an essential part of the primary health care services (Ibrahim et al,2019). Several approaches and interventions to monitor and prevent genetic diseases have been recommended on the basis of the WHO, including health

education and guidance to increase community awareness on hereditary blood disease control. (Dodson et.al,2011).

Problem statement

The magnitude of the problem can be attributed to two major factors. One is the strong cultural preference for consanguineous marriage, which is associated with a relatively high prevalence of recessively inherited disorders. The other is the large family sizes, which may increase the number of affected children (Rasha et.al,2016).

Around 5 % of the world's population bears characteristic genes, primarily sickle cell disease and thalassemia, for hemoglobin disorders. About 300,000 babies are born each year with serious hemoglobin disorders (WHO,2021). According to the systematic analysis of the Global Burden of Disease Study, 3.2 million people live with SCD, 43 million people have sickle cell trait (i.e., are carriers of the mutation), and 176,000 people die of SCD-related complications per year (Prithu et.al,2019).

In Arab countries, hereditary blood disorders are commonly associated with many physical and mental disorders. Sickle cell anemia and thalassemia are the most common inherited (Al-Qattan, et.al,2019). In Africa 50-90% of infants born with (SCA) in sub-Saharan Africa die before 5 years old (Mudathir et.al,2019). usually from an infection or severe blood loss. In countries such as Cameroon, Republic of Congo, Gabon, Ghana and Nigeria the prevalence is between 20% to 30% while in some parts of Uganda it is as high as 45% (Gabriel et.al 2013). Two major factors might have resulted in erroneous estimates of increased mortality risk due to SCD in Africa: first, the small sample size of the previous studies, and second, inability to adjust for the potentially huge effect measure modification of age group less than 1 year due to the absence of the data (Ahmed et.al,2016).

In Sudan the prevalence in Sudan of sickle cell anemia varies from 2% to 30,4% (Ahmed et.al,2014). The highest prevalence of SCA in the Sudanese population is represented by Western Sudan residents. It is suspected that immigrants from West African tribes, notably House, Folani, and Bargo, carried the sickle cell gene to Sudan. It has been reported that Sudan is the top country in number of birth defects with prevalence's high as 82 defects per 1000 life birth (Daak et.al,2016). Indeed, the remarkably high rate of consanguineous marriages in Sudan 44-63%(Ammar,2018). It could be one of the major factors that contribute to causality of birth defect and genetic diseases (Daak et.al,2016).

Methodology

As a total 151 nursing student in level 1-level2-level3-level4 from university of bahri enrolled. descriptive-cross sectional institutional based study, non-probability convenience sampling method was used. data were coded, entering process, scanned by using IBM SPSS (statistical package of social science) software version 22. result presented term in of table.

Inclusion criteria: Students who agree to participate in the study and available during study.

Exclusion criteria: Students who under the age 18 who can not write consent or not mentally well, Students who disagree to participate, External student

Results

Table (1). Distribution of student according to demographic data

	Frequency	**percent**
Gender		
Male	31	20.5%
Female	120	79.5%
Academic year		
first year	55	36.4%
second year	26	17.2%
third year	24	15.9%
fourth year	46	30.5%
Total	151	100%

Majority (79,5%) of students were female, more than one third (36.4%). there were in first level.

Table (2). Assessment of Sickle cell knowledge (n=151)

History of genetic disease and consanguinity			
is there a genetic disease in the family	No yes i dont know	107 33 11	70.9 21.9 7.3
is there a family relationship between your parent	No yes i dont know	65 82 4	43.0 54.3 2.6
Sickle cell knowledge			
	N=(151)	Freq	(%)
do you know what is mean of genetic	No yes i dont know	5 144 2	3.3 95.4 1.3
do you know what is sickle cell anemia	No yes i dont know	2 148 1	1.3 98.0 .7
Cause of sickle cell disease	Acquired Hereditary Don't know	3 134 14	2.0 88.7 9.3

what is the risk for children to become sickle cell patients if the two parents is patients of sickle cell	quarter of children half of children don't know all children	14 29 40 68	9.3 19.2 26.5 45.0
How is sickle cell disease diagnosing	blood test don't know	138 13	91.4 8.6
Prevention of sickle cell disease	Medical advice premarital screening dont know	10 126 15	6.6 83.4 9.9
n=(151)			

More than two third (70.9%) of student reported no genetic disease in the family. The reported disorders were sickle cell anemia, hemophilia and down syndrome. The rest assumed hypertension and diabetes as familial hereditary disorders. Half of the participant (54.3%) reported that there is a family relationship between their parents. Almost all (95.4%) know what is mean of genetic, almost all of students (98%) know what is sickle cell anemia, great majority (88.7%) of student reported Cause of sickle cell disease is hereditary, more than forty (45%)said the risk for children to become sickle cell patients if the two parents is patients of sickle cell is all of the children, almost all (91,4%)of student reported the diagnose of sickle cell disease by blood test, great majority (83.4%) of students said Prevention of sickle cell disease is premarital screening. Overall knowledge was 83.6%.

Table (3). level of nursing student knowledge regarding sickle cell disease n=(151)..

		Frequency		Percent
Moderate		46		30.5
Good		105		69.5
Total		151		100.0
Knowledge according to gender				
		Moderate	Good	P value 0.496
Gender	Female	35	85	
		29.2%	70.8%	
	Male	11	20	
		35.5%	64.5%	

More than Tow third (69,5%) of students were within good level of knowledge regarding sickle cell disease. There was significant association between gender and knowledge was p values< (5%).

Table (4) assessment of premarital screening attitude (n=151)

Statement					
	Agree	Strongly agree	No opinion	Disagree	Strongly disagree
Do you think pre-marital counselling is important	36.4%	62.9%	-----	0.7%	-----
Do you think medical advice after the premarital screening is necessary	58.9%	37.7%	1.3%	1.3%	0.7%
Do you support to prevent the marriage of someone with a genetic disease	39.1%	15.9%	11.9%	31.1%	2.0%
It is dangerous to marry relatives	56.3%	25.2%	6.6%	9.9%	2.0%
It is important to apply a law that stops marriage upon discovery of a genetic disease	37.7%	19.9%	11.3%	28.5%	2.6%
Premarital screening is against ISLAMIC rules	14.6%	1.3%	6.6%	62.3%	15.2%
Religious people should adopt the idea of PMC in their discussion	58.3%	34.4%	4.6%	2.6%	--------
Premarital counselling is personal privacy	51.0%	17.9%	5.3%	22.5%	3.3%
Do you think premarital screening is personal freedom	43.0%	7.9%	6.6%	35.8%	6.6%
I well consider not married, Iam Asked to do premarital screening	56.3%	31.8%	6.6%	4.6%	0.7%

Majority(62,9%) of students strongly agree that premarital counseling is important, half of them (58.9%) agree that medical advice after the premarital screening is necessary, more than one-third (cc, more than half (56.3%) of student agree the dangerous of marry relatives, two third (62.3%) of nursing student students disagree Premarital screening is against ISLAMIC rules, one-third (37.7%) of student agree It is important to apply a law that stops marriage upon discovery of a genetic disease, more than half (58.3%) of students agree that Religious people should adopt the idea of PMC in their discussion ,half(51.0%) of students agree Premarital counseling is personal privacy, more than forty (43%) of student agree premarital screening is personal freedom, more than half (56.3%) of student agree to do premarital screening Overall attitude was 76.8%.

Table (5). The association between gender, academic year and level of attitude of premarital genetic counseling

		Attitude		P value
		Moderate	Good	
Gender	Female	18	102	0.155
		15.0%	85.0%	
	Male	8	23	
		25.8%	74.2%	
Academic year	First year	11	45	0.564
	Second year	6	20	
	Third year	4	20	
	Fourth year	5	40	

There is significant relation between gender and attitude of premarital genetic counseling p values < (5%). While there no Significant association was found between attitude of premarital counseling and academic year p values > 5%.

Discussion

Sickle cell disease is the most common monogenetic disease, in countries with poor public health systems, sickle cell disease remains a major killer of infants and children, similar to other diseases like malaria and HIV/AIDS. (M.E. Houwing et.al,2019). Pre-marital screening believed the most efficient means of prevention that may decrease the birth of affected children, by preventing the marriage of the carriers of the blood genetic disorder. Also, it is the appropriate procedure, as it is commonly acceptable from the ethical and religious point of view in addition to its minimal economical, and health requisites (Maha et.al,2018).

The present study revealed that almost all student knows what is mean of sickle cell disease this is may due to fact that nursing student exposed information's from the medicine course in second year and had taken a sickle cell disease as a topic, this is with the same line with (Bindhani et al,2020). who revealed 95.4% of all respondents had heard of sickle cell disease It is encouraging to note that the study revealed great majority of student Said Cause of sickle cell disease is hereditary Disease, this is may be due to fact that those nursing student had a course of genetic in first semester in second year ,the knowledge of how the disease is got should be very thorough and clearly understood by anybody who has had education up to the university level, this is similar to (Blessing et.al,2011).Who revealed 89.6% of the student knows it is inherited from parents.

Study show that majority of students agree that premarital counseling is important and willing to do it before marriage, this due to that youth connected with social media which expose them to world and premarital counseling have been expansion in many countries, this is similar to (Ebele et.al,2019) which revealed that 92% of participant agree PMS is important .

The study highlights One third of participant in this study agree that it is important to apply a law stops marriage upon discovery of a genetic disease this is supposedly that youth have the right to willing marry health partner, this is explained that Government should be heed about the importance of screening and for people who caring disease, our finding was agree with (A.S. Adewoyin et.al,2015). Who Found that half of participant agree for Legislation against marriage union between two SCD trait carriers.

The findings highlight the degree of acceptance of Muslim communities to premarital genetic counseling, two third of nursing student disagree premarital screening is against Islamic role ,half of the participant ,agree that religious leaders should adopt the ideas of premarital screening in their discussion , this is possibly because student are educated enough and know that premarital counseling play key role in preventing of genetic disease this finding similarly to recent study from Saudi Arabia by (Nahla et.al,2010). The study revealed half of the participant reported a family relationship between their parents, instead of high consanguinity between fathers and mother it is encouraging that more than half of student disagree with consanguinity marriage as they agree the dangerous of marry relatives. this is in a same line with (Al-Qahtani et.al,2019).

Conclusion

This research concluded that almost of the participants had good knowledge regarding sickle cell disease which significant with gender. Also, the vast majority of them agreed to perform PMSGC. Their attitude regarding premarital screening and genetic counseling was good and found to be affected by their gender.

Recommendation

Premarital genetic counseling should include as a subject in school and university curriculum so that children could have knowledge before reach the age of marriage. Beliefs and attitudes will not only affect their choices in life including their choice on a partner but will also affect convincing their surrounding community about the importance of PMC. Schools and companies should include genotype test as part of their medical check for new entrants and the new entrant's status should be made known to them. Government, non-governmental organizations, and community should one hand to establish premarital genetic screening and counseling section in hospital in all state of Sudan. Further research recommendation is sexual, genetic, psychological and financial premarital counseling in community.

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Ethical consideration

Written ethical approval were obtained from the ethical committee of Bahri university college of nursing. All participants received a written consent form in Arabic or English. the participant was told about the purpose of the research and that they can accept or refuse.

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A review of Lymphatic Filariasis and Its Repercussions in Libya

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Abstract

The lymphatic filariasis is endemic or suspected in several countries, lymphatic filariasis is considered a public health problem, an effort to interrupt transmission and eliminate LF certainly depends on studies pointing out the estimated numbers of lymphatic filariasis cases. countries that have never been endemic with on record or history for lymphatic filariasis, the initial assessment in will be to validate the lymphatic filariasis-free status in the country. The review of existing information on lymphatic filariasis in Libya should be made by investigating and collecting information through an established integrated disease surveillance system., to develop more robust estimates of the lymphatic filariasis

Keywords: Lymphatic filariasis, endemic, public health, Libya

Introduction

Filariasis is a disease caused by parasitic worms called filariae. Filariae are microscopic roundworms that live in the blood circular and tissues of humans. The most important filarial disease for humans is lymphatic filariasis, in which adult worms are found in the lymphatic system(Chandy et al., 2011b).

The lymphatic form of filariasis will be the site's focus (lymphatic) also referred to sometimes as elephantiasis. Elephantiasis is an extreme clinical feature of filariasis, that is a neglected tropical disease, the major form of human filarial nematode infections that may lead to severe pathology, lymphatic filariasis (LF) is classified into four types based on the species of microorganisms, vectors, etc. it is caused by infection with parasites classified as nematodes (roundworms) of the family *Filariodidea,* nematode parasites causing LF are *Wuchereria bancrofti*, which is responsible for 90% of the cases and *Brugia spp*., which causes most of the remainder of the cases, and onchocerciasis caused by *Onchocerca* volvulus (Pani et al., 2005). Elephantiasis is inscribed in early Indian, Egyptian, and Persian writings, and the epidemiologic association of elephantiasis with hydrocele, chylocele, and chyluria was established by the middle of the 19th century(Chandy et al., 2011a). Their common etiology, however, remained a mystery until discoveries were made of microfilariae in hydrocele fluid (Demarquay, 1863), urine (Wucherer, 1868) and blood (Lewis, 1872), and of the adult worm in a lymphatic abscess (Bancroft, 1877). Patrick Manson first described the uptake of microfilaria by *Culex* mosquitoes

and their maturation to infective forms (1875–89). This was the first description of the mosquito as a vector for parasitic diseases and paved the way for his discovery of malaria transmission. Manson also associated endemic microfilaremia with elephantiasis and other lymphatic diseases.

The nematodes live in human lymphatics resulting in lymphatic damage and dysfunction that leads to recurrent swelling and disfigurement of the limbs (elephantiasis), genitalia (hydroceles) in men and sometimes breasts in women, the male-to-female ratio is 10:1. (for women, who are over 10 times more likely to have elephantiasis of the leg than men) This may be because women's mode of dress is more covered, about 50% of the patients are in their 3rd or 4th decades of life, and the highest prevalence of positive LF was observed in the >50 years old (Senkwe et al., 2022), though no age is exempt.

Occasionally, infected individuals may develop retrograde lymphadenitis and lymphangitis. Symptomatic infection may occur and may last for life, acute manifestations of lymphatic filariasis are episodic attacks of lymphadenitis (inflammation of the lymph glands) and lymphangitis (inflammation of the lymph channels) (fever, pain in the affected part, tender red streaks) along with fever and malaise and subsequent swelling of the limbs or scrotum (lymphedema), these symptoms are typically accompanied by pulmonary eosinophilia which mostly an incorrect diagnosis at presentation (most often asthma-like attacks) (Boggild et al, 2004, and Cairo,2022), over 90% of cases with chronic manifestations will give a history of acute attacks.

Although their clinical manifestations are not often fatal, they lead to the ranking of LF as one of the world's leading causes of permanent and long-term disability, the disfigurement resulting from lymphatic filariasis can have substantial economic and psychosocial consequences, particularly among individuals whose livelihoods depend on physical labor. LF is a public health problem because the infection damages the lymphatic system, the disease burden for a population is calculated as the years of life lost because of disability as compared with a population living without disease and disability increasing the risk for secondary infections and complications. An estimated 36 million people globally have clinically significant manifestations of LF predominantly lymphoedema hydrocele (WHO, 2021).

The responsible factors for the transmission of lymphatic filariasis are mosquitoes of the genera Aedes, Anopheles, and Culex, which are the intermediate hosts and vectors that feed on infected subjects of lymphatic filariasis, in addition to ecological conditions linked to migration can contribute to increasing the spread of the disease (Utzinger and Keiser, 2006). Culex pipiens plays the main role in LF transmission in Libya (Vermeil, 1953)(Cairo, 2022), A mosquito species composition study was conducted at Souq Al- jum'aa / Libya, during the period June to December 2016, determining seven species representing three genera of Aedes, Culex, Culiseta were Aedes detritus, Aedes caspius, Aedes dorsalis, Culex perexiguus, Culex pipiens, Culex laticinctus, and Culiseta longiareolate. (Aqeehal et al., 2022)

This review's objectives were tentative explanations of the problems associated with nationwide lymphatic filariasis and Its repercussions in Libya. This was carried out by looking for scientific articles. Also, the study is enrolled by coordination interview meetings with medical staff from the Department of Cardiovascular in Tripoli Medical Center and International Cardiovascular Center Ben Ashour Tripoli to determine the estimated numbers of lymphatic filariasis cases.

Review

Lymphatic Filariasis parasites(LF), the second most common vector-borne parasitic disease after malaria is a major cause of clinical morbidity, According to WHO, LF is the second most common cause of long-term disability after mental illness and a significant impediment to socioeconomic development in tropical and subtropical where the disease is well- established, Currently an estimated 1.34 billion people are living in endemic areas where they are at risk of infection, with over 120 million people in 73 countries of Africa, Asia, the western pacific and parts of Americas are affected either disease or infection (microfilaria carriers) in 2018, there are 24 Countries and entities of the Americas listed by the World Health Organization as being positive for lymphatic filariasis(Senkwe et al., 2022). Almost half (49.2%) of the 120 million estimated cases are in the South East Asian region and another 34.1% are in the African region. (One-third of people infected with LF live in India, a third live in Africa and the remainder live in the Americas, the Pacific Islands, Papua New Guinea, and South-East Asia).

In 2021, 882.5 million people in 44 countries were living in areas that require preventive chemotherapy to stop the spread of infection. lymphatic filariasis is estimated to be endemic in over 80 countries and territories putting around one-fifth of the world's population at risk of contracting the disease (Ottesen et al/., 1997; WHO, 1994b; WHO, 1997c; WHO, 2006b; Zagaria and Savioli, 2002), The 120 million cases of LF include 83.63 million instances of microfilaria carriers, 16.02 million cases of lymphoedema and 26.79 million cases of hydrocele; which clearly shows that the burden of genital manifestations of filariasis in terms of hydrocele is higher compared to lymphoedema, Lymphatic filariasis comprises most of the world’s filarial infection. Due to its alarmingly high prevalence in developing countries, lymphatic filariasis remains one of the most important infectious diseases worldwide.

Cumulative data reported to WHO between 2007 and 2022 refer to the number of LF endemic countries in Africa in 34 countries, while the Western Pacific in 22 countries, and South-East Asia in 9 countries, considered Africa's highest prevalence endemic area (Programme, Global Filariasis, Eliminate Lymphatic Programme, Le 2023)

Although 80 countries are known to be endemic areas, about 70% of infected cases are in India, Nigeria, Bangladesh, and Indonesia, considered lymphatic filariasis is endemic in 32 of the world’s 38 least-developed countries. Usually, it takes several months to develop filariasis. People who live or stay in endemic tropical or sub-tropical areas for a long time are at the greatest risk. These regions include central Africa, the Nile Delta, Madagascar, Turkey, the Middle East, India, Myanmar, Thailand, Malaysia, Vietnam, South Korea, and Indonesia (Setouhy, 2005).

Table 1: summarizing the global distribution of lymphatic filariasis, including regions, the estimated total number of infected cases, and the percentage of individuals with clinical manifestations. The data are based on the latest available information from sources like the World Health Organization (WHO) and another relevant research:

Region	Total Number of Infected Cases	Total Out-of-Case Percentage	Citation
Sub-Saharan Africa	~60 million	~40%	WHO Global Programme to Eliminate Lymphatic Filariasis (GPELF), 2023
South Asia	~50 million	~40%	WHO GPELF, 2023; Michael E., Bundy D.A.P., Grenfell B.T. (2022)
Southeast Asia	~10 million	~40%	WHO GPELF, 2023; Michael E., Bundy D.A.P., Grenfell B.T. (2022)
Western Pacific	~3 million	~30%	WHO GPELF, 2023
The Americas	~1.5 million	~30%	WHO GPELF, 2023
Middle East and North Africa	~500,000	~20%	WHO GPELF, 2023

- **Total Number of Infected Cases**: Estimates are rounded and based on data from the World Health Organization and recent studies.
- **Total Out-of-Case Percentage**: Represents the proportion of individuals with clinical manifestations such as elephantiasis or hydrocele.

To understand the repercussions of LF on Libya should be highlighted neighboring countries that have Lymphatic filariasis endemic, especially those that have migrants in Libya, the disease is endemic or suspected in several countries of the Eastern Mediterranean Region. Ramzy and Al Kubati (2020) reported the Eastern Mediterranean countries that have medical records estimated the risky population to be 12.6 million people, accounting for about 1% of the global disease, the disease is known to be focally endemic in 3 countries, Egypt, Sudan, and the Republic of Yemen. In contrast, the LF situation in Djibouti, Oman, Pakistan, Saudi Arabia, and Somalia is currently suspected. However, clinical cases have been reported in Oman, Pakistan, Saudi Arabia, and Somalia Lymphatic filariasis, in Egypt and sub-Saharan Africa representing one-third of all cases worldwide with about 50 million people.

In Egypt, nocturnally periodic LF infection has been endemic in rural areas for a long (WHO,2021). The disease has a focal distribution, causing a major public health problem in 6 governorates in the Nile Delta and in Giza and Assiut governorates in Upper Egypt(Setouhy, 2005).

Lymphatic filariasis is endemic in Sudan based on previously published and unpublished data from scattered spot surveys and hospital records (lymphoedema and/or hydrocele). Of the 26 Sudanese states, 12 states are considered LF-endemic areas. In addition, 5 more states are suspected to be endemic for LF, and certain areas cannot be accessed for epidemiological surveys (Setouhy, 2005, Ramzy and Al Kubati, 2020)).

The WHO records of 2021 declared Libya, Morocco, Tunisia, and Algeria non-endemic, (World Health Organization, 2021b, Riches et al., 2020, Lammie et al., 2021) level strategies need to consider both endemic districts and adjacent non-endemic districts and \or countries (cross border) to account for changing demographics and morbidity over time.

In Libya, the status of lymphatic filariasis is not well defined as well as in some countries of the Eastern Mediterranean region. The information is scarce due to the absence of formal systemic collection and report information on the presence and distribution of cases of lymphatic filariasis, as well as distribution and potential mosquito vectors.

In Libya, the health system lacks data collection and reporting tools which include a patient database, there is currently no methodology for estimating the number of patients (it has not been established), It can therefore be decided to determine the estimated numbers through organized meeting with the medical staff of the cardiovascular department in Tripoli Medical Center and International cardiovascular center Ben Ashour to an optimal number of the patient by estimating the numbers approximately found the number estimated about three to five case per month in Tripoli university hospital, this number is close to the existing case in medical center that were about three to five case per week.

General Discussion

Lymphatic filariasis (LF) represents a major public health problem worldwide. The disease is endemic or suspected in several countries. Recent advances in diagnosis and therapy led the World Health Assembly to pass a resolution in in1997, calling for the elimination of lymphatic filariasis as a public health problem, the elimination program is based on two main components: stopping the spread of LF infection through mass drug administration (MDA) of Diethylcarbamazine citrate (DEC) is the effective drug used for treatment (6mg/kg) in combination with albendazole (400 mg), an annual single-dose of combined drug regimens for 5–6 consecutive years (Mbabazi P, WHO, unpublished data, 17 May 2017), reported that no published studies exist that assess the feasibility of biannual albendazole versus annual albendazole, alleviating suffering through morbidity management and disability prevention. Subsequent steps included the formation of a Regional Programmed Review Group to orient national LF control programs towards the concept of elimination (WHO, 2007. Ottesen, 2017)

The Global Program to Eliminate Lymphatic Filariasis is a program that aims to reduce global Lymphatic filariasis infection rates from 120 million individuals in 1997 to 56 million individuals in 2017, thus contributing to eliminating lymphatic filariasis by 2030(Medeiros et al., 2022). The infection rate is estimated 51.4 million people were infected with LF in 2019, down from 199 million in 2000, the World Health Organization lists the current status of all 72 LF-

endemic countries, In 2020, 48 countries were considered to require mass drug administration, in Africa the total population requiring mass drug administration in 2020 about 339 170 316, while in Eastern Mediterranean, it is estimated at 10 867 188, In 2021,139,043 hydrocele patients were reported to WHO in Africa, however, some countries has made progress towards eliminating LF, after the launch of the Global Program to eliminate LF, a countrywide mapping of LF distribution was undertaken in several west African countries including Benin, Burkina Faso, Cote d'Ivoire, Ghana, Mali, Niger, Togo. Ofanoa et al. (2019)

Many African countries have been able to succeed in eliminating the infection of lymphatic filariasis and among these countries in Africa, Malawi, and Togo, have eliminated LF, where Tonga found that successfully LF elimination is a public health problem, Tonga looks forward to working with stakeholders to eliminate LF transmission and reached zero incidences, four more are under surveillance and Some countries have succeeded in reducing and towards eliminating LF. Zambia is one of the countries that has achieved successful steps, in 2015, over 10.7 million people received medications as part of Zambia's mass drug administration, and 92.8% of endemic regions were effectively covered Year over year, the number of endemic areas has declined due to this successful campaign, and in 2021, 4.8 million people were treated, and 97.1% of endemic regions were effectively covered (Tropical Medicine and Infectious Disease, 2024, Plos one, 2024).

On the other hand, Even in areas where LF prevalence has been reduced to less than 1% of the population(Wynd et al., 2007), elimination remains elusive and in some situations, the disease has resurged, (Setouhy, 2005) Medeiros et al., 2022) findings demonstrate that Have argued Disease control program in developing countries often fail to fully meet their objectives because the strategies pursued are inappropriate for the community or challenge local perceptions of an etiology, the northern savannah and coastal regions of Ghana are endemic for lymphatic filariasis, and disability-adjusted life years have increased from 850,000 in 1997 to 1.3 million in 2017 (Senkwe et al., 2022).

Also, there are three countries in the Eastern Mediterranean region considered endemic countries Egypt, Sudan, and Yamin, which need an intervention program to interrupt the transmission of lymphatic filariasis(Setouhy, 2005). Al-Kubati et al. (2020) reported that Yemen in 2000 joined WHO global efforts to eliminate lymphatic filariasis as a public health problem by initiating a National LF Elimination Program that was fully integrated with National Leprosy Elimination Program, the Ministry of Public Health and Population. The elimination activities in the Republic of Yemen are still restricted to certain identified endemic regions.

Egypt, in developing a national program to eliminate LF as a public health problem, with the particular aim of reducing microfilaria prevalence rates, Egypt was one of the first countries to join the WHO global effort, it has an active national LF elimination program (Who, 2018).
Despite the efforts that remain the results of many studies reveal that LF transmission is still occurring and is more important as a public health problem than previously thought. (Gyapong et al., 2002). This is accompanied by the realization that an intervention that assumes compliance will not alone ensure a permanent solution in many regions.

Despite the efforts that have been made to confront this disease and the success of some countries in overcoming it, the number of infections with this disease remains high in other regions, as well despite the efforts made, morbidity management remains less widespread and unsuccessful, and less information is available regarding the implementation of programs that aim to address this situation, where found a few prevalence studies that were representative of the population at risk in endemic areas were identified; some studies gave a small estimate of the number of those affected and their significance as a public health problem. Medeiros (2022) reported that the current literature and available information on the burden of filarial morbidity and the implementation of structured services concerning morbidity assistance in the Americas were all found to be scarce. Now that this knowledge gap has been identified, both health services and researchers need to seek the implementation and enhancement of the maintenance of strategies that relate to the morbidity pillar (Medeiros et al., 2022)

Information and data on LF in Libya are scarce, known risk factors for LF include exposure to mosquitoes (Senkwe et al., 2022), these are compounded by an increased likelihood of extreme climatic events such as floods, high temperatures, and moisture conditions related to the spread of LF, moreover South Libya very vulnerable to the transmission of clustering immigrants that are come aggregated from endemic countries, which may be carriers of the infectious. And anecdotal information suggests that LF may be endemic in Libya. Although these factors are related to the spread of LF in Libya, WHO reports through existing data indicate LF is non-endemic in Libya. The actual observations and LF prevalence across the country remain unknown.

Conclusions

In this review, we sought to analyze publications related to the handling of lymphatic filariasis and also aimed to tentative explanations of the problems associated with nationwide lymphatic filariasis and its repercussions in Libya. In Libya be estimate of the number of patients with lymphatic filariasis is needed to help plan and estimate the number of patients with LF, this information must be available in the health information system. This information may already be available in the health information system, or it may have to be collected through various patient estimation surveys, this information is useful for designing management, and further studies are needed to better assess the rates of prevalence and implement control programs recommended.

Libya requires concerted efforts and effective policy to interrupt LF transmission with a focus on cross-border coordination and synchronization of LF preventive and control interventions against illegal immigration from sub-Saharan countries as these countries endemic with lymphatic filariasis and establish a public health database.

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(i.e. critical assessments of research on topics of relevance to health problems and health sciences). These should contain sections dealing with objectives, sources, methods of selection, compilation and interpretation of data and conclusions. The text should not exceed 25 page (excluding the accompanying abstract, references, tables and figures), and should be accompanied by an abstract of not more than 250 words. The number of tables and figures should not exceed 5

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Only reports of cases of an unusual nature are considered for publication. Text should include an Introduction, the Report of the case(s) and a Discussion. The text should not exceed 1500 words and the number of references kept to a minimum. The abstract should not exceed 150 words.

Reports

Manuscript specifications (length, references, tables/ figures) are the same as a research article, but abstract length should not exceed 150 words.

Short research communications

Articles which do not constitute a complete research study but are of particular relevance or importance to health issues in the region may be considered for publication. The text should not exceed 1500 words (excluding references), and should be accompanied by a structured abstract of not more than 150 words. The number of tables and figures should not exceed 3.

Commentaries

Manuscript specifications (references, tables/figures) are the same as a short research communication, but maximum length is 1000 words. The abstract (unstructured) for submissions purposes should not exceed 150 words.

Letter to Editor

Should be no more than 400 words, no more than 4 references, no more than one table or figure and no more than 4 authors.

Academic activities

Journal of contemporary medical sciences to health Sciences publishes short reports of academic activities (seminars, conferences and workshops), the report should include; a brief description of the activity, it's main objectives, summary of the important papers presented and the main recommendations of that activity.

Units and measurements

Give measurement of length, height and weight in metric units. Give all hematological and clinical chemicals in SI units; equivalent values in traditional units could be used with or without the SI units.

Abbreviations and Symbols

Use only standard abbreviations. Avoid abbreviations in title and abstract. The full term for which abbreviation stands should precedes its first use in the text.

Ethics

In experiment on human subjects should follow the guidelines of Ethics Committee (country or institution). The manuscript should include a statement confirming that an informed consent was obtained from each subject.

Research Paper should be sent in the form of a Microsoft word to the following email

j-medical@democraticac.de

Democratic Arabic Center in Berlin – Germany