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Original Article

Estimated serum Interleukin-2 levels in beta-thalassemia patients infected with Hepatitis B and C viruses

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ABSTRACT

Beta thalassemia is the most common inheritable disease worldwide. Thalassemia patients are more susceptible to blood-borne viral infections, such as hepatitis B (HBV) and hepatitis C (HCV), because of the frequency of blood transfusions. This study aimed to detect HBV and HCV infections among patients with beta-thalassemia and estimate interleukin-2 (IL-2) levels in the serum of infected patients compared with a control group. The study was conducted on a total of 151 patients (83 males and 68 females). HBs antigen and anti-HCV antibody tests were performed for the detection of HBV and HCV infections using enzyme-linked immunosorbent assays (ELISA). Of the 151 thalassemia patients, 12 (7.9%) had HCV (10 males [6.6%] and 2 females [1.3%]), and none of them was HBsAg positive. There were significant increases in IL-2 levels (Mean 159.33 pg/ml) in HCV-infected thalassemia patients compared with the control group (38.67 pg/ml), ($P < 0.05$). A statistically significant relationship was found between HCV infection and the frequency of transfusions and splenectomy in patients with thalassemia ($P < 0.05$). No statistically significant relationship was found between HCV infection and either type of beta-thalassemia or residence ($P > 0.05$). The current study demonstrated that there were significant increases in serum IL-2 levels in patients with thalassemia infected with HCV compared with the healthy control group. There were statistically significant relationships between transfusion frequency, splenectomy, and HCV infection in thalassemia patients. The types of beta-thalassemia and residency were not statistically significantly related to HCV infection in thalassemia patients.

Keywords: *Beta-thalassemia, HBV, HCV, IL-2*

Introduction

Thalassemia is a group of genetic diseases categorized as alpha-thalassemia and beta-thalassemia [1]. Beta thalassemia is a prevalent global genetic condition that follows an autosomal recessive pattern. It is characterized by a deficiency or absence of the beta-globin chain [2]. Thalassemia is highly prevalent in African, Mediterranean, and Southeast Asian populations. A globin variant is present in 5% of the global population, whereas 1.7% exhibit either an alpha-thalassemia or beta-thalassemia trait, according to research. Thalassemia has an equal impact on males and females [3]. Thalassemia patients have been on the rise in various parts of the world,

such as North America, Northern Europe, and Northeast Asia, primarily because of an increase in immigrant populations. Nevertheless, there is a dearth of thorough knowledge regarding the epidemiological characteristics of thalassemia in these areas. Accurate assessment of the frequency and occurrence rates of thalassemia is essential for effective patient care, advancement of prenatal diagnostic methods, and efficient allocation of healthcare resources [4]. The worldwide occurrence of babies with symptomatic thalassemia, such as HbBart hydrops fetalis, HbH disease, β-thalassemia major, and β-thalassemia/HbE disease, is 0.46 per 1,000 births. The most severe type of thalassemia is HbBart hydrop fetalis, which results in the death of the fetus in the uterus. As a result, most people worldwide with severe thalassemia have either β-thalassemia or β-thalassemia/HbE homozygosity. Approximately 40,618 infants per year are predicted to have β-thalassemia, with 25,511 (62.8%) experiencing severe anemia and requiring blood transfusions. However, only 11.7% of patients who require blood transfusion can really receive the necessary treatment [5] . Hepatitis B virus (HBV) and hepatitis C virus (HCV) are significant viruses that affect a significant percentage of people globally [6]. The World Health Organization (WHO) estimates that the global prevalence of HBV is 3.84%, which corresponds to 295.9 million individuals. In addition, chronic HCV infection affects 57.8 million patients globally, with a prevalence of 0.75 percent [7]. Both HBV and HCV are blood-borne pathogens that can be transmitted through many methods, including blood transfusions, injections, surgical tools, dental procedures, piercings, tattoos, toothbrushes, razors, pedicure devices, manicure devices, and other invasive practices [6]. Moreover, it has been shown that both viruses can be passed from an infected mother to her newborn, a phenomenon referred to as vertical transmission [8,9] . Additionally, both viruses can also be spread through sexual intercourse [10] . Both Hepatitis B virus (HBV) and Hepatitis C virus (HCV) result in liver damage, resulting in the development of chronic liver disease, liver cirrhosis, hepatocellular carcinoma (HCC), end-stage liver disease, and ultimately, death. In addition, they are the most prevalent viruses transmitted by blood transfusions [11,12]. Regular blood transfusions, however, save the lives of patients with thalassemia, but they also increase the risk of contracting blood-borne viruses such as HBV and HCV [13] . Interleukin-2 (IL-2) is a cytokine that promotes the growth and division of T cells and is necessary for the development of effector and memory T cells. It was the first cytokine to be molecularly cloned [14]. Furthermore, IL-2 has the capacity to regulate natural killer cells (NK), maintaining their ability to kill and promoting the proliferation of B cells. IL-2 has also been shown to reduce the pathogenicity of viruses [15].

Materials & Methods

A cross-sectional study was conducted on diagnosed cases of beta-thalassemia patients who received regular blood transfusions as part of their treatment. The study was conducted from October 2023 to March 2024 in the Thalassemia Management Center of Al-Hadbaa Specialized Hospital for Blood Diseases and Bone Marrow Transplantation in Mosul city, Iraq. The study population comprised 151 beta-thalassemia patients, including 83 male and 68 female beta-thalassemia patients, ranging in age from 1 to 41 years, who received regular blood transfusions. The control group, which was matched with the patients, consisted of 30 apparently healthy individuals of nearly identical age and gender who exhibited no obvious disease. Appropriate official agreements were obtained from the Nineveh Health Directorate prior to initiating the study, as documented by letter No. 40289 dated October 8, 2023.

Sample collection

A five ml syringe was used to collect five milliliters of blood via vein puncture. Blood samples were placed in gel tubes and allowed to clot for 30 min at 37°C, then subjected to centrifugation for 15 min at a speed of 4000 revolutions per minute. The sera were extracted and placed into Eppendorf tubes, which were subsequently stored in a freezer at 20°C for late serological testing using ready-made analysis kits from the manufacturer for detection of HBs Ag, specific HCV antibodies, and human IL-2. Additionally, a researcher-designed questionnaire was administered to the patients; the questionnaire included many variables, some of which were: frequency of transfusions, type of beta-thalassemia, place of residence, and whether they had undergone splenectomy.

Biomarker Analyses

A. HBs-antigen detection

An ELISA kit from *(Qingdao Hightop Biotech Co., China)* used the double antibody sandwich ELISA principle. A microplate is coated with purified hepatitis B surface antibody (HBsAb). The HBsAg in the sample will first combine with the HBsAb and then with the enzyme-labeled HBsAb complex. If the result is positive, the microplate will turn dark blue. No color or very light blue indicate a negative result. Following the addition of the stop solution, which turns yellow when the reaction is stopped, the intensity of the reaction is determined photometrically. The kit was designed to detect hepatitis B surface antigen (HBsAg) in human serum or plasma.

B. Anti-HCV antibody detection

Following the manufacturer's guidelines, serum samples were collected from all participants using an ELISA kit *(Bioneovan Co., Ltd., Beijing)* to determine the presence of anti-HCV antibodies. The microtiter wells contained several HCV protein epitopes (Core, NS3, NS4, and NS5) bound to them. HCV antibodies attach to the solid phase and interact with recombinant proteins when present in the test sample. Antibodies that do not react are eliminated by the wash buffer. Anti-human IgG peroxidase conjugate reacts with human IgG bound to the antigen, and the result is visible using a chromogenic substrate. Medium to dark blue is the result of a positive sample. Either no color or a very light blue indicates a negative result. Following the addition of the stop solution, which turns yellow when the reaction is stopped, the intensity of the reaction is determined photometrically.

C. Estimation of human IL-2 levels

The method used in the ELISA kit from *(SunLong Biotech Co.,LTD, China)* is Sandwich-ELISA. The kit's Microelisa stipulate is pre-coated with an IL-2-specific antibody. The appropriate Microelisa stipulate wells are filled with standards or samples, which are then mixed with the designated antibody. Subsequently, each Microelisa strip plate was thoroughly coated with an IL-2-specific horseradish peroxidase (HRP)-conjugated antibody and allowed to incubate. Free components are washed away. TMB substrate solution is added to each well. Wells containing interleukin-2 (IL-2) and horseradish peroxidase (HRP)-conjugated IL-2 antibody will exhibit a blue color, which will change to yellow when stop solution is added. The spectrophotometric determination of the optical density (OD) was performed at a wavelength of 450 nm. The OD value is directly proportional to the IL-2 concentration.

Results & Discussion

The results indicated 12 patients (7.9%) were positive for anti-HCV antibodies, while 139 patients (92.1%) were negative. In addition, all the thalassemia patients under study were negative for HBsAg (Table 1). This may be due to a success-based vaccine management system for patients, in which all patients under study were vaccinated against HBV.

Table 1. Detection of HBV and HCV infection among thalassemia patients and control group.

Tests		Thalassemia patients N=151(100%)		Control group N = 30 (100%)		P-values
		Frequency	Percentage	Frequency	Percentage	
Anti-HCV Ab (ELISA)	Positive	12	7.9%	0	0%	0.000*
	Negative	139	92.1%	0	0%	
HBs Ag (ELISA)		0	0%	0	0%	

**P<0.05= Significant*

Hepatitis C virus (HCV) infection is a major cause of liver diseases and is considered a global health concern [16]. The current study found that only 12 (7.9%) of 151 thalassemia patients were anti-HCV positive. The study done in Mosul City by Mohammed *et al.* reported that the anti-HCV positives were only 17% of the 200 thalassemia patients [17]. The study performed in Kirkuk City by Jamal *et al.* reported that 33.5% of the 254 major thalassemia patients had anti-HCV positivity [18]. A systematic review performed by Zainab *et al.* showed that the pooled HCV prevalence in beta thalassemia major in Iraq was 20.49% [19]. The results of the present study are nearly in agreement with the data recorded by previous Iraqi studies. The HCV-infected thalassemia patients had the highest mean IL-2 level (159.33 pg/ml) compared with the control group (38.67 pg/ml) (Table 2). These results were statistically significant, as indicated by a P-values = 0.000.

Table 2. Estimation of IL-2 levels in the serum of HCV-infected thalassemia patients.

Levels of IL-2 (pg/ml)	Patients with HCV infection N=12	Control N= 30	P-values
Mean± Standard deviation	159.33±86.12	38.67±3.99	0.000*

**P<0.05= Significant*

Interleukin-2 (IL-2) is one of the first cytokines discovered and stimulates lymphocyte proliferation. This cytokine is primarily produced by activated T cells and serves as an autocrine and paracrine growth factor for both T cells and natural killer cells (NK). IL-2 plays a

significant role in immunity [20]. The present study revealed that HCV-infected patients had a significantly higher mean ± standard deviation (SD) level of IL-2 (159.33 ± 86.12 pg/ml) compared to the healthy controls (38.67 ± 3.99 pg/ml). The study by Riyad *et al.* in Thi Qar city demonstrated that there was a statistically significant increase in the concentration of IL-2 observed in patient samples infected with HCV, as opposed to in the healthy group [21] confirming the present study. In addition, the study by Al-Ma'amouri *et al.* reported that HCV patients and health controls had different levels of IL-2, but these differences were not considered statistically significant [22], which was in disagreement with the present study.

Table 3 shows the association between HCV infection and blood transfusion frequency among thalassemia patients. The results indicate a statistically significant relationship (p=0.01) between transfusion frequency and HCV positivity. Specifically, a higher proportion of HCV-positive patients (3.3%) received blood transfusions every 2 weeks compared to HCV-negative patients (16.5%). Similarly, a lower proportion of HCV-positive patients received less frequent transfusions (every 3-6 weeks) compared to HCV-negative patients.

Table 3. Association between HCV infection and blood transfusion frequency among thalassemia patients.

		Transfusion frequency				P-value
		Every 2 weeks	Every 3 weeks	Every 4 weeks	Every 6 weeks	
HCV (Anti_HCV Ab)	Positive	5 (3.3%)	3 (2.0%)	3 (2.0%)	1 (0.6%)	0.01*
	Negative	25 (16.5%)	93 (61.6%)	18 (12%)	3 (1.9%)	
Total (151;100%)		30 (19.8%)	96 (63.6%)	21 (14%)	4 (2.6%)	

**P<0.05= Significant*

Thalassemia patients require regular blood transfusions to remain alive. Multitransfusion is associated with an increased risk of transfusion-transmitted infections (TTIs) [23]. The findings from the current study are consistent with previous research that has reported a significant association (p=0.01) between transfusion frequency and HCV infection in patients with thalassemia. It has been reported that the relationship was significant (P ≤ 0.01) between monthly blood transfusion frequency and anti-HCV [24]. The prevalence of HCV was noticeably higher in patients who received blood transfusions more frequently [25].

Table 4 shows the association between HCV infection and splenectomy among thalassemia patients. The results indicate a statistically significant relationship (p=0.001) between splenectomy and HCV positivity. Specifically, a higher proportion of HCV-positive patients (5.3%) had splenectomy compared to non-splenectomized HCV-positive patients (2.6%).

Table 4. Association between HCV infection and splenectomy among thalassemia patients.

		Splenectomy		Total	P-values
		Yes	No		
HCV (Anti-HCV Ab)	Positive	8 (5.3%)	4 (2.6%)	12 (7.9%)	0.001*
	Negative	31(20.5%)	108 (71.5%)	139 (92.1%)	
Total		39 (25.8%)	112 (74.2%)	151 (100%)	

**P<0.05= Significant.*

The findings from the current study are consistent with previous research that has reported a significant association between splenectomy and HCV infection in patients with thalassemia. A highly significant (P ≤ 0.01) relationship between splenectomy and anti-HCV positivity has been indicated [24], and most patients with viral hepatitis C-positive status had a monthly blood transfusion and splenomegaly; 5 of 12 (20.7%) had been splenectomized [26]. In Egypt, Amal *et al.* showed that thalassemic patients with HCV had splenomegaly. There was a statistically significant difference (P = 0.001) [27].

Table 5 shows the association between HCV infection and the type of beta-thalassemia among thalassemia patients. The results indicate no statistically significant relationship (p=0.061) between the type of beta-thalassemia and HCV positivity. Specifically, all HCV-infected thalassemia patients 12 (7.9%) had a major type of beta-thalassemia.

Table 5. Association between HCV infection and type of beta-thalassemia.

	The type of beta- thalassemia		Total	P-values
	Major	Intermedia		

HCV (Anti-HCV Ab)	**Positive**	12 (7.9%)	0 (0%)	12 (7.9%)	
	Negative	107 (71.1%)	32 (21%)	139 (92.1%)	0.061**
Total		119 (79%)	32 (21%)	151 (100%)	

***P>0.05= Not significant.*

A major type of beta-thalassemia (homozygous state) is a significant form of the condition and is marked by profound anemia and mortality before the age of 3 years. Nevertheless, the life expectancies of thalassemic patients have been extended because of the accessibility of regular blood transfusions. The blood transfusion protocol for individuals with beta-thalassemia major is employed to manage anemia and enhance their overall well-being. However, this treatment carries adverse consequences and heightens the susceptibility to transfusion-transmitted infections (TTIs), specifically hepatitis B and hepatitis C viruses (HBV and HCV)[28]. This study revealed that all HCV-infected thalassemia patients had a major type of beta-thalassemia. This is similar to the study by Mostafa *et al.* in Iran, which showed that patients with major thalassemia had three times the risk of HCV infection compared with patients with intermediate thalassemia [29] , but different from a previous study by Tariq *et al.* in Wassit City, which revealed that the highest percentage of intermediate thalassemia patients (17.8%) were positive for HCV antibody in comparison with 6.4 % in thalassemia major and positive for HCV antibody [30] .

Table 6 shows the association between HCV infection and residency among thalassemia patients. The results indicate no a statistically significant relationship (p=0.599) between residency and HCV positivity. Specifically, of the 12 (7.9%) HCV-infected thalassemia patients, 9 (6%) resided in urban areas and 3 (1.9%) were rural.

Table 6. Association between HCV infection and residency in thalassemia patients.

		Residence		Total	P-values
		Urban	**Rural**		
HCV (Anti-HCV Ab)	**Positive**	9 (6%)	3 (1.9%)	12 (7.9%)	
	Negative	94 (62.1%)	45 (30%)	139 (92.1%)	0.599**
Total		103 (68.1%)	48 (31.9%)	151 (100%)	

***P>0.05= Not significant.*

This study demonstrated that residencc is not a risk factor for HCV infection in patients with thalassemia. This finding was in agreement with data reported from a previous study by Hassan *et al.* in Najif city, which revealed that residency and seropositivity to anti-HCV do not directly correlate ($P \geq 0.05$) [24]. The study by Tariq *et al.* in Wassit City showed that HCV antibody seropositivity did not significantly correlate with residence [30], which is also in agreement with the present study.

Conclusion

This study found that thalassemia patients infected with the hepatitis C virus had significant increases in serum IL-2 levels compared to the control group. Frequent blood transfusions and splenectomy were significantly associated with HCV infection in patients with thalassemia. In contrast, the type of beta-thalassemia and places of residence were not associated with HCV infection. These results suggest that immunological factors, as indicated by increased IL-2, and certain clinical variables, namely transfusion frequency and splenectomy status, may be more important determinants of HCV susceptibility in thalassemia patients than genetic or environmental factors. Nonetheless, the findings underscore the importance of comprehensive monitoring of thalassemia patients, especially those with histories of repeated transfusions and splenectomy, to enable early detection and management of HCV infection and mitigate the substantial morbidity associated with this co-morbidity.

Conflict of Interest

The authors declare that there are no competing interests.

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References

[1] Yang T, Luo X, Liu Y, Lin M, Zhao Q, Zhang W, Chen Z, Dong M, Wang J, Wang Q, Zhang X. Next-generation sequencing analysis of the molecular spectrum of thalassemia in Southern Jiangxi, China. Human Genomics. 2023 Aug 17;17(1):77.

[2] AL O, EX F, RIMENTAL P, ICAL BM, IEN S. Revisiting Gene Therapy Approaches for Beta-Thalassemia. Journal of Experimental and Basic Medical Sciences. 2023;4(2):104-9.

[3] Ferih K, Elsayed B, Elshoeibi AM, Elsabagh AA, Elhadary M, Soliman A, Abdalgayoom M, Yassin M. Applications of Artificial Intelligence in Thalassemia: A Comprehensive Review. Diagnostics. 2023 Apr 26;13(9):1551.

[4] Lee JS, Rhee TM, Jeon K, Cho Y, Lee SW, Han KD, Seong MW, Park SS, Lee YK. Epidemiologic Trends of Thalassemia, 2006–2018: A Nationwide Population-Based Study. Journal of Clinical Medicine. 2022 Apr 20;11(9):2289.

[5] Chaichompoo P, Svasti S, Smith DR. The Roles of Mitophagy and Autophagy in Ineffective Erythropoiesis in β-Thalassemia. International Journal of Molecular Sciences. 2022 Sep 16;23(18):10811.

[6] Mahmood M, Aslam M, Zaman N, Parvaiz F, Muhammad A, Irshad N, Pervez K, Ahmad KS. Identification of HBV and HCV transmission in multi-transfused Thalassemia patients of Azad Jammu and Kashmir, Pakistan. medRxiv. 2022 Jan 25:2022-01.

[7] Cui F, Blach S, Mingiedi CM, Gonzalez MA, Alaama AS, Mozalevskis A, Séguy N, Rewari BB, Chan PL, Le LV, Doherty M. Global reporting of progress towards elimination of hepatitis B and hepatitis C. The Lancet Gastroenterology & Hepatology. 2023 Apr 1;8(4):332-42.

[8] di Filippo Villa D, Navas MC. Vertical Transmission of Hepatitis B Virus—An Update. Microorganisms. 2023 Apr 27;11(5):1140.

[9] Ades AE, Gordon F, Scott K, Collins IJ, Claire T, Pembrey L, Chappell E, Mariné-Barjoan E, Butler K, Indolfi G, Gibb DM. Overall vertical transmission of Hepatitis C Virus, transmission net of clearance, and timing of transmission. Clinical Infectious Diseases. 2023 Mar 1;76(5):905-12.

[10] Javed H, Bano A, Fatima W, Khan R, Akhtar A. Sexually transmitted infections and associated risk factors among the transgender population of Pakistan. BMC Infectious Diseases. 2023 Sep 19;23(1):618.

[11] Ornos ED, Murillo KJ, Ong JP. Liver Diseases: perspective from the Philippines. Annals of Hepatology. 2023 Mar 6:101085.

[12] Sabir N, Ghafoor T, Fatima S, Lodhi R, Mehmood A, Zaman G. Prevalence and Association of Transfusion-Transmissible Infections with Age of Blood Donors: A Regional Transfusion Centre Study in Northern Pakistan. Journal of the College of Physicians and Surgeons--Pakistan: JCPSP. 2023 Sep 1;33(9):978-82.

[13] Nsekuye O, Uwayo HD, Simbi CM, Habtu M, Ntaganira J. Risk factors of transfusion transmissible infections among blood donors at Karongi Regional Centre for blood transfusion in Rwanda. Rwanda Journal of Medicine and Health Sciences. 2023 Aug 10;6(2):143-53.

[14] Wheeler BD, Gagnon JD, Zhu WS, Muñoz-Sandoval P, Wong SK, Simeonov DR, Li Z, Debarge R, Spitzer MH, Marson A, Ansel KM. The lncRNA Malat1 inhibits miR-15/16 to enhance cytotoxic T cell activation and memory cell formation. bioRxiv. 2023 Apr 17.

[15] Zhao K, Li X, Lei B, Han Y, An T, Zhang W, Zhang H, Li B, Yuan W. Recombinant porcine Interferon-α and Interleukin-2 fusion protein (rPoIFNα+ IL-2) shows potent anti-pseudorabies virus activity in vitro and in vivo. Veterinary Microbiology. 2023 Apr 1;279:109678.

[16] Liu CH, Kao JH. Acute hepatitis C virus infection: clinical update and remaining challenges. Clinical and Molecular Hepatology. 2023 Feb 20.

[17] Khalid MD, Abdullah BA. Prevalence of anti-HCV antibodies among thalassemia patients in Mosul City, Iraq. Journal of Life Sciences. 2012 May 1;6(5).

[18] Qassim Welli J, Najii Agha M, Saleem Mohammed M. Transfusion-Related Infections in Thalassemia Major Patients: Kirkuk Thalassemia Center Experience. Kirkuk Journal of Medical Sciences. 2021 Sep 30;3(1):22-8.

[19] Aljumaili Z. Hepatitis C Virus Prevalence among Patients with Beta Thalassemia Major in Iraq: A Systematic Review.: HCV in Thalassemia Major. International Journal Of Medical Sciences. 2021;4(3):6-16.

[20] Damoiseaux J. The IL-2–IL-2 receptor pathway in health and disease: The role of the soluble IL-2 receptor. Clinical Immunology. 2020 Sep 1;218:108515.

[21] Abed RE, Salman AN, Issa AH, Al-Salih M. Evaluation of the level of IL-2 in the HCV patients in the Thi Qar Province Southern Iraq. InAIP Conference Proceedings 2023 Sep 13 (Vol. 2845, No. 1). AIP Publishing.

[22] Al-Ma'amouri MY, Abdul Kareem MK. Analysis of some cytokines in patients infected with hepatitis c virus. Biochemical & Cellular Archives. 2021 Oct 1;21(2).

[23] Şahinoğlu EP, Karakoyun M. Evaluation of Transfusion-Related Infections in Patients with Beta Thalassemia Major in Southeast Turkey. Journal of Pediatric Academy. 2023 Dec 1;4(4).

[24] Abd HM, Atiyah MN, Faisal MM, Nasrawi AJ, Al-faydawi AH. Factors Affecting the Transmission Hepatitis C Among the Thalassemic Patients in Al-Najaf Al-Ashraf governorate. Kufa Medical Journal. 2022 Jun 15;18(1):24-34.

[25] Syed FN, Kashif N, Khan MR, Memon FA, Jabbar MZ, Palleti SK. Hepatitis C Virus Infection in Patients with Beta Thalassemia after Multiple Transfusions at a Tertiary Care Hospital. Pakistan Journal of Medical & Health Sciences. 2023 Feb 26;17(01):363-.

[26] Al-Sharifi LM, Murtadha J, Shahad A, Mohammed Y, Sura J, Waleed Z, Raheeq M, Sura A, Ehab H, Shahad M, Abbas Q. Prevalence of hepatitis B and C in thalassemic patients and its relation with type of thalassemia, frequency of blood transfusion, and spleen status. Medical Journal of Babylon. 2019 Apr 1;16(2):108-11.

[27] Mohamed AO, Hany AM, Fayed HM, Ahmed SA. Frequency and risk factors for Hepatitis C virus seropositivity in blood transfusion-dependent thalassemic patients in Qena hospitals. SVU-International Journal of Medical Sciences. 2023 Jul 1;6(2):489-500.

[28] Waheed U, Saba N, Wazeer A, Ahmed S. A systematic review and meta-analysis on the epidemiology of hepatitis B and hepatitis C virus among beta-thalassemia major patients in Pakistan. Journal of Laboratory Physicians. 2021 Sep;13(03):270-6.

[29] Sadeghi M, Soltani M, Etemad K, Abdollahi M, Sayyadi M, Barzegar M, Salehnasab C, Rahmatinejad Z, Rezaei M, Valadbeigi T, Hajipour M. The prevalence of anti HCV infection and its related factors in patients with Beta-Thalassemia in Shiraz-Iran. Pharmacophores. 2018 Jan 1;9(1):80-4.

[30] Muslim TM. Epidemiologic study of hepatitis b and c virus among thalassemia patient in wassit governorate/iraq. Journal of Techniques. 2014;27(2).