ISSN 2664-4169 (Print) & ISSN 2664-7923 (Online)
South Asian Research Journal of Biology and Applied Biosciences
Abbreviated Key Title: South Asian Res J Bio Appl Biosci

| Volume-5 | Issue-4 | Jul-Aug -2023 | **DOI: 10.36346/sarjbab.2023.v05i04.003**

Original Research Article

Prevalence of Hepatitis B Virus among Renal Dialysis Patients in Diyala Province, Iraq

Zainab Shawqi Ali[1*], Ansam Dawod Salman[1], Iman Abbas Ali[1]

[1]Assistant Professor Dr. in Department of Biology, Collage of Sciences, Diyala University, Diyala, Iraq

***Corresponding Author:** Zainab Shawqi Ali
Assistant Professor Dr. in Department of Biology, Collage of Sciences, Diyala University, Diyala, Iraq

Article History
Received: 28.05.2023
Accepted: 01.07.2023
Published: 11.07.2023

Abstract: ***Background*:** One of the main public health challenges in the globe is the prevalence of the hepatitis B virus (HBV). ***Objectives*:** The curent study aimed to determine the prevalence of hepatitis B virus in renal dialysis patients in Diyala province, Iraq. ***Methods*:** Ninety of renal dialysis patients attended Ibn-Sina Dialysis Center were screened for HBsAg, HBc IgG, HBc IgM and HBe Ab using ELISA technique, and then positive results were retested by conventional PCR for detection of *HBs* gene and *HB core* gene. Demographic data of study population were recorded. Simple statistical analysis was done using SPSS Version 25. ***Results*:** Among studied samples, the seroprevalence of HBsAg**,** Anti-HBc Ab, Anti-HBc IgM and Anti-HBe Ab were 4.4%, 17.8%, 2.2% and 5.6% respectively. The results of conventional PCR showed that only 1(1.1%) of *HBs* gene at 417 bp in renal dialysis patients were positive. Whereas, the *HBc* gene at 791 bp was not detected in all included study population. ***Conclusion*:** The prevalence of HBV was relatively high among renal dialysis in Diyala province/Iraq.

Keywords: Renal dialysis, HBV, HBs Ag, ELISA, PCR and Diyala province.

INTRODUCTION

Viral hepatitis is a systemic sickness particularly involving the liver [1, 2]. Hepatitis B virus (HBV) is a particle portion double-stranded DNA virus, a species about the square Orthohepadnavirus yet a quantity about the Hepadnaviridae family. It is the motives touching passionate yet persistent HBV contamination international [3].

HBV was the one of the common type in viral hepatitis or the fully form causing continual hepatitis because of anybody a note was once available. HBV intent acute and power hepatitis frequently growth of consequence along everlasting job governance and Hepatocellular carcinoma (HCC) [4]. HBV ancient in imitation of remain transmitted via capacity about contact which includes gore afterwards organism fluids over an infected people [5].

Hepatitis B virus infection constitutes a fundamental pecuniary then populace fitness trouble all via the world, especially within the flourishing countries which consists of the Middle East, the place so much is responsible due to the fact enormous illness afterwards mortality but the enchantment on a continual service state, mettle cirrhosis in imitation of that total may also increase within imitation along hepatocellular carcinoma [6, 7]. A ball comparison concerning country-level incidence atop continual HBV contamination observed up to expectation the HBs Ag seroprevalence was once 3.61% global collectively along ultima auspicious endemicity among countries over African, West Pacific and South America areas [8].

Acute or chronic over HBV infections are a frequent health problem inside Iraq. In the pre-vaccine era, the prevalence regarding the HBs Ag used to be once 3-4 p.c amongst normal wholesome population, instituted Iraq amongst the global places involving intermediate region upstairs endemicity [9]. However, closing learning decided up to hope the countrywide prevalence atop HBsAg dropped under according to 0.6% afterward correlated positively which include youth [10]. In incomplete ignoble full-size populace study performed of Basrah, reportedly 2.3% gore donors had

serological proof for HBV infection; on hence 0.2% tested a exceptional end result because each anti-HBc antibody yet HBs Ag [11].

In Diyala province, the advance seroepidemiological discipline touching HBV infection amongst blood donors yet unstable agencies used to be once observed protecting the length beside 1989 afterward 2002. It back to keep placed up according to hope the chance about HBV positivity content among gore donors used to be 1.5%, then the superior feasible positivity dimensions was amongst hemophilia sufferers (42.5%) [12]. Previous study used to be received outdoors concerning in 2018 between Diyala priesthood used after lie reported the amount HBV high-quality used to be (5.12%) [13]. Another seroprevalence learning in accordance in imitation of explore the HBV infections within Diyala government whole through the duration by 2003 into consequence about 2008, executed that the occurrence regarding HBs Ag was once 3.9% [14].

HBV is vindicated among 4 fundamental serotypes (adr, adw, ayr, ayw) based on floor antigenic epitopes. Genotypes vary via the use of at least 8% respecting the adjunct below maintain amazing geographical distributions below that has been associated alongside anthropological history. Within genotypes subtypes bear been described: such fluctuate by means of capability on 4–8% over the genome. There are eighth acknowledged genotypes labeled A thru H [15].

There is no seasonal fashion because HBV infection but no excessive predilection due to the fact concerning any person dominance group, though at that place are specific high-risk organizations secure namely parenteral remedy abusers, institutionalized persons, fitness seriousness personnel, accomplish transfused patients, organ transplant patients, hemodialysis patients yet staff, surprisingly promiscuous persons, then current child toddlers nee among conformity together with mothers including hepatitis B [16, 17].

METHODS

Study population (Renal dialysis patients):

The present study was conducted in Diyala province for the period from 1/ 1 / 2019 to 30 / 12/ 2021. The current study included 90 patients of renal dialysis. The patients were attended Ibn-Sina Dialysis Center. Sexty of patients were males and 30 were females. The age range was <10 years to =>60 years. Special questionnaire was pre-constructed to collect information regarding age, sex, residence, education level and vaccinated with HBV vaccine. The information was collected through short personal interview with patients.

Blood samples collection:

Five milliliters of venous blood was collected from each participant using 5 milliliters sterile disposable plastic syringes after cleaning the area of aspiration by 70% ethyl alcohol. Serum samples were aspirated and poured in new plastic disposable tubes using 250 microliters automatic pipette and disposable tips. Serum samples were divided into aliquots 250 microliter each in Eepindorff plastic tubes. These tubes were arranged in a rack in upright position and kept at -20 C till use.

Detection of serological markers HBsAg, Anti-HBc Ab, Anti-HBc IgM and Anti-HBe Ab by ELISA test:

This test was performed using commercially available kit (Dia.PRO, Italy HBs Ag ELISA).

Nucleic Acid Extraction:

Genomic DNA was isolated from serum samples according to the protocol of QIAamp® MinElute® Virus Spin Kit.

Primer:

Sets of PCR primers for Surface (*S*) Hepatitis B virus genes and *HBcore* gene has been used in the conventional PCR amplification in order to get PCR products used in the sequencing method for genotyping of the virus and phylogenetic tree analysis [(18)]. These primers were used for positive samples detected by ELISA test for detected (HBs Ag and HBc Ab). Primers were provided by (Macrogen/ Korea) and sequences, table (1).

Table 1: Primers for detection of *HBV- core* gene and *HBV- S* gene

Primer Name	Seq.	Annealing Temp. (^{o}C)	Product size (bp)
HBV_CoreF1	5`-CAG GTC TTG CCC AAC GTC TTA-3`	56	976
HBV_CoreR1	5`-CTG TCA GAG GGC CCA CAT ATT -3`		
HBV_CoreF2	5`-GAC CGA CCT TGA GGC ATA TTT-3`	65	790
HBV_CoreR2	5`-TCC CAC CTT ATG AGT CCA AGG-3`		
HBV_SF	5`-CGTGGTGGACTTCTCTCAATTTTC-3`	56	417

HBV_SR	5`-GCCARGAGAAACGGRCTGAGGCCC-3`		

Assay Optimization:

After optimizing of primer, PCR detection HBV was performed. To determine the optimum annealing temperature, gradient PCR was set at 56°C and 65°C. The best condition for the virus was obtained and then samples along with negative (water) and positive controls (previously known PCR-positive samples of HBV) were amplified.

***HBs* gene and *HBcore* gene PCR detection:**

Molecular detection of HBV DNA was set as follows: initial activation at 95°C/5 minutes, 40 cycles at 94°C/30 seconds, 56°C/30 seconds, 72°C/30 seconds, and a final extension step of 72°C/10 minutes. Semi-nest PCR amplifications were performed similar to the first step with different reverse primers. Hepatitis B virus genotyping was carried out with the same conditions, but using other primer pairs which targeted the S gene on HBV DNA-positive samples.

All reactions were performed in duplicate and in the presence of negative and positive controls. The final products were detected by electrophoresis on 2% agarose gel and the size of the PCR products were estimated by the migration pattern of a 1000-bp DNA ladder.

Statistical analysis:

Analysis of data was carried out using the available statistical package of SPSS-25 (Statistical Packages for Social Sciences- version 25).

RESULTS

Renal dialysis group

Ninety patients under renal dialysis were enrolled. The majority of them were male with 60 years and older. All (100%) of them were vaccinated with HB vaccine, as shown in table (2).

Table 2: Variables of the renal dialysis group

Variables	No.	%
Age (Ys)		
<10 years	-	-
10---19	5	5.6
20---29	8	8.9
30---39	9	10.0
40---49	13	14.4
50---59	18	20.0
=>60 years	37	41.1
Gender		
Male	60	66.7
Female	30	33.3
Residence		
Urban	13	14.4
Rural	77	85.6
Level of education		
Illiterate	20	22.2
Primary	33	36.7
Intermediate	31	34.4
Secondary and above	6	6.7
Vaccination with HBV		
Yes	90	100
No	-	-

Distribution of serological markers according to study population

Table 3 revealed that the HBsAg positivity rate among renal dialysis were 4.4%. Regarding the anti-HBc IgG antibody, the results showed that the anti-HBc IgG Ab among renal dialysis patients was (17.8%).

The results of the anti-HBc IgM Ab revealed that the positivity rate among renal dialysis were 2.2%. The HBe IgG Ab positivity rate among renal dialysis patients were 5.6%.

Table 3: Distribution of study population according to serological markers

Education	Renal Dialysis
	No. (%)
HBsAg	
Positive	4 (4.4)
Negative	86 (95.6)
Anti-HBV core IgG	
Positive	16 (17.8)
Negative	74 (82.2)
Anti-HBV core IgM	
Positive	2 (2.2)
Negative	88 (97.8)
Anti-HBVe IgG	
Positive	5 (5.6)
Negative	85 (94.4)
Anti-HCV Ab	
Positive	29 (32.2)
Negative	61 (67.8)

Distribution of detected genes according to study population

Regarding the HBV, two genes were detected namely the *HBs* gene at 417 bp and *HBc* gene at 791bp among 45 patients with renal dialysis. The detection rate of *HBs* gene at 417 bp in renal dialysis patients was 1 (1.1%), while the gene was not detected in 44 (48.9%). Whereas, the *HBc* gene at 791 bp was not detected in all included study population, table 4.

Table 4: Distribution of detected genes according to study population

Education	Renal Dialysis	
	No.	**%**
***HBs* gene at 417bp**		
Detected	1	1.1
Not-detected	44	48.9
Not done	45	50.0
***HBcore* gene at 791bp**		
Detected	-	-
Not-detected	45	50.0
Not done	45	50.0

DISCUSSION

Virus infections transmitted through the blood (HBV and HCV) was showed as an important risk for patients and staff in rental dialysis units, blood center unit and blood bank, this due to multible blood transfusion and nosocomial transmission [19].

In the present study, HBs Ag was detected in 4.4% of renal dialysis patients. The present results were higher than that found in the previous study conducted in Baghdad to evaluate the prevalence of HBV among HD patients was (1.3%) [20]. While, other lonely previous study conducted in Duhok among hemodialysis patients to evaluate the prevalence of HBV was (3.2%) [21]. This result is also higher than other studies from surrounding countries was evaluated seroprevalence of HBs Ag among HD patients [22-24].

The current study found that the HBc IgM and HBc IgG positivity rate in renal dialysis patients 2.2%, 17.8% respectively, the present results were converging with other Iraqi result reported by [25] was reported a positivity rate for anti-HBc IgG 16.5% among renal dialysis patients in Nineveh government/Iraq. While, others Iraqi researchers were inconsistent with current results [26, 27] who reported 8.3%, 3.49% positivity rate of HBc IgG among renal dialysis patients. Nevertheless, worldwide studies have founded different anti-HBc Ab rate among renal dialysis patients; for instance, in Iran [28] reported an anti-HBc Ab rate 23.5%, while in Turkey, the anti-HBc Ab rate was 2% [29].

Regarding the prevalence of anti-HBe Ab, the present results was 5.6% among dialysis, these results which were disagreement to Iraqi study was conducted in Nineveh governorate / Iraq by [30] reported 32.07% among renal dialysis patients. Other Iraqi study was also conducted in Nineveh / Iraq by [31] reported (29.3%) anti-HBe Ab positive among

risky groups (renal dialysis patients, thalassemia patients and blood donors) and disagreement with the present results. Additionally, global studies reported results disagreement with present results in positivity rate of anti-HBe Ab among renal dialysis patients [32-34].

This difference between the current results in population of the study with different serological markers and other results perhaps due to other study were done on different age group (adult and children), and variation of the sensitivity of kits (ELISA) and its type of generation were used in detection of HBV in different studies. This difference may also be attributed to inadequate hematological investigations such as PCR for HBV mainly on blood and blood products as the mean number of blood transfusions in HBV infected group is significantly higher than the non-HBV group [35]. Renal dialysis patients have required long-term and multiple blood transfusions, this recurrent blood transfusion increases the risk of transmission of transfusion-related viruses – hepatitis B virus [36]. The low rate of different serological markers of HBV in renal dialysis patients can be due to the improvement of knowledge about HBV transmission routes, HBV vaccination among renal dialysis patients. Additionally, a good vaccination program may help to decrease the transmission of infection in these groups [37, 38].

The current study showed that the detection rate of *HBs* gene among renal dialysis was 1.1%, this result was in agreement with result of other study in Baghdad governorate reported 1.1% detection rate of *HBs* gene among renal dialysis [39]. Previous global studies reported different results in the detection rate of *HBs* gene in renal dialysis, for instance, in Iran [22] reported a detection rate 2.9%, while in Turkey [40] reported detection rate 38% and in US [41] 4% was the detection rate of *HBs* gene among renal dialysis. The *HBc* gene was not detected in all included study populations in the present study. Previous study was conducted in Iran reported similar result with the present result 0.0% detection rate of *HBc* gene in the renal dialysis patients [42] Since, other studies had reported differ results, so the present results were disagreement with some of these studies, like that conducted in Nineveh government/Iraq which reported a detection rate 13% of *HBc* gene among renal dialysis [25]. However, it was lower than that reported by other studies; for instance, in Alexandria, Egypt, HBc gene detected in 32% for renal dialysis patients [43]. It is important to mention that the *HBc* gene and antigen (protein) is remaining restricted in the hepatocytes of the infected liver and only its antibodies; the IgM and Total were appeared in the circulation [44]. So, the successful detection of *HBc* gene is solely depend on the type of specimen used. Therefore, the study suggests further molecular studies for detection of this gene using other specimens chiefly liver biopsy.

The disparity in the results of different studies may be occurred by following reasons: geographical distribution, spontaneous viral clearance and low levels of virus DNA in the Blood that is not determined by PCR and technical condition [45].

One of the limitation in viral detection by genetic amplification techniques is the false negative results, which may be the result of poor quality RNA / RNA extraction due to insufficient initial specimen quantity and degradation of the genetic material of the virus due to improper specimen storage or loss of it during extraction, the other explanation may be the presence of inhibitors of the enzyme mixture (reverse transcriptase and DNA polymerase) in the samples [46].

CONCLUSION

Hepatitis B virus prevalence in this study were relatively high among patients with renal dialysis in Diyala province, Iraq.

RECOMMENDATIONS

Thus, we recommend similar molecular and serological studies on detection and genotyping of HBV other risky groups in the community, since the HBc genes are restricted to the hepatocytes, further molecular studies on the detection of these genes in liver biopsies especially for the risky groups and a special molecular and serological study on detection and genotyping of HBV strains causing these mysterious conditions.

REFERENCES

1. Arie, R., Eugene, R. S., Howard, T. H., Stanley, L., & Arie, Z. (2005). Clinical Aspects of Viral Hepatitis. In: Viral Hepatitis. 3th Ed. 2005. Blackwell Publishing. pp 33-78.
2. Ifeanyi, O. E., Chinedum, O. K., Uzoma, O. G., Ndidiamaka, E. I. G., Stella, E. I., & Florence, O. (2018). A review on hepatitis and haemostasis. *Int J Compr Res Biol Sci*, *5*(2), 24-46.
3. Ryu, W. (2017). Molecular Virology of Human Pathogenic Viruses. Academic Press. pp. 247–260.
4. Seeger, C., & Mason, W. S. (2000). Hepatitis B virus biology. *Microbiology and Molecular Biology Reviews*, 64(1), 51–68.

5. Dieter, G., Nora, G., Chris, L., & Stefan, S. (2021). HBV evolution and genetic variability: Impact on prevention, treatment and development of antivirals. *Antiviral Researc*, 186, 104973.
6. Bineeta, K., Urvashi, T., & Anupam, P. (2018). Hepatitis B virus transmission and health care workers: Epidemiology, pathogenesis and diagnosis. *Indian Journal of Medical Specialities*, 9(1), 30-35.
7. Chang, M. H. (2007). Hepatitis B virus infection. *Seminars in Fetal Neonatal Medicine*, 12(3), 160-167.
8. Abiola, A. H. O., Agunbiade, A. B., Badmos, K. B., Lesi, A. O., Lawal, A. O., & Alli, Q. O. (2016). Prevalence of HBsAg, knowledge, and vaccination practice against viral hepatitis B infection among doctors and nurses in a secondary health care facility in Lagos state, South-western Nigeria. *Pan African Medical Journal*, *23*(1), 160.
9. Schweitzer, A., Horn, J., Mikolajczyk, R. T., Krause, G., & Ott, J. J. (2015). Estimations of worldwide prevalence of chronic hepatitis B virus infection: a systematic review of data published between 1965 and 2013. *The Lancet*, *386*(10003), 1546-1555.
10. Omer, A. R., & Al-Douri, S. (1984). Viral hepatitis in Iraqi normal population. In *Proceedings of the 6th international congress of virology, Sendai-Japan* (pp. 32-36).
11. Ataallah, T. M., Hanan, K. A., Maysoun, K. S., & Sadoon, A. A. (2011). Prevalence of hepatitis B and C among blood donors attending the National Blood Transfusion Center in Baghdad, Iraq from 2006-2009. *Saudi medical journal*, *32*(10), 1046-1050.
12. Al-Rubaye, A., Tariq, Z., & Alrubaiy, L. (2016). Prevalence of hepatitis B seromarkers and hepatitis C antibodies in blood donors in Basra, Iraq. *BMJ open gastroenterology*, *3*(1), e000067.
13. Shafiq, A. R., Omer, A. R., & Al-Mashhadani, J. I. (2006). Seroepidemiological study of hepatitis B virus infection among blood donors and risky groups in Diyala. *Iraqi Journal of Community Medicine*, *19*(1), 14-18.
14. Hasan, A. S., & Mustaf, M. M. (2018). The efficacy of hepatitis B vaccine among children in Baquba-Diyala province. *Iraqi Journal of Communication and Medicine*, 30(3 & 4), 176-181.
15. Al-Jebori, A. S., Hasan, A. S., & Al-Duliami, A. A. (2010). Seroprevalence of hepatitis B and hepatitis C virus infections in Diyala province during 2003-2008. *Diyala Journal for Pure Sciences*, 6(1), 292-302.
16. Olinger, C. M., Jutavijittum, P., Hübschen, J. M., Yousukh, A., Samountry, B., Thammavong, T., ... & Muller, C. P. (2008). Possible new hepatitis B virus genotype, southeast Asia. *Emerging infectious diseases*, *14*(11), 1777-1780.
17. Chen, Y., & Tian, Z. (2019). HBV-Induced Immune Imbalance in the Development of HCC. *Frontiers in Immunology*, 10, 2048.
18. Zhang, Z., Wang, C., Liu, Z., Zou G., Li, J., & Lu, M. (2019). Host Genetic Determinants of Hepatitis B Virus Infection. *Front generation*, 10, 696.
19. Tabasi, M., Makvandi, M., Teimoori, A., Dastoorpoor, M., Moradzadegan, H., Parsanahad, M., ... & Karami, C. (2018). Epidemiology and Molecular Detection of HAV, HBV, and HCV in Patients with Acute Hepatitis Symptoms in Ahvaz. *Jundishapur Journal of Microbiology*, *11*(5), e63317.
20. Elizabeth, G., Veena, R., Sam, D., Simon, L., Kay, T., & James, E. (2019). Clinical practice guideline management of blood borne viruses within the haemodialysis unit. *BMC Nepherology*, 20-399.
21. Nasir, N. A., & Abdilazeem, M. A. (2019). Prevalence of Viral Hepatitis B, C, and human immunodeficiency virus among end stage renal disease patients on hemodialysis. *Iraqi Postgraduate Medical Journal*, *18*(2), 79-86.
22. Nashwan, M. R., Zana, S. M., & Nawfal, R. H. (2017). The Prevalence of HIV, HCV, and HBV among Hemodialysis Patients Attending Duhok Hemodialysis Center. *International Journal of Infection*, 5(1), e63246.
23. Rastegarvand, N., Makvandi, M., Samarbafzadeh, A., Rasti, M., Neisi, N., Pouremamali, A., ... & Shabani, A. (2015). Molecular characterization of pre-core/core and s region of hepatitis b virus in hemodialysis patients with occult hepatitis b infection. *Jundishapur Journal of Microbiology*, *8*(10), e23686.
24. Anwar, K., Imran, M., Shahzad, F., Noreen, M., Atif, M., & Ahmad, F. (2016). Prevalence of Hepatitis B and Hepatitis C infection among patients undergoing dialysis. *J Hum Virol Retrovirol*, *3*(3), 94.
25. Kizilates, F., Berk, H., Coban, M., Seyman, D., Sarikaya, M., Sari, F., & Oztoprak, N. (2016). Seroprevalence of hepatitis B and C virus in patients who undergo hemodialysis in Antalya province, Turkey. *Asian Biomedicine*, *10*(4), 339-344.
26. Mohammed, D. K. (2014). Detection of Anti-HBc and HBV-DNA in Blood Donors Negative for Hepatitis B Virus Surface Antigen in Mosul Central Blood Bank-Iraq. *Iraqi Journal of Biotechnology*, 13(2), 119-127.
27. Ghusoon, S. H., & Mohammed, D. K. (2020). Prevalence of Hepatitis B Virus in Hemodialysis Patients Infected with Hepatitis C Virus in Mosul District / Iraq. *American Scientific Research Journal for Engineering, Technology, and Sciences*, 63(1), 1-6.
28. Saleem, Z. S. M., Naqid, I. A., Hussein, N. R., Mohammad, S. J., Noaman, J. S., Haji, R. S., ... & Mohammed, P. H. (2020). The prevalence of hepatitis B and C virus in patients with end-stage kidney disease on regular hemodialysis in Duhok, Iraq: A brief report. *Avicenna Journal of Clinical Microbiology and Infection*, *7*(1), 31-33.
29. Samadi, E., Mirshahabi, H., Motamed, N., & Sadeghi, H. (2020). Prevalence of occult hepatitis B virus infection in hemodialysis patients using nested PCR. *Reports of biochemistry & molecular biology*, *9*(1), 82-88.
30. Ramezani, A., Aghasadeghi, M. R., Ahmadi, F., Razeghi, E., Eslamifar, A., Banifazl, M., ... & Aghakhani, A. (2015). Isolated anti-hbc and occult HBV infection in dialysis patients. *Nephro-urology monthly*, *7*(1), e22674.

31. Salim, R. W., & Abdullah, B. A. (2014). The prevalence of hepatitis B virus in high risk groups in Nineveh governorate/Iraq. *Baghdad Science Journal*, *11*(2), 888-893.
32. Ergunay, K., Balaban, Y., Cosgun, E., Alp, A., Simsek, H., Sener, B., ... & Hascelik, G. (2015). Epidemiologic trends in HBV infections at a reference centre in Turkey: an 11-year retrospective analysis. *Annals of hepatology*, *11*(5), 672-678.
33. Sallam, T. A., El-Bingawi, H. M., Alzahrani, K. I., Alzahrani, B. H., & Alzahrani, A. A. (2020). Prevalence of hepatitis B and hepatitis C viral infections and impact of control program among blood donors in Al-Baha region, Saudi Arabia. *Prevalence*, *9*(1), 56-60.
34. Mansour-Ghanaei, F., Joukar, F., Naghipour, M., Hassanipour, S., Yeganeh, S., Sepehrimanesh, M., & Fathalipour, M. (2021). Epidemiologic profile of viral hepatitis B and C in North of Iran: results from PERSIAN Guilan Cohort Study (PGCS). *BMC Research Notes*, *14*, 1-7.
35. Telaku, S., Fejza, H., Elezi, Y., & Bicaj, T. (2009). Hepatitis B and C in dialysis units in Kosova. *Virology Journal*, *6*, 1-4.
36. Al-Sharifi, L. M., Murtadha, J., Shahad, A., Mohammed, Y., Sura, J., Waleed, Z., ... & Abbas, Q. (2019). Prevalence of hepatitis B and C in thalassemic patients and its relation with type of thalassemia, frequency of blood transfusion, and spleen status. *Med J Babylon*, *16*(2), 108-111.
37. Hussein, N. R., Saleema, Z. S., & Abd, Q. H. (2019). Direct acting antiviral treatment for patients with end-stage kidney disease with acute HCV infection. *Mediterranean journal of hematology and infectious diseases*, *11*(1), e2019034.
38. Sinjari, H. Y. A., & Bakr, K. A. (2018). Prevalence and risk factors of hepatitis b and c virus infections among patients undergoing hemodialysis in kurdistan, Iraq. *Hepatitis Monthly*, *18*(5), e11776.
39. Fahmi, N., Faruoq, Z., Hassan, W. F., & Ahmed, A. (2018). Prevalence of viral hepatitis (B and C) in pediatric hemodialysis centers in Baghdad. *Iraqi Postgraduate Medical Journal*, *17*(4), 366-372.
40. Sayan, M., & Dogan, C. (2012). Genotype/subgenotype distribution of hepatitis B virus among hemodialysis patients with chronical hepatitis B. *Annals of hepatology*, *11*(6), 849-854.
41. Apata, I. W., Nguyen, D. B., Khudyakov, Y., Mixson-Hayden, T., Rosenberg, J., Zahn, M., ... & Moorman, A. C. (2019). Hepatitis B virus mutant infections in hemodialysis patients: a case series. *Kidney Medicine*, *1*(6), 347-353.
42. Hatatian, F., Babakhani, F., Gudarzi, H., Momenifar, N., Norouzi, M., Shafieifar, M., ... & Yaghoobi, M. H. (2020). Relative frequency of blood-borne viruses in hemodialysis-dependent and kidney transplant recipients in iran. *Iranian Journal of Public Health*, *49*(11), 2136-2143.
43. Helaly, G. F., El Ghazzawi, E. F., Shawky, S. M., & Farag, F. M. (2015). Occult hepatitis B virus infection among chronic hemodialysis patients in Alexandria, Egypt. *Journal of infection and public health*, *8*(6), 562-569.
44. Martinez, M. C., Kok, C. C., Baleriola, C., Robertson, P., & Rawlinson, W. D. (2015). Investigation of occult hepatitis B virus infection in anti-hbc positive patients from a liver clinic. *PLoS one*, *10*(3), e0117275.
45. Khalid, M. D., & Abdullah, B. A. (2012). Hepatitis C virus genotypes in Iraq. *Iraqi Journal of Biotechnology*, *11*(2), 475-480.
46. Kouni, S., Karakitsos, P., Chranioti, A., Theodoridou, M., Chrousos, G., & Michos, A. (2013). Evaluation of viral co-infections in hospitalized and non-hospitalized children with respiratory infections using microarrays. *Clinical Microbiology and Infection*, *19*(8), 772-777.