Viral Load of HBV and HCV Correlation to Torque Teno Virus (TTV) Co-Infection in Iraqi Patients

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Abstract

Background: While hepatitis viruses A–E are established, emerging evidence points to additional, novel viral hepatitis agents. The torqueteno virus (TTV) has garnered interest due to its prevalence among patients with hepatitis, suggesting potential hepatotropism. **Aim:** This study was conducted to detect TTV antigens in individuals infected with chronic hepatitis B (HBV) and/or C (HCV) using molecular diagnostics and to explore any associations between TTV presence and demographic characteristics of the cohort. **Materials and Methods:** The current study was conducted from the period of September 2022 to April 2023, the investigation involved Iraqi patients aged between 32 and 83 years and diagnosed with HBV and HCV in National Iraqi Central Laboratories, Virology Section. We employed real-time PCR to quantify HBV and HCV viral loads and conventional PCR for TTV DNA detection. **Results:** TTV DNA was detected in 17 of the 60 patients (28.3%) with chronic HBV and HCV infections. Viral load distribution was found to be similar for both HBV and HCV. An age-related increase in viral load was noted, suggesting a trend where older individuals exhibited higher concentrations. However, no significant variation was observed that could link alterations in HBV and HCV viral loads to TTV status directly. Statistical analysis indicated a positive correlation between age and the likelihood of presenting with the virus, aligning with observed trends in other age-associated chronic conditions, such as Type 2 diabetes mellitus and chronic kidney disease. **Conclusion:** The presence of TTV in patients with enduring HBV or HCV infection could implicate the virus as a contributing factor to hepatitis pathology. Studies such as this are pivotal for elucidating the age-associated risk profiles for various pathologies and shaping corresponding public health policies and preventative approaches.

Keywords: HBC, HCV, molecular diagnostics, TTV, viral load

Introduction

Torque teno virus (TTV), classified within the Anellovirus genus of the Circoviridae family and possesses a circular DNA genome of approximately 3.8 kilobases that is, single-stranded. The absence of a suitable culture system for TTV remains a challenge. Despite the detection of TTV in over 90% of the global adult population, its pathogenic potential in humans remains unverified. Notably, elevated viral loads of TTV have been noted in individuals with lupus, malignancies, and severe idiopathic inflammatory myopathies, findings that are intriguing and warrant further investigation. High TTV levels have also been reported in infants with acute respiratory illnesses. Research is underway to evaluate TTV's utility as an immunological marker in patients with inflammatory diseases and immunological deficiencies. Given its omnipresence and immune system-regulated replication, TTV is often studied in the context of human virology.[1] TTV load in plasma is now being recognized as a potential indicator of an individual's immune suppression. This association is underscored by numerous studies that have observed a correlation between heightened TTV levels and an increased risk of infectious diseases, as well as a reduced risk of organ rejection posttransplantation, making TTV quantification a promising tool in transplant medicine.[2]

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As a member of the transfusion-transmitted (TT) viruses and originally identified in the blood,[1,2] TTV's transmission routes have expanded in understanding to include parenteral,[3,4] sexual,[5,6] and vertical (mother to child) transmissions,[7,8] according to recent studies. The virus's role in autoimmune reactions is an emerging theory still under investigation.[9] The heterogeneity of TTV genotypes presents a substantial challenge in the development of universal PCR primers, complicating the understanding of TTV distribution among humans despite over a decade of research.[10]

Cervical cancer, with its significant global burden and high prevalence in low-income countries, has demonstrated a higher TTV presence in high-risk human papillomavirus infections, highlighting the importance of continued cancer screening programs.[11,12] The implication of TTV in a variety of diseases, including respiratory and oncological conditions, is an area of ongoing research.[13]

The global health impact of hepatitis B virus (HBV) and hepatitis C virus (HCV) infections, with hundreds of millions affected, is substantial.[14] Although HBV and HCV share hepatotropic characteristics, their replication cycles differ significantly, potentially influencing co-infected cells' viral expression and serologic patterns. In HBV-HCV co-infections, the clinical progression and risk of hepatocellular carcinoma are enhanced. Treatment guidelines specific to this co-infection are lacking, as typically one virus is dominant in co-infected individuals, repressing the replication of the other, or in some cases, both viruses replicate concurrently.[14]

Contrary to the presence of both viral nucleic acids in the serum during co-infection, not every hepatocyte is co-infected. In patients with chronic HCV and occult HBV infection, the distribution of infected cells is variable. The implications of these findings, however, are constrained by the small study scale and the inclusion of individuals with occult HBV infection only.[15] Despite its status as a leading cause of cancer mortality, the relationship between non-viral factors and viruses such as hepatitis G virus and TTV in liver cancer remains under-explored, with TTV suggested as a potential risk factor for hepatocellular carcinoma in patients with HCV-related liver disorders.[16] TTV infection rates are remarkably high in individuals with liver disease, HIV, intravenous drug users, thalassemia patients, and those undergoing hemodialysis. Initial studies have associated TTV with liver disease indicators such as alanine transaminase (ALT) levels, and liver tissue TTV DNA levels exceed those in serum, pointing to the liver as a replication site for the virus. The prevalence of TTV viremia in Qatar spans across healthy individuals and those with HBV or HCV infections.[17,18]

This investigation seeks to ascertain the prevalence and distribution of TTV viremia in the context of concurrent hepatitis B and C virus infections.

Materials and Methods

Sixty blood specimens of Iraqi patients detected to be infected with HBV and HCV in National Iraqi Central Laboratories, Virology section, were included in this study. They were collected between September 2022 to April 2023. Their age ranges between 32 and 83, 30 sample was taken from female and 30 from male patients. A total of 5 mL of venous blood for serum separation was withdrawn, viral load was detected for the genetic material of HBV and HCV by real-time PCR, and then the detection of TTV DNA was done by using conventional PCR test.

Ethical approval

The study was conducted in accordance to with the ethical principles practical and the laboratory work was accomplished in private research laboratories. This study was approved by the Ethics Committee, Department of Biology and according to the document number CSEC/0124/0010 on Jan 24, 2024.

HBV and TTV DNA extraction

DNA was extracted from patients plasma using ZR Viral DNA Kit,

ZYMO/USA and the procedure was done according to the kit instructions.

Real-time quantitation of hepatitis B virus

Viral load was detected using HBV Real-TM Quant kit. The PCR was done according to the kit instructions.

Extraction of HCV RNA by using Ribo virus (K-2/C)

Extraction of HCV RNA by using Ribo Virus (K-2/C) and the extraction was done according to the kit directions.

HCV viral load determination by Real-time PCR

Viral load was detected using Kit of HCV Real-TM Quant and the test was done according to the kit instructions.

Amplification of the TTV-specific gene

The primers were lyophilized, they dissolved in the free DdH_2O to give a final concentration of 100 pmol/μL as stock solution and keep a stock at −20 to prepare 10 pmol/μL concentration as work primer suspended, 10 μL of the stock solution in 90 μL of the free DdH_2O water to reach a final volume 100 μL, was investigated by IDT (Integrated DNA Technologies company, Canada) [Tables 1 and 2].

The thermalcycler was programed, initiation denaturation at 95°C for 3.00 min, denaturation at 95°C for 00:45 min, then annealing and synthesis at 58°C for 00:20 min (40 cycles), extension at 72°C for 7.00 min.

Results

This analysis [Tables 3–5] displays the findings of a study that examined the connection, between the presence of TTV and HCV status with the severity of diseases. The severity of diseases was categorized in a manner, where higher categories indicate greater severity.

The estimate for TTV predictor is 1.263 with an error (SE) of 0.519. The z value, which indicates how many standard errors away the estimate is from 0 measures at 2.432. This suggests that the estimate is 2.432 times higher than the error itself. Moreover, the *P* value associated with TTV is 0.015, which's below the alpha level of 0.05. This implies that there is a association between the presence of TTV and an increased severity of chronic diseases within the considered ordered categories.

On another note in terms of HCV results (versus negative) its estimate stands at 0.315 alongside an error of 0.465. The corresponding z value is calculated as 0.679, which falls within one error away from zero.

Furthermore, its *P* value equates to 0.497—higher than 0.05—which indicates no association between HCV status and the severity of chronic diseases, within this sample [Figures 1 and 2].

Table 1: The primers used for TTV detection

Primer	Sequence	Tm (°C)	GC (%)	Product size
Forward	5′-GTGGAACGTGGTTGCACAAA-3′	59.9	60	1308 bp
Reverse	5′-ACAGTTGGGAAGCATCTGGG-3′	56.4	50	

Table 2: The Results of the patients in this study. Gender distribution of responses and chi-square analysis of independence between gender and response category

Gender	Stat	Negative	Positive	Chi-square	*P* Value
Male	Observed	17	13	0.066	0.793
	% within column	48.6%	52.0%		
Female	Observed	18	12		
	% within column	51.4%	48.0%		

Table 3: Statistical summary of age in relation to negative and positive response groups

Group descriptive	Group	*N*	Mean	Median	SE	*P* value
Age	Negative	35	58.3	59	2.84	0.695
	Positive	25	56.6	55	2.93	

Distribution of HBV viral load

This chart depicts the distribution of Hepatitis B Virus (HBV) viral load among the individuals in the dataset [Figure 3].

This study shows that the distribution of HBV is similar to HCV, the distribution of HBV viral load is right-skewed, indicating that a majority of individuals have lower values, while a few have very high viral loads. The skewness and the presence of a long tail suggest that while the condition is generally less severe among most individuals, there are exceptions with extreme cases. This distribution can be crucial for understanding the epidemiology of HBV and for planning treatment strategies [Figure 4].

The distribution is fairly uniform but shows a slight skew to the right, indicating a modest concentration of older individuals. Age distribution is a fundamental

Table 4: Comparison of HCV test results across various chronic diseases

Chronic diseases	Stat	HCV result		*P* value
		Negative	Positive	
Chronic kidney disease	Observed	6	4	0.522
	% within column	17.1%	16.0%	
Hepatocellular carcinoma (Liver cancer)	Observed	6	2	
	% within column	17.1%	8.0%	
Chronic kidney disease	Observed	5	2	
	% within column	14.3%	8.0%	
Type 2 diabetes mellitus	Observed	3	5	
	% within column	8.6%	20.0%	
Essential hypertension	Observed	5	4	
	% within column	14.3%	16.0%	
Fatty liver disease (nonalcoholic steatohepatitis, NASH)	Observed	6	2	
	% within column	17.1%	8.0%	
Cardiovascular disease	Observed	4	6	
	% within column	11.4%	24.0%	

demographic characteristic and can be important for various analyses, including the prevalence of diseases and healthcare planning.

The relationships between viral loads, TTV status, and chronic diseases

The results [Figure 5] show the relationship between HCV (Hepatitis C Virus) viral load and TTV status. The aim is to see if there's a notable difference in HCV viral load among individuals with and without TTV.

Table 5: Ordinal logistic regression analysis of TTV presence and HCV status on severity of chronic diseases

Predictor	Estimate	SE	Z	p
TTV	1.263	0.519	2.432	0.015
HCV result				
positive–negative	0.315	0.465	0.679	0.497

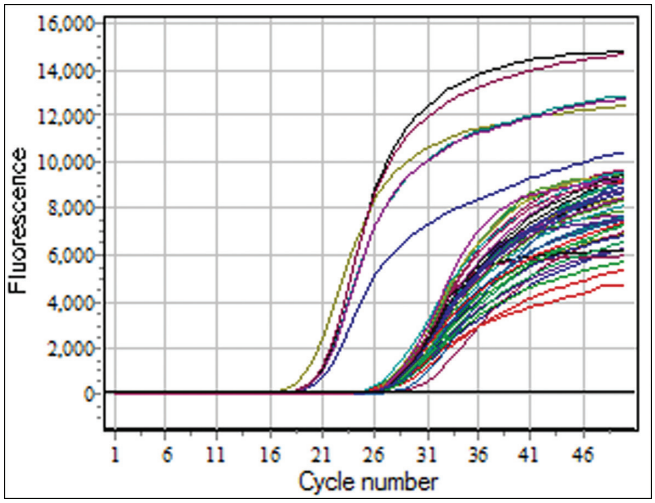

Figure 1: Results of HCV amplification curves

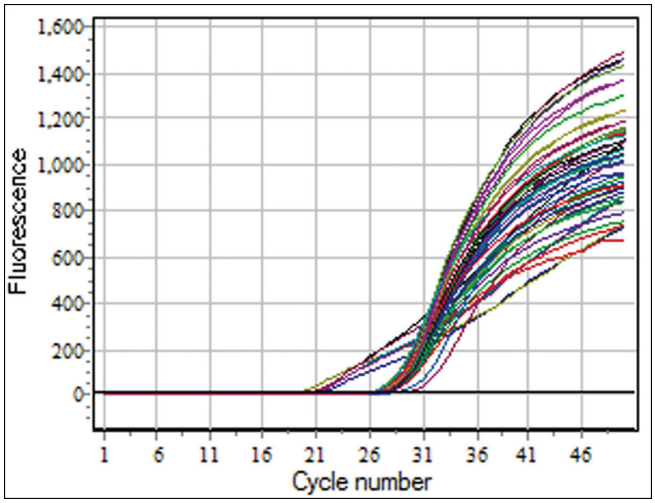

Figure 2: Results of HBV amplification curves

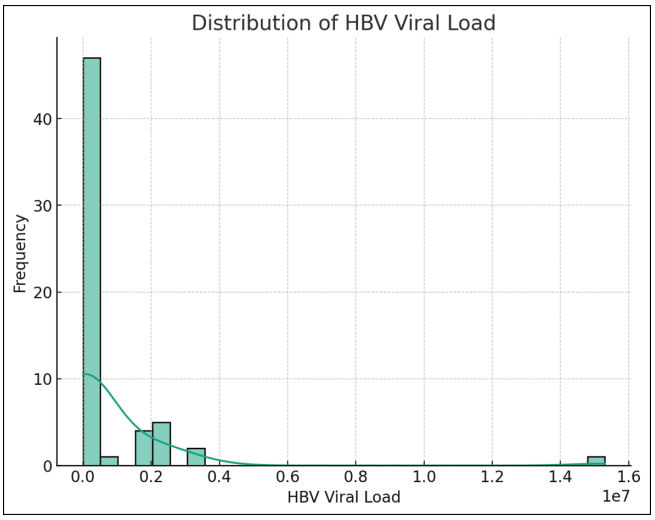

Figure 3: Histogram of HBV viral load distribution

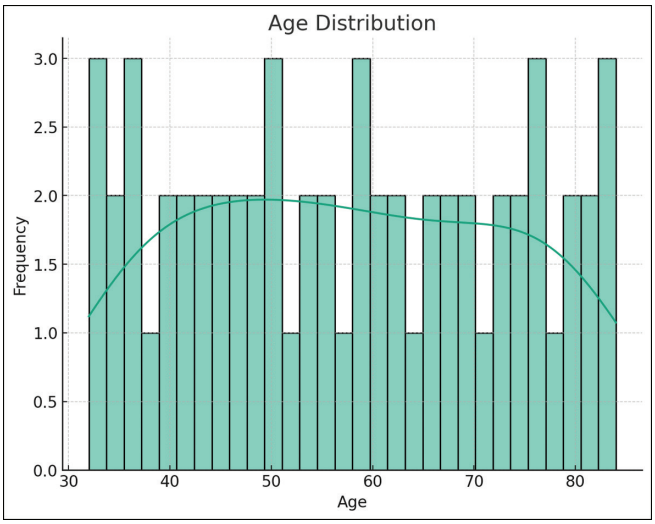

Figure 4: Histogram of age distribution

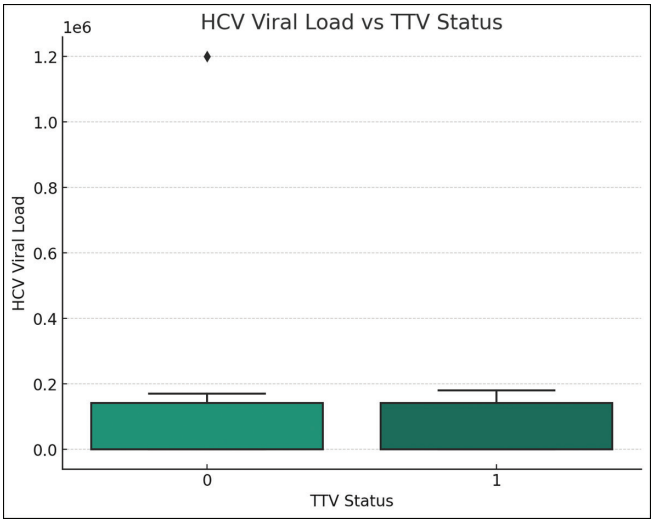

Figure 5 : Boxplot of HCV viral load vs. TTV status

The boxplot compares the HCV viral load distributions between individuals with TTV (TTV = 1) and those without TTV (TTV = 0) meaning that both groups show a similar range of HCV viral load values. The median viral load appears comparable in both groups. There is no significant differences observable, suggesting that TTV status might not be strongly associated with variations in HCV viral load.

HBV viral load vs. TTV status

In Figure 6, this chart looks at the relationship between HBV (Hepatitis B Virus) viral load and TTV status. The goal is to determine if TTV status influences HBV viral load. This boxplot compares the distributions of HBV viral load for individuals with and without TTV. Similar to the HCV analysis, both groups (TTV = 1 and TTV = 0) show overlapping ranges of HBV viral load values. The medians are also similar, indicating no marked difference in HBV viral load based on TTV status. These observations suggest that the presence of TTV may not have a substantial impact on HBV viral load.

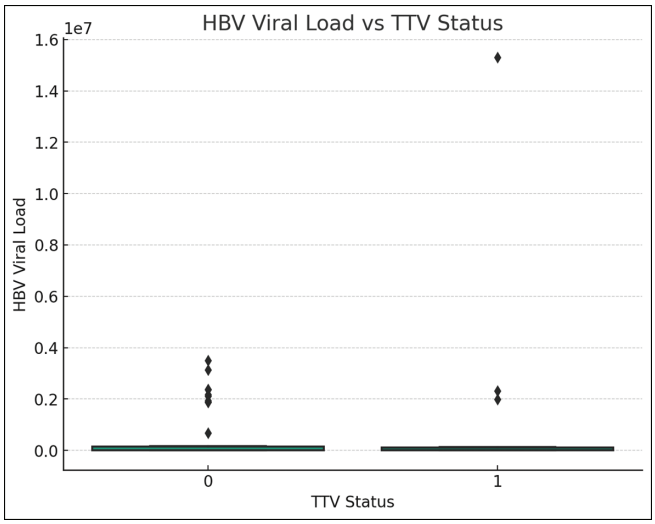

Figure 6: Boxplot of HBV viral load vs. TTV status

Prevalence of chronic diseases

In Figure 7, this chart displays the prevalence of various chronic diseases within the datasets. It helps in understanding which chronic conditions are more common among the individuals studied. The bar plot illustrates the number of cases for each chronic disease in the dataset. Conditions like essential hypertension, cardiovascular disease, and chronic kidney disease are more prevalent compared to others such as hepatocellular carcinoma (liver cancer) or type 2 diabetes mellitus (DM). Understanding the prevalence of these diseases can help in medical research, resource allocation.[19,20]

Correlation between age and chronic diseases

The results [Figure 8] show that there is positive values indicate a positive correlation, meaning that as age increases; the likelihood of having the disease also increases. Some chronic diseases show a positive correlation with age, suggesting their prevalence may rise with increasing age. This type of analysis is crucial in understanding the age-related risk factors for different diseases, which can inform healthcare strategies and preventive measures.

In Figure 9, viral load of HCV shows that there is several individuals have significantly higher HCV viral loads compared to the rest.

HBV viral load

In Figure 10, the distribution of HBV viral load values is similar to the HCV, some values extend to the most extreme values within 1.5 times the IQR, indicating the presence of outliers. These outliers represent individuals with exceptionally high HBV viral loads. Identifying outliers is important as they can represent severe cases. In medical contexts, understanding the range and outliers in viral loads can inform diagnostic and treatment approaches.

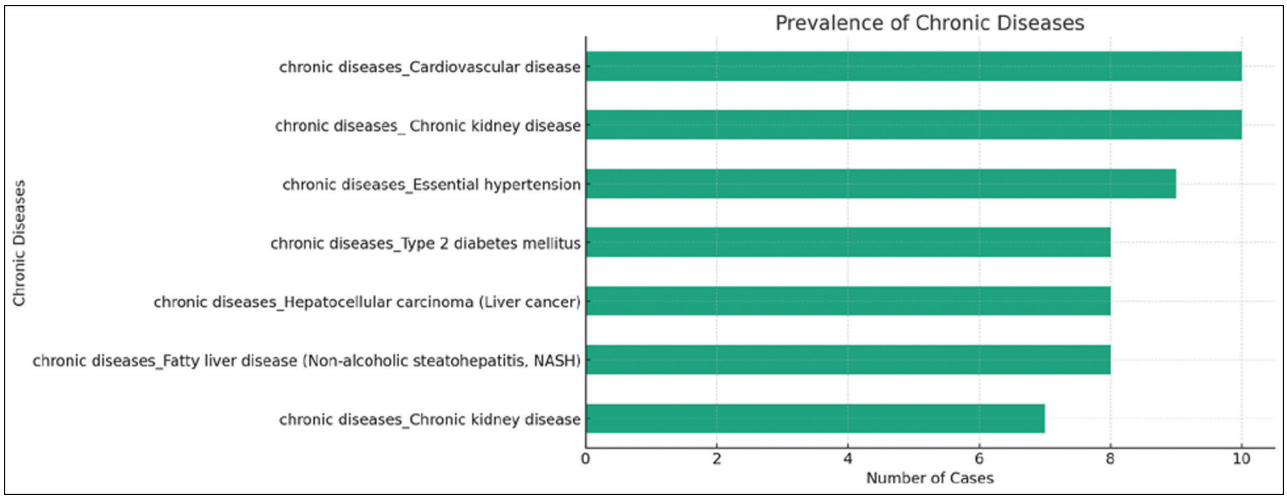

Figure 7: Bar plot of the prevalence of chronic diseases

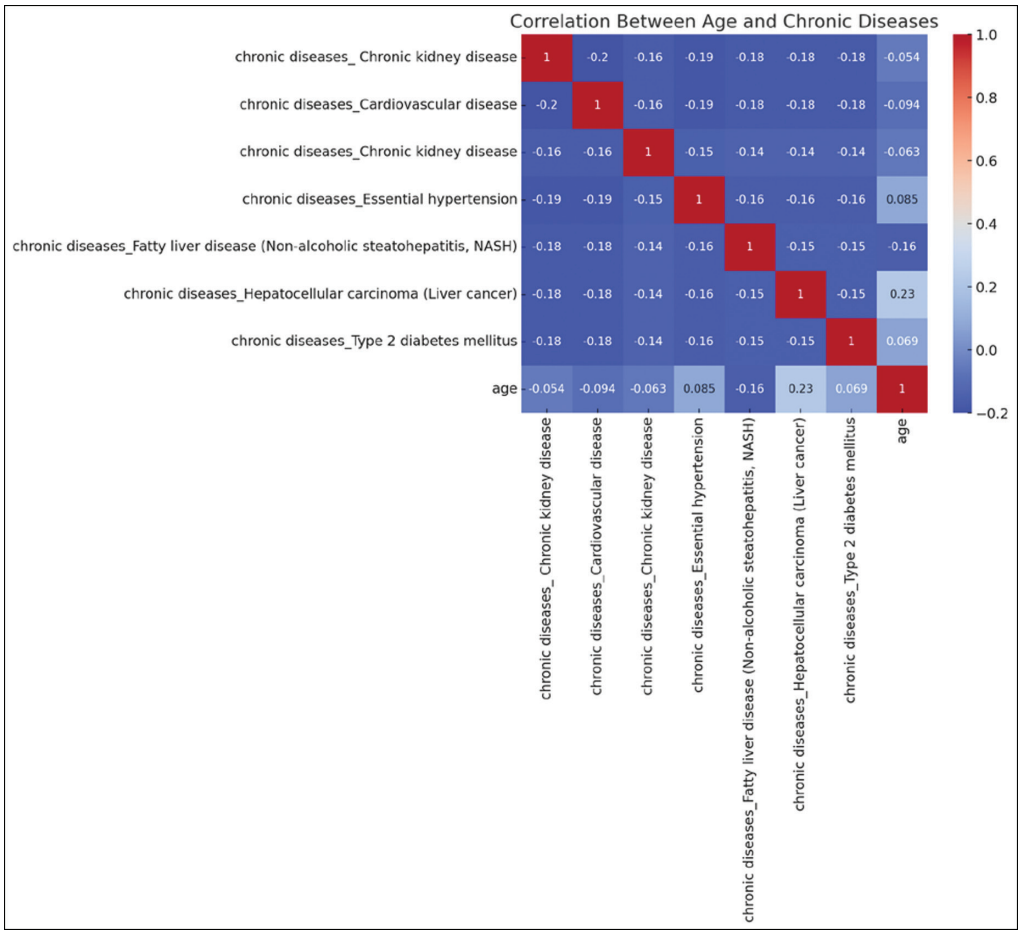

Figure 8: Heatmap of correlation between age and chronic diseases

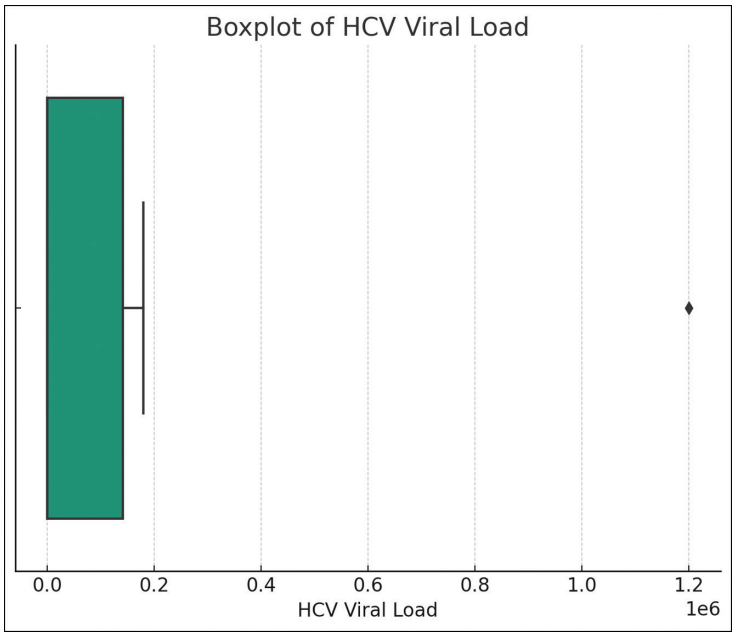

Figure 9: Boxplot of HCV viral load

Discussion

Hepatitis B and C are common viral infections in Iraq.[21] Within the show ponder including 60 people with HBV and HCV disease pointed to clarify the affiliation of TTV with HBV and HCV diseases. In 2016 the WHO set the objective to significantly dispose of HBV and HCV

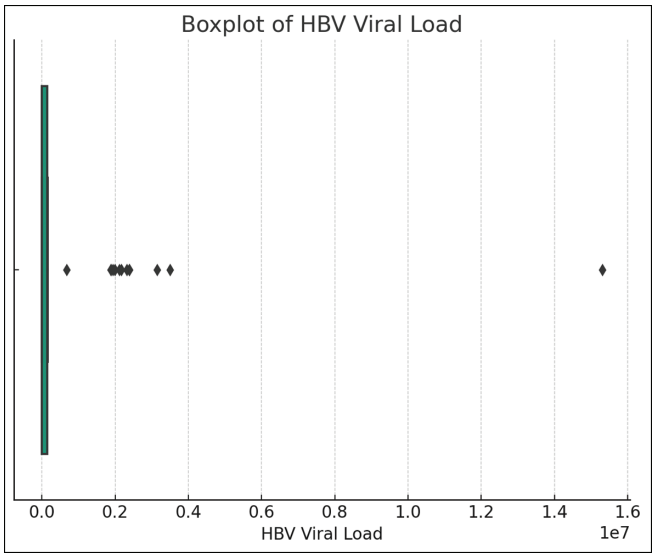

Figure 10: Boxplot of HBV viral load

diseases and their related mortality by 2030, and most nations got to be a portion of the challenge. However, the objectives may not be accomplished in a few nations since there's small financing accessible for these infections at a worldwide level, in expansion to a need of devoted hepatitis budgets or programs in numerous nations, particularly those with restricted assets.[22,23] In expansion, a need of solid epidemiological information can block accomplishing the objective.[22,24] As already expressed, the lion's share of HCV contaminations are asymptomatic for a long time; hence, numerous people are ignorant that they are tainted and don't look for screening and treatment.[25]HBV and HCV infections may be a serious health issue. Many men, 40–80%, are constantly infected with HBV or HCV. Subsequent data show that about 350 million are chronically contaminated with HBV and 200 million with HCV.[26] In later a long time, increasingly information has been created in creating nations and high/intermediate endemic zones where the foremost common course of contamination is still vertical transmission from mother to child, and level transmission between children, especially kin.[27] According to historical information from 2006 to 2009, hepatitis B and C contamination is mu predominant in Baghdad, the capital of Iraq.[28] A detailed comparison can be made in Basra. HBV and HVC are predominant in Erbil.[29] The HBV DNA evaluation is utilized to start and screen antiviral treatment.[30] The GeneXpert HBV-VL test, which has the potential for near-point atomic treatment testing, appeared fabulous execution and demonstrated to be a solid instrument for measurement of HBV DNA.[31] The inquire about found a seroprevalence rate of 13.3% of HBeAg among imminent asymptomatic blood benefactors.[32] For the most part, viral diseases can have an extraordinary part within the pathogenesis of DM. The precise component of HBV and HCV within the pathogenesis of DM isn't well caught on. A few consider found an affiliation between affront resistance and parenteral viral hepatitis, extraordinarily more conspicuous with HCV infection.[33] Patients with DM have the next chance of securing hepatitis B and C infections since they are more uncovered to restorative intercessions and are regularly hospitalized.[34] Hazard variables such as blood transfusions, dental mediations, hemodialysis, liquor utilization, etc., are specifically involved to an expanding predominance of HBV and HCV contaminations in diabetic patients.[35,36] Episodes of HBV diseases were detailed among DM patients who have had shared blood glucose gadgets. This might be connected to the moo level of instruction with respect to tall hazard of hepatitis B and C viruses' transmission amid finger adhere testing.[37]

Hepatitis B infection contamination was moreover a common well-being issue influencing patients on hemodialysis; be that as it may, a awesome decrease within the frequency rate of HBV in those patients has been taken note over that last decades. This lessening within the rate of HBV has been attributed to inoculation, the utilize of erythropoietin rather than visit blood transfusion for treatment of frailty and screening of blood benefactors. By the by, the chance of procuring HBV by hemodialysis patients is still tall since of a few hazard variables such as shared hemodialysis devices, increased presentation to blood and blood items, immunodeficiency state and visit skin breaching. Undoubtedly, intense disease with HBV in dialysis patients is ordinarily mellow and regularly asymptomatic; in any case, noteworthy extent of patients may advance to carrier state or unremitting hepatic malady, expanded chance of cirrhosis, and indeed liver cancer.[38] Here in Mid-Euphrates locale of Iraq, there are restricted assets with regard to hemodialysis unit in Adiwaniayh Instructing Healing Center at Adiwaniayh territory. The number of hemodialysis gadgets and related types of gear is constrained and inoculation against HBV is regularly inaccessible. For these reasons, we anticipate to discover a generally tall predominance rate of HBV and HCV contamination in Iraqi patients on hemodialysis. In this manner, the current ponder was arranged and carried out pointing at investigating the predominance rates of both HBV and HCV in patients and hemodialysis.[39] In one study, 89.2% (33 of 37) of the patients with HBV positive and 30.8% (4 of 13) of the patients with HCV positive had TTV, compared to 23.3% of the patients (10 out of 43) with healthy blood. This observation shows that compared to HCV patients and healthy blood donors, TTV prevalence is typically higher in patients with HBV infection. In terms of risk factors, it was discovered that TTV inspiration and catastrophic ALT levels had a significant genuine association, but TTV closeness was not connected to AST, gender, age interactions, or a history of blood transfusions, surgery, or ink. TTV Ag recurrence rates were often greater in HBV patients compared to HCV patients and solid blood donors, and this difference

may be significant in the hepatitis. The identification of TTV in blood donors raises the possibility that these illnesses can spread through methods other than blood transfusions.[40,41]

Conclusion

This study indicates that HBV and HCV remain significant public health challenges in Iraq, compounded by the potential co-infection with TTV. The study underscores the need for enhanced public health strategies, better resource allocation, and increased awareness to tackle these infections effectively. Further research is warranted to explore the implications of TTV infection with HBV and HCV to develop comprehensive approaches to mitigate these viral infections' impact on public health in Iraq.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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