

Pathological and Diagnostic Implications of Hepatitis C Viral Load and Torque Teno Virus ORF1 Gene Variability

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ABSTRACT

Background: The management of liver disorders, many of which are attributed to Hepatitis C Virus (HCV) infection, has been complicated by co-infections such as Torque Teno Virus (TTV). This study explores the impact of HCV and TTV co-infection, focusing on TTV's genetic variability in the ORF1 gene and its implications for disease progression and treatment outcomes. **Methods:** This study analyzed blood and serum samples from sixty individuals across two medical institutions. HCV viral loads were quantified using real-time PCR, while TTV genetic diversity was evaluated through sequencing of the ORF1 gene. **Results:** No significant correlations were found between age or gender and the presence of TTV or HCV. A minor negative association was observed between TTV positivity and chronic renal disease. Notably, HCV viral loads varied across the study cohort. Sequencing of TTV ORF1 revealed high genetic similarity with specific mutations, suggesting possible viral adaptations. These findings highlight the complex interaction between HCV and TTV in co-infected patients and underscore the potential influence of TTV ORF1 genetic variability on the clinical management of HCV. **Conclusion:** The genetic diversity observed in TTV ORF1 and its interaction with HCV underscores the need for comprehensive diagnostic strategies that consider co-infection dynamics in liver disease management. Future research should focus on expanding sample sizes and diversifying participant demographics to better understand the clinical implications of TTV ORF1 variability and inform more effective treatment protocols for HCV.

Keywords: Hepatitis C Virus, Torque Teno Virus, Viral Load, Genetic Variability, Coinfection Dynamics.

INTRODUCTION

The Hepatitis C virus (HCV) is responsible, for liver diseases such, as cirrhosis and hepatocellular carcinoma which're health concerns worldwide [1]. Hepatitis C virus (HCV) has a material composed of RNA. It is part of the Flaviviridae family. Exhibits characteristics that can result in long term infections [2]. Since many conditions may develop silently early detection and treatment play a role [3]. The emergence of acting antivirals (DAAs) has brought about a revolution, in the treatment of Hepatitis C virus (HCV) leading to substantial improvements, in patient outcomes (Adil et al., 2021). However challenges still exist in managing HCV infection due to factors such as genotypes, viral resistance and the risk of reinfection [4]. It crucial to keep track of the load in order to effectively manage HCV. By doing we can gain insights, into the effectiveness of treatments and make informed decisions, about therapy [5]. This aspect emphasizes the significance of having protocols that can effectively manage HCV for both developed and developing nations [6]. HCV differs from TTV that it lacks a membrane. Which is one of the Anelloviridae family. And it contain double stranded circular DNA [7, 8]. Despite being a prevalent infection source, we don't fully understand its effects on the body, and heath care takers are unsure of its effects [9, 10]. Researchers studying virus evolution and health effects face uncertainty about the infection's effects. Researchers typically detect the virus using PCR. This approach targets viral genome ORF1 [11]. Another challenge for researchers is to be perusing the coinfection between HCV and TTV [12, 13]. And this coinfection is crucial to understanding disease development for the treatment options considerations [14]. There are several studies proved that the treatment of the disease can be highly complicated by the presence of the co-infection with those two viruses [15]. Studying the coinfection of HCV and TTV exceeding the virology field since more health implications can occur and mainly the liver damage [16, 17]. Understanding epidemiology and dynamics of

transition for those viruses is essential to prevent disease [18]. Moreover, the recent techniques in biological field improved our understanding of TTV and HCV infections, which helps manage them [19]. Cost is one of the main concerns that it should be taken during examining coinfection with these viruses [20, 21]. In this study the HCV viral loads were the main focus in order to understand the disease's severity, development, and behavior, and explore the viral load associated with TTV coinfection and the link between those parameters and other chronic diseases. As we embark on this project one of our goals is to sequence the ORF1 gene of Torque Teno Virus (TTV) in samples. The ORF1 gene has a diversity that can provide insights into the specific virus we are studying. Our objective is to deepen our understanding of TTV behavior and progression and how these traits may impact the bodys defense system, against this virus. By examining the differences, in TTV levels among individuals who're co infected our goal is to identify factors that may play a significant role in understanding its potential impact on human health. Moreover, this research aims to investigate the interactions between individuals who have both TTV and HCV infections. This study could provide insights into how these viruses interact in terms of their progression, life cycle and overall impact on an individual's wellbeing. Gaining an understanding of co infection dynamics is crucial for developing treatment and care strategies, for patients dealing with multiple infections.

Methods

Samples Collection

In this study we collected serum and blood samples, from a group of 60 individuals. These participants were carefully selected from two medical institutions in Iraq; Baghdad Medical City and Kadhimiya

Educational Hospital. We chose these locations because they provide access to a range of patients allowing us to obtain a representative sample from the community. Before being included in the study, the samples that were included in this study were diagnosed previously using serological techniques. Participants' varying lengths of time from diagnosis allowed for a more comprehensive understanding of HCV dynamics throughout the course of infection and reflected a range of disease stages. Respecting standards we obtained consent from each individual before proceeding with the sample collection process. Our approach adhered to both national guidelines and it was closely monitored and approved by the relevant institutional review boards. To gather the samples, we drew three milliliters of blood into gel tubes for each participant.

After allowing the blood to coagulate at room temperature for 30 minutes we subjected it to centrifugation at $2000 \times g$ for ten minutes. This process helped us isolate the serum which was then stored under conditions, for analysis. Furthermore, we also collected 2 ml of blood using EDTA tubes for each participant. EDTA serves as an anticoagulant that prevents blood clotting while preserving the integrity of blood cells. We took care in ensuring mixing so that the EDTA was well integrated throughout. After transferring 100 microliters of blood from this mixture to each of the 200 Eppendorf tubes containing the RNA/DNA Shield it was essential to stabilize and preserve the nucleic acids in the blood samples to maintain the integrity of DNA and RNA, for subsequent analytical procedures.

Next the blood is subjected to centrifugation at a speed typically ranging between 1500 and $2000 \times g$ for a duration of ten to fifteen minutes. Following centrifugation great care is taken while pipetting out the liquid referred to as plasma into a new sterile tube in order to avoid any disruption or harm to other layers. Both serum and blood samples are assigned identification numbers to ensure confidentiality. Subsequently these samples are preserved by storing them at 80°C until analysis. To ensure conditions for scheduled tests such, as detection of HCV viral load and sequencing of TTV ORF1 gene utmost care was taken during collection and storage of these samples. The group of people being studied, which consisted of individuals, from age groups, genders and health conditions offered a foundation, for conducting a comprehensive examination of how viruses behave. This was made possible by the sample set available.

Detection of the HCV

RNA Extraction: RNA was extracted from cell-free biological fluids using the Ribo Virus (K-2/C) kit according to the manufacturer's protocol. The viral RNA was then stored at -20°C until qRT-PCR working day. **HCV Real-Time Quantitative PCR:** The HCV Real-TM Quant kit was employed for quantitative detection of HCV RNA. This method includes the simultaneous detection of HCV-specific Internal Control (IC) via dual color detection. The procedure began with the thawing, vortexing, and brief centrifugation of reagent sets. The reaction mix was prepared by adding RT-PCR-mix-1, RT-PCR-mix-2, Hot Start Taq Polymerase, and M-MLV Revertase to the tube. Afterward each reaction tube received 12.5 μl of the reaction mix through aliquoting as described by Warkad et al.[22]. Upon preparing the reaction tubes or PCR plate the corresponding tube holding the reaction mix was supplemented with 12.5 μl of the extracted RNA sample. Before adding them extra care was taken to re-centrifuge the RNA samples and ensure that the pellet remained undisturbed while using the supernatant. To perform PCR, a set of standards and a negative control were created using the provided Quantitation Standards, for HCV and IC along with TE buffer for the PCR Negative Control. Once sealed these reaction tubes were placed into a Real Time PCR apparatus to undergo amplification. The amplification process followed cycling conditions which entailed an initial reverse transcription step at 50.0°C for 15 minutes followed by a denaturation step at 95.0°C for 15 minutes. This was succeeded by five cycles consisting of denaturation at 95.0°C for 5 seconds annealing at 60.0°C for 20 seconds and extension at 72.0°C for 15 seconds. Subsequently there were forty cycles involving denaturation at 95.0°C for 5 seconds annealing at 60.0°C for 30 seconds and extension at 72.0°C , for another seconds. The researchers then analyzed the samples to check for the presence and amount of HCV RNA. They used controls. Followed strict PCR setup protocols to ensure accurate measurement of HCV viral load. This is crucial, for assessing prognosis and monitoring their response to therapy. Sustained virological response was defined according to standards as the absence of HCV RNA six months after stopping therapy. A standard curve was created to quantitatively determine levels of HCV RNA using RT-PCR. The linear regression analysis provided an equation representing the line along with information about the assay's efficiency and standard deviation. The high R^2 value demonstrates that the assay is reliable, for determining load (see Figure 1).

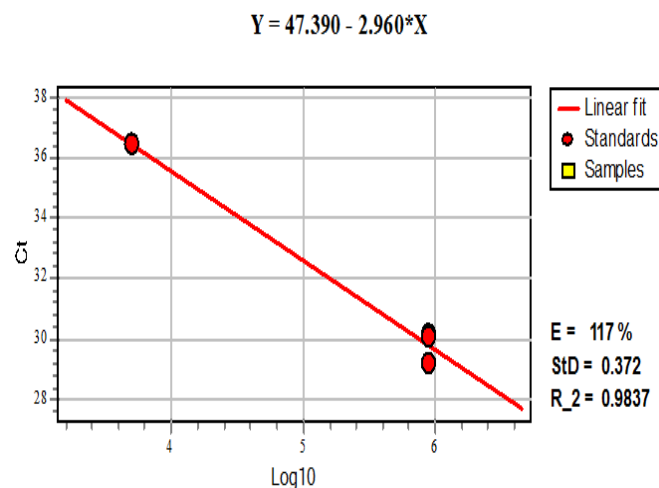


FIGURE 1: Standard Curve for the Quantitative Determination of HCV RNA Levels by RT-PCR. The linear regression analysis Provides the Equation of the line, with the Efficiency and Standard Deviation of the Assay Indicated. The high R^2 Value Demonstrates the Assay's Reliability for Accurate Viral Load Determination.

Detection of the TTV

TTV DNA Extraction

For the recovery of viral DNA, a standardized extraction protocol was employed. Plasma was chosen as the biological fluid for extraction, considering its high yield and quality of viral DNA content. The extraction commenced with the addition of 4 volumes of Viral DNA Buffer to 1 volume of plasma (e.g., 800 µl Viral DNA Buffer to 200 µl plasma) in a 1.5 ml microcentrifuge tube. The mixture was briefly vortexed and then allowed to stand at room temperature for 5-10 minutes. For samples that contained particulate matter, a centrifugation step was conducted at 10,000-16,000 x g to remove any potential clogging material before applying the supernatant to a Zymo-Spin™ IC Column placed in a Collection Tube. Following the transfer, the samples were centrifuged, and the flow-through was discarded. The DNA bound to the column was washed with the prepared DNA Wash Buffer to remove impurities. After washing, the purified DNA was eluted in a minimal volume of DNA Elution Buffer or water to ensure a high concentration, with a final elution step performed after a 1-minute incubation at room temperature.

Amplification of TTV DNA

The extracted TTV DNA underwent amplification using the Maxime PCR PreMix kit (i-Taq), which includes all necessary components for PCR in a convenient master mix solution. Specific primers for the TTV ORF1 gene were utilized:

Forward Primer

5'- GTGGAACGTGGTTGCACAAA-3'

Reverse Primer

5'-ACAGTTGGGAAGCATCTGGG-3'

These primers were designed to amplify a product size of 1308 bp, with the primer melting temperatures (T_m) optimized for the reaction conditions. The primer mix was prepared with a final working concentration of 5 picomols/µl for both forward and reverse primers. The PCR reaction was prepared with a final volume of 25 µl per sample, containing 5 µl of Taq PCR Pre Mix, 1 µl each of forward and reverse primers, 1.5 µl of extracted DNA, and 16.5 µl of distilled water. The amplification conditions were set as follows: Initial denaturation at 95°C for 3 minutes. The process involves repeating the steps; heat, at 95°C for 45 seconds cool down to 58°C for 1 minute. Then increase the temperature to 72°C for another 45 seconds. Finally, there is a step where the temperature is maintained at 72°C, for a duration of 7 minutes.

Sequencing and Sequence Alignment

After amplification the PCR samples were separated using a 2% agarose gel. We then observed them under UV light after staining with ethidium bromide or Red Stain [23]. The bands corresponding to the TTV ORF1 gene were extracted from the gel, and the DNA was purified for sequencing. Sequence data obtained were subjected to homology search using the BLAST program available at the National Center for Biotechnology Information (NCBI) and aligned using the BioEdit

software. The expectation value (E-value) and bit score from the BLAST results were used to assess the degree of similarity between the sequences, with lower E-values indicating higher similarity and higher bit scores indicating higher degrees of sequence homology.

Statistical Analysis

For the study analysis this study utilized SPSS version 24. To assess the significance of the association, between variables a square test was employed, a commonly used nonparametric test. In our study we used the test to examine whether there was a meaningful relationship, between gender (male or female) and the presence of the viral infection or specific markers being investigated. We conducted a regression analysis to evaluate the impact of TTV presence and HCV status, on illnesses severity. We classified the severity levels of these illnesses based on their significance with levels indicating severe diseases. Our model considered TTV presence (confirmed through test results). Hcv status (determined by serological findings), as predictor variables.

Results and Discussion

The Torque Teno Virus (TTV) is a type of virus that has a stranded DNA and lacks an envelope. Its known for its diversity especially within the open reading frame 1 (ORF1) gene [24, 25]. This gene contains the protein of the virus. Has various functional domains that play roles in replication and impacting the immune responses of the host [26]. The genetic variations observed in ORF1 are primarily due, to a mutation rate, which has led to the existence of genotypes and subtypes of TTV in human populations [9, 27]. According to the World Health Organization (WHO) hepatitis C virus (HCV) infection poses a public health concern worldwide particularly in countries, with lower incomes [28]. In 2019 its estimated that there were 290,000 deaths caused by HCV related complications [29, 30]. To address this issue comprehensively WHO launched the Global Health Sector Strategy for Viral Hepatitis 2016–2021 with the goal of eliminating the transmission of hepatitis B and C by 2030 [31, 32]. The data analysis reveals no significant association between gender and the presence of the viral infection or marker under investigation, shown in table (1). Specifically, the distribution of negative and positive test results across male and female participants shows a relatively even split. Among males, 17 were observed to test negative, accounting for 48.6% of the negative results, while 13 tested positive, representing 52.0% of the positive results. Conversely, females exhibited a close distribution, with 18 testing negative (51.4% of negatives) and 12 testing positive (48.0% of positives). The chi-square test yielded a value of 0.066, corresponding to a P-value of 0.793. This P-value, being significantly above the standard threshold of 0.05, indicates a lack of statistical significance in the relationship between gender and test results. Consequently, we can infer that within the confines of our study, gender does not significantly influence the likelihood of testing positive or negative for the condition or marker in question. This finding suggests that the variable of gender does not play a discernible role in the prevalence or detection of the viral infection or marker among the examined population.

Table 1: 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 %		

In reviewing the age distribution of the study participants with respect to their test results, the data suggests minimal variation between the age of individuals who tested negative and those who tested positive for the condition in question. For the group that tested negative, the sample size (N) was 35, with a mean age of 58.3 years and a median age of 59 years. The standard error (SE) of the mean in this group is 2.84. In contrast, the positive group comprised 25 individuals with a slightly lower mean age of 56.6 years and a median age of 55 years, with a standard error of 2.93 years. The P-value for the

age difference between the groups is 0.695, which is well above the conventional threshold for statistical significance (0.05), indicating that the observed age differences between the negative and positive groups are not statistically significant. This result implies that age, within the sample under study, does not appear to be a determining factor for testing positive or negative for the condition being assessed. It is important to note that the close proximity of the mean and median values within each group also reflects a fairly symmetrical age distribution without skewness.

Table 2: 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	

In the investigation of the correlation between chronic diseases and HCV infection status, table (3), the data presented do not indicate a statistically significant association, as evidenced by a P-value of 0.522. Chronic kidney disease was observed in 10 individuals, with a nearly even distribution between HCV negative (6 individuals, 17.1%) and HCV positive (4 individuals, 16.0%). A similar pattern emerged in the case of hepatocellular carcinoma (liver cancer), where it was noted in 6 HCV negative individuals (17.1%) and 2 HCV positive individuals (8.0%), as well as for chronic kidney disease with 5 negative (14.3%) and 2 positive (8.0%) occurrences. Interestingly, Type 2 diabetes mellitus was more common in the HCV positive group (5 individuals,

20.0%) compared to the HCV negative group (3 individuals, 8.6%). Essential hypertension and fatty liver disease (NASH) were also reported among the participants but showed no significant skew towards either HCV negative or positive status. In contrast, cardiovascular disease was reported in 4 HCV negative individuals (11.4%) and 6 HCV positive individuals (24.0%), suggesting a higher prevalence in the HCV positive group. However, without a significant P-value, the data does not support a definitive link between HCV infection status and the prevalence of these chronic diseases within the study sample.

Table 3: 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 (Non-Alcoholic Steatohepatitis, NASH)	Observed	6	2	
	% within column	17.1 %	8.0 %	
Cardiovascular Disease	Observed	4	6	

Torque Teno Virus (TTV) infection. Chronic kidney disease appears infrequently among TTV positive patients, shown in table (4), with only one case (5.9%) compared to nine cases (20.9%) in TTV negative individuals, which is on the threshold of statistical significance with a P-value of 0.05. This may indicate a potential inverse association between TTV presence and kidney disease, albeit further research is necessary to clarify this relationship. Hepatocellular carcinoma and chronic kidney disease were more commonly observed in TTV negative subjects, with 16.3% for each condition compared to 5.9% and 0.0% respectively in TTV positive subjects, suggesting that TTV infection might not be a contributing factor to these conditions.

Type 2 diabetes mellitus presented slightly more in TTV positive individuals (17.6%) than negatives (11.6%). Interestingly, essential hypertension was significantly more prevalent among TTV positives at 29.4%, versus 9.3% in negatives. For fatty liver disease (NASH) and cardiovascular disease, the differences were less pronounced, with fatty liver disease presenting slightly less in TTV positives (11.8% vs. 14.0%) and cardiovascular disease showing an equal distribution across TTV statuses (29.4% for both). The data indicates that while some chronic diseases may have an association with TTV status, others do not, underscoring the complexity of the relationship between chronic conditions and viral infections.

Table 4(a): Comparison of TTV Test Results Across Various Chronic Diseases

Chronic Diseases	Stat	TTV		P-Value
		Negative	Positive	
Chronic Kidney Disease	Observed	9	1	0.05
Hepatocellular Carcinoma (Liver Cancer)	% Within Column	20.9 %	5.9 %	
	Observed	7	1	
Chronic Kidney Disease	% Within Column	16.3 %	5.9 %	
	Observed	7	0	

Table 4(b): Comparison of TTV Test Results Across Various Chronic Diseases

	% Within Column	16.3 %	0.0 %
Type 2 Diabetes Mellitus	Observed	5	3
	% within column	11.6 %	17.6 %
Essential hypertension	Observed	4	5
	% within column	9.3 %	29.4 %
Fatty liver disease (Non-alcoholic steatohepatitis, NASH)	Observed	6	2
	% within column	14.0 %	11.8 %
Cardiovascular disease	Observed	5	5
	% within column	11.6 %	29.4 %

In this analysis Table (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 an 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.

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

The table (6) presents the correlation between two different tests for Hepatitis C Virus (HCV) infection, showing whether a secondary test (possibly another diagnostic method or a confirmatory test) aligns with initial HCV test results. In this dataset, among those who initially tested negative for HCV, 26 individuals (60.5%) also tested negative in the subsequent test, while 9 individuals (52.9%) who initially tested negative were found to be positive in the follow-up. On the other hand, for those who had an initial positive HCV test result, 17 individuals (39.5%) tested negative and 8 (47.1%) tested positive on the subsequent test. The P-value of 0.594 indicates that there is no

significant statistical association between the initial test results and the subsequent test results. This high P-value suggests that the follow-up test does not significantly confirm or contradict the initial HCV test results. This could imply that the reliability of either or both tests may be in question, or there could be variability in the virus's detectability at different stages or due to different testing methods. It's also possible that the two tests have different sensitivities or specificities, which could account for the discrepancies observed between the initial and subsequent testing.

Table 6: Correlation Between HCV Laboratory Results and HCV Rapid Strip Test Outcomes

HCV Result	Parameter	HCV Strip Negative	HCV Strip Positive	P-Value
Negative	Observed	26	9	0.594
	% Within Column	60.5 %	52.9 %	
Positive	Observed	17	8	
	% Within Column	39.5 %	47.1 %	

Figure (2) displays the amplification curves for the detection of HCV RNA by RT-PCR. Each curve corresponds to a different clinical sample tested for HCV. The x-axis represents the cycle number of the RT-PCR, while the y-axis shows the fluorescence intensity, indicative of the amount of DNA amplified during each cycle.

The threshold line is set at the level where the amplification of the product can first be reliably detected, which corresponds to the Cycle Threshold (Ct) value for each sample. Samples with lower Ct values indicate a higher initial concentration of HCV RNA, and vice versa. The various colors represent individual amplification reactions from different samples or standards, depicting the range of the viral loads present in the test cohort.

The alignment details provided in figure (3) and table (6) highlight a comparison between a query sequence from Torque Teno Virus (TTV) ORF1 clone D-59nt and a reference sequence from GenBank with the accession number AJ309728.1. The alignment showcases a significant degree of similarity, with a score of 79.7 bits and an expect value of $1e-14$, indicating a highly significant match between the two sequences.

The sequence identity stands at an impressive 98%, with only a single gap representing 2% of the aligned sequence. Specifically, the query sequence from positions 181 to 228 matches almost perfectly with the corresponding subject sequence from positions 246 to 292, save for a single nucleotide gap [33].

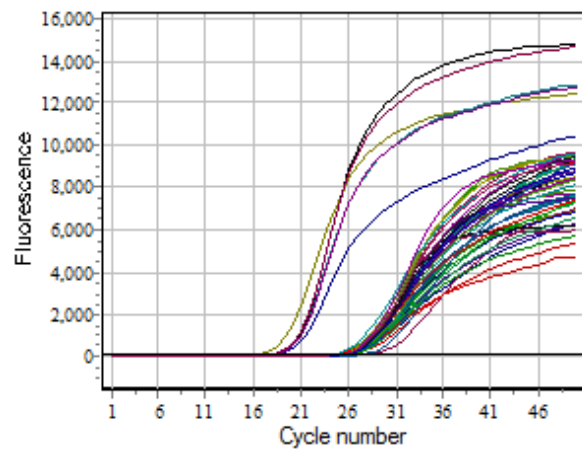


Figure 2: RT-PCR Amplification Curves for HCV RNA Detection. The Exponential Phase of Amplification is visible, Where the Fluorescence Intensity Increases with Each Cycle Number. The Ct values are determined at the Point where Each Curve Crosses the threshold line, Allowing for the Quantification of Viral RNA in the Samples.

This gap does not disrupt the overall high level of sequence conservation observed [34]. Additionally, the query stretch from positions 206 to 243 aligns with the subject sequence from 246 to 282, again with high fidelity and a single nucleotide gap [35]. These results underscore a strong conservation of the ORF1 gene sequence among TTV samples, which could be indicative of critical functional regions

within the gene. The plus/plus strand orientation indicates that the sequences are aligned in the same direction, reflecting their similar genomic orientations [36]. The high level of sequence identity and the significant alignment score are suggestive of a conserved function or structure in the protein encoded by this gene, which may be important for the virus's life cycle or interaction with the host [37].

TT virus ORF1, clone D-59nt

Table 7: Alignment Statistics for Match #1

Sequence ID: AJ309728.1, Length: 292, Number of Matches: 2				
Range 1: 246 to 292		Gen Bank Graphics	Next Match	Previous Match
Alignment statistics for match #1				
Score	Expect	Identities		Strand
79.7 bits(87)	1e-14	47/48 (98%)		Plus/Plus
Query	181	A A C T T T G G A A A T G G T A A A A T G C C A G A C T T T G G A A A T G G T A A A A T G C C		228
Subject	246	A A C T T T G G A A A T G G T A A A A T G C C A G - A C T T T G G A A A T G G T A A A A T G C C		292
Query	206	A A C T T T G G A A A T G G T A A A A T G C C A G A C T T T T G G A A A T G		243
Subject	246	A A C T T T G G A A A T G G T A A A A T G C C A G A C - T T T G G A A A T G		282

The table (8) and figure (3) presents a statistical summary comparing Hepatitis C Virus (HCV) viral load between two groups labeled as 'negative' and 'positive'. It is not immediately clear what the

'negative' and 'positive' groups refer to—possibly they relate to a response to treatment, or perhaps they indicate the presence or absence of another condition or a different viral strain.

Table 8: Mutational Analysis of Torque Teno Virus ORF1 Gene: Sequence Variations and Their Predicted Impacts

Source	Identities	Expect	Score	Sequence ID	Range of Nucleotide	Predicted Effect	Amino acid Change	Nucleotide Change	Nucleotide	Location	Type of Substitution	No. Of Sample
Torque Teno Virus ORF1 Gene	98%	1.00E-14	79.7	AJ309728.1	246 to 282	Missense	Glutamic > Aspartic Acid	GAA>GAC	A	272	Gap	1
						Silent	Leucine	CTT> CTT	T	271	Gap	
Torque Teno Virus ORF1 Gene	98%	1.00E-14	79.7	AJ309728.1	246 to 278	Missense	Glutamic Acid > Aspartic Acid	GAA>GAC	A	271	Gap	2
						Missense	Leucine> Tryptophan	TTG>TGG	T>G	276	Transition	

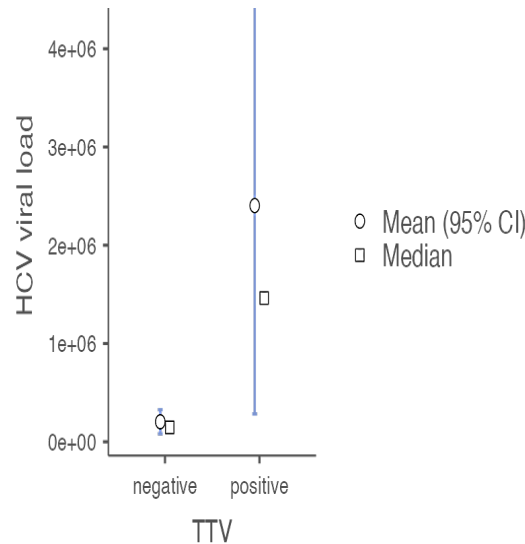
In the 'negative' group, there were 17 individuals with a mean HCV viral load of 203,594, a median of 146,780, and a standard error (SE) of the mean at 62,437. For the 'positive' group, comprising 8 individuals, the mean viral load was significantly higher at approximately 2.40 million, with a median of 1.46 million and a very high standard error of about 1.08 million [38, 39]. The comparison, between groups yielded a P value of 0.006 which's below the used significance threshold of 0.05. This suggests that there is a difference

in the levels of HCV load between the two groups. Specifically, the 'positive' group exhibited an average viral load Figure 3.

This significant disparity in load could have implications in terms of treatment effectiveness or the natural progression of the virus. Notably within the 'positive' group there appears to be variation in load as indicated by the high standard error. Exploring this variability further may provide insights, into the factors contributing to this variation.

Table 9: Group Statistical Summary of HCV Viral Load in Negative and Positive Cases

Group Descriptive	Group	N	Mean	Median	SE	P-Value
HCV Viral Load	Negative	17	203594	146780	62437	0.006
	Positive	8	2.40E+06	1.46E+06	1.08E+06	

**Figure 3:** HCV Viral Load Distribution in Patients with Negative and Positive TTV Status

Through research, on the TTV ORF1 gene it was discovered that there is a level of sequence conservation [40]. Additionally, certain nucleotide modifications can result in both missense and mutations. The discovered mutations, with their predicted values and high sequence identity of 98 percent indicate that they are not variations but potentially linked to the functional adaptations of the virus [41]. While one mutation from glutamic acid to acid is conservative it raises concerns about modest effects on protein function [42]. Additionally, the substitution of Leucine with Tryptophan suggests an impact on the proteins structure. Consequently, on the behavior of the virus [24]. In the population under study there was no correlation found between chronic diseases and infection status regarding TTV or HCV [43]. This implies that these viruses might function separately from long term illnesses or it could suggest that the sample size was insufficient to establish a relationship [37]. Interestingly there is a correlation between TTV status and chronic kidney disease. This indicates that further research is necessary to determine whether TTV has an effect or if it is simply an artifact, in our sample. In our multivariate analysis when we used ordinal logistic regression to examine the data we found a connection, between TTV status and the severity of chronic illnesses. This discovery contradicts our observations where we didn't see any correlation. By including factors in our analysis we gained a nuanced understanding of how different elements interacted revealing that TTV might indeed have an impact on the progression of chronic conditions. However, when it comes to HCV status and the severity of illnesses our

multivariate analysis didn't find an association. This suggests that HCV may have influence on disease progression compared to TTV or that other confounding factors might be overshadowing its impact. It's also possible that limitations in our sample size or specific demographic characteristics within our study cohort hindered us from detecting a relationship for HCV. The multivariate analysis highlighted a finding; there is a correlation, between TTV status and chronic kidney disease. This points towards a link that should be explored further in studies. Considering the nature of interactions and their influence, on the well-being of the host our research strongly suggests that a thorough exploration is necessary to differentiate the effects of TTV from any possible distortions present, in the sample. It is possible that HCV infection is not directly affecting the prevalence of these disorders in the population under study [40, 44], as evidenced by the absence of statistical significance in the distribution of chronic conditions across HCV positive and negative results. However, the increased risk of cardiovascular illness in the HCV positive population may indicate a connection worth pursuing in bigger populations [17].

Conclusion

The results of the study, in summary, add to a more sophisticated knowledge of the relationships between HCV and TTV and how they affect viral pathogenesis. The stark variations in viral loads highlight the need for tailored strategies to treat HCV infection.

References

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