

Research Article

Torque Teno Virus and Hepatitis: A review on correlation

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Abstract

Torque teno virus (TTV) is one of the “orphan” viruses that have been discovered almost two decades ago, with little information on the relationship of the infection to any diseases. It is one of the 45% of the commensal virus which is found throughout the population and becoming one of the most extensively studied viruses on its prevalence among the various level of health status. From healthy blood donors to patients who suffered severe illness, TTV infection level seems to be high and the findings have triggered interest from the researcher. Even though the study on TTV prevalence is actively performed, the actual pathogenesis of TTV to any specific diseases is yet to be ascertained. Many suggestions on the possible association of TTV infection with severe diseases such as acute respiratory diseases, liver-related diseases and even cancer have been discussed. However, one type of diseases which might have an association with TTV is hepatitis. Albeit, it remains a theory as the actual pathogenicity of TTV is not fully understood.

Keywords: Torque teno virus; hepatitis; non-hepatitis; prevalence; continent; transmission

1.0 Introduction

Torque teno virus or TTV is a small, non-enveloped virus which is non-pathogenic and being extensively studied throughout the world and hypothesised to be a newly emerging infectious agent. “Torque teno” words derived from Latin name and defined as ‘thin necklace’ is given based on the molecular structure of TTV which is in circular form.

Other than that, TTV is also known to be a negative polarity of a single-stranded DNA genome. TTV was first known as “floating virus” as it does not match with the available group of viruses. Later, TTV was first classified as *Circovirus* before being reassigned to a new formation of virus group which is known as *Anellovirus* as it was disclosed to be larger in size compared to other *Circovirus* members (Hino & Miyata, 2007). The analysis of TTV was first started with the finding of unfamiliar 500 nucleotides of N22 region isolated from sera of unknown etiology of Japanese hepatitis patients. Subsequent findings produced additional data of the actual TTV size which is 3.8kb on average (Nishizawa et al., 1997 & Peng et al., 2002).

The TTV genome can be divided into two main parts which are non-coding region and coding region. The non-coding region or also known as untranslated (UTR) region is conserved in all isolates and the size is approximately 1.2kb of the total genome. It is responsible for the regulation of viral replication (Bostan et al., 2013). Apart from the UTR region, there is GC rich region which consists of 117 nucleotides, a downstream poly A sequence and a TATA box (Heller et al., 2001; Bostan et al., 2013) in the conserved region. Meanwhile, for the coding region, the sequence of approximately 2.6kb is not conserved and consists of 3 - 5 open reading frames (ORFs) (Okamoto et al., 2002; Hino & Miyata, 2007; Mi et al., 2014; Wei et al., 2015). The molecular structure of TTV is illustrated in Figure 1.

Every single isolate has a different sequence in the coding region as proof that TTV is a heterogeneous genome virus (Spandole et al., 2015). The position of ORFs is different in every isolates but with a similar role in protein translation and gene expression (Bostan et al., 2013). In brief, ORF1 is the largest ORF and encoding a capsid protein in DNA binding during packaging of viral DNA into the capsid. Meanwhile, ORF2 which encodes proteins of almost 200 amino acids can be divided into two smaller parts; ORF2a and ORF2b which is depending on stop codon in certain isolates. The protein encoded is useful in the involvement of cellular or viral protein regulation during infection (Peters et al., 2002). Lastly, ORF3 encoded a protein which responsible in cell cycle regulation and suppression of antiviral resistance. It is believed that the intra-genomic arrangement in the sequence of TTV with the alternative splicing has increased the number of ORFs and the variability of TTV isolates (Leppik et al., 2007).

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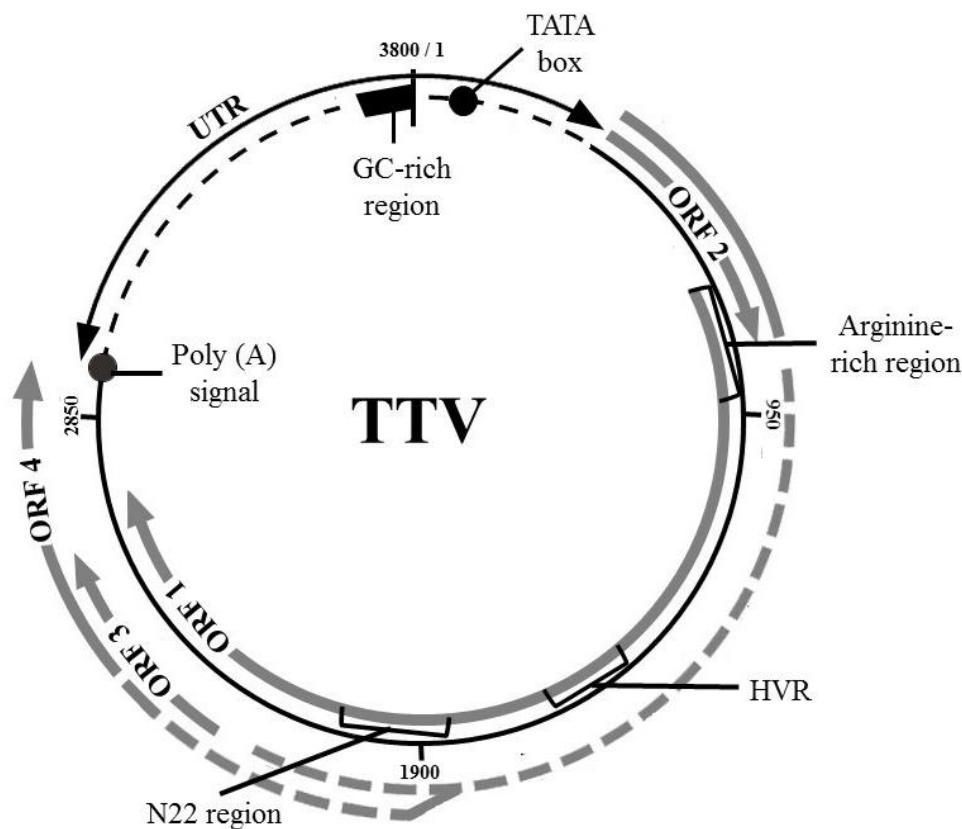


Figure 1: The diagram illustrate the molecular and genomic organization of TTV (Hsiao et al., 2016). Adapted from Jarkasi et al., 2017.

The hypervariability of TTV is well known and has been a unique characteristic of the virus genome. There are 29 species of TTV detected with more than 39 genotypes isolated across the continent (Irshad et al., 2008). There are three main species for Anelloviruses and TTV is categorised under *Alphatorquevirus* species meanwhile for *Betatorquevirus* and *Gammatorquevirus* species is belongs to Toque teno midi virus (TTDV) and Torque teno mini virus (TTMV), respectively (as shown in Figure 2). The classification was based on ORF1 region with the cut value of 30% by the International Committee on the Taxonomy of Viruses report in 2011. The current genotypes isolated were assigned into 5 major phylogenetic groups with 30% divergence from one another and more than 50% difference between the groups (Mi et al., 2014). However, in 2016, Hsiao et al have discovered a new genotype which is distinct from all of known TTV groups and was proposed for the robust criterion of major phylogenetic TTV classification (Hsiao et al., 2016). The discovery of new genotype is correlated with more population detected with TTV infection as the genetic heterogeneity among human being.

Numerous studies on TTV prevalence are well conducted worldwide in order to determine the actual source of infection, the pathogenesis, and TTV association to any current diseases. There was an abundance of information on nature as well as the molecular characterization of TTV which has been discovered, but the actual mechanism of TTV infection towards the population remains unclear. Many studies have been conducted to observe the prevalence pattern of TTV infection in many types of diseases, however, the most common disease which believed to have a strong acquaintance with TTV genome is hepatitis.

TTV was first detected in the serum of non-A non-G hepatitis patient as an unfamiliar 500 nucleotide DNA sequence (Nishizawa et al., 1997). Hence, many researchers were postulated TTV infection have a strong relationship with hepatitis diseases. Hepatitis is a liver inflammation disease which leads to various kind of fatal complications such as hepatocellular carcinoma (HCC), liver cirrhosis and liver failure. Hepatitis infection has become one of the upsetting public health problems worldwide and it was known that HBV and HCV infection are the most prevalent cases in the world population (Lozano et al., 2012). According to the World Health Organization (WHO) report, viral hepatitis was responsible in causing 1.34 million deaths which triumph the number of death caused by HIV infection in 2015 (World Health Organization, 2017). On top of that, 96% out of 1.34 million deaths were caused by HBV and HCV infection with 66% and 30% respectively (Figure 3).

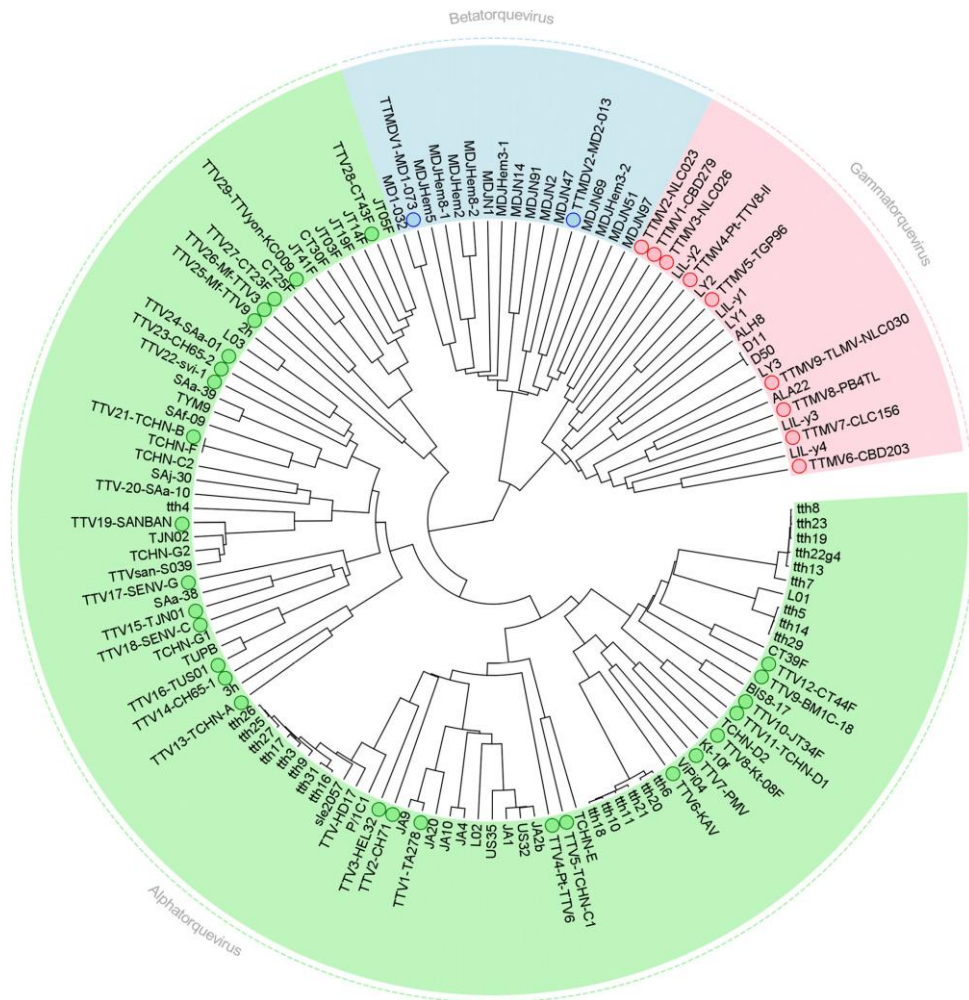


Figure 2: The diagram demonstrates the three main species of Anelloviruses which are: *Alphatorquevirus* for TTV, *Betatorquevirus* for TTMDV and *Gammatorquevirus* for TTMV. All three Anelloviruses are different in terms of the size (bp). The image is taken from Spandole et al.,2015.

Mortality Percentage in 2015

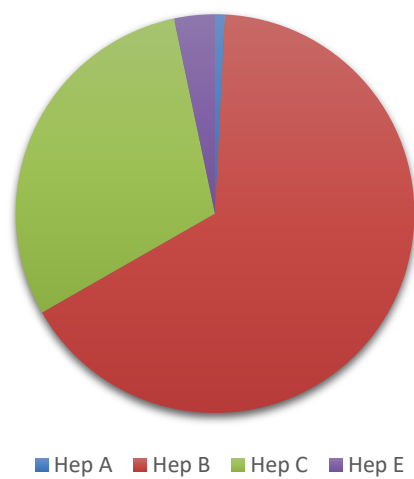


Figure 3: The pie chart represents the data for the mortality percentage of four type of hepatitis; Hep A, Hep B, Hep C and Hep E. The data retrieved from the Global Hepatitis Report,2017 (World Health Organization, 2017)

2.0 Molecular detection of TTV

The reliability of prevalence data of TTV infection gathered among a population with different level of health status are based on the sets of primer chosen as well as the type of method used for screening. The most common and standard technique used for the detection of TTV is Polymerase Chain Reaction (PCR). The most suitable sets of primer should be selected based on the highest percentage of detection. It was shown that the sets of primer which amplified the non-coding region or untranslated region of TTV give higher prevalence compared

to ORF region (Table 1) (Okamoto et al., 1999). It is due to the unique characteristics of TTV genome sequence which is heterogeneous and consists of hypervariable (HVR) region in coding area which makes TTV as one of the most diverse viruses (Okamoto et al., 1999; Nishizawa et al., 1999; Kakkola et al., 2008 & Hsiao et al., 2016).

TARGET REGION	Type of Primer	Percentage of Detection
UTR A	NG054/NG049	53
	NG054/NG132	93
	NG133/NG147	93
	NG133/NG147; NG134/NG132	93
UTR B	NG005/NG009	13
	NG148/NG135	70
	NG148/NG135; NG149/NG136	87
	NG148/NG065; NG149/NG021	70
	NG022/NG021	10
CODING (N22) REGION	NG016/NG065	30
	NG057/NG058	10
	NG015/NG012	10
	NG059/NG063; NG061/NG063	17
	NG061/NG063	17
	RD037/RD038	10

Table 1: Table retrieved from Okamoto et al., 1999 listed types of primers with the percentage of detection. The higher the percentage of detection, the higher the sensitivity of primer used in amplifying specific region.

3.0 Prevalence of TTV in Hepatitis diseases

TTV infection among hepatitis patients

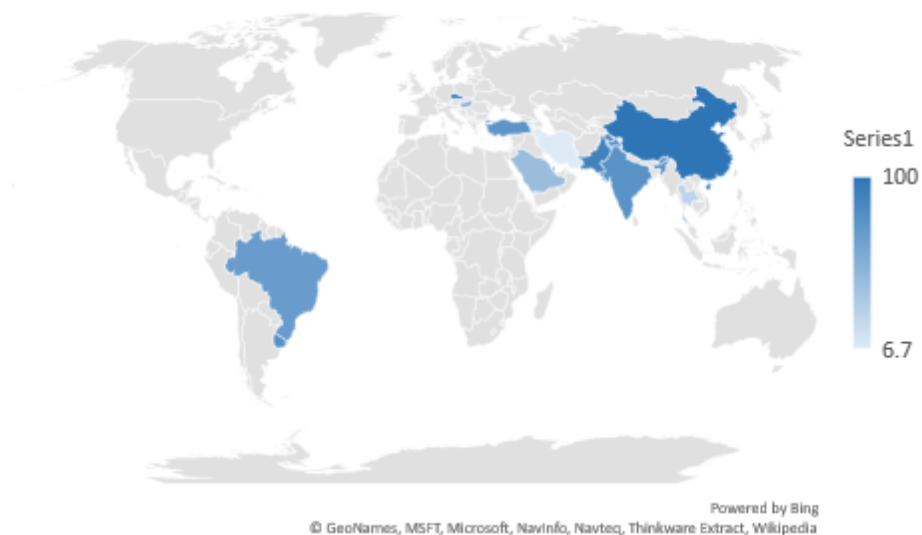


Figure 4: The heat map above shows the prevalence of TTV infection among hepatitis patients across different continent. The data gathered from previous journals are in percentage and the map was constructed by using Microsoft Excel version 2010.

The most common type of disease believed to have an association with TTV infection is hepatitis. One of the reason is TTV was first discovered from non-A to non-E hepatitis Japanese patients (Nishizawa et al., 1997). However, the studies conducted hitherto shows collateral with the theory as the percentage of TTV infection among hepatitis patients is in high number in term of infection (majority with more than 50%) throughout the world (**Figure 4**) (Koochi et al., 2012; Abuodeh et al., 2015; Peng et al., 2015). There are many studies conducted to justify the relationship between TTV infection with hepatitis diseases and many findings showed positive association in term of prevalence.

Table 2: List of TTV prevalence among hepatitis and liver-related diseases patients. The blue-coloured tables are the prevalence among non-hepatitis patients but liver-related diseases patients (Jarkasi et al., 2018).

Continent	Countires	References	Subjects	Prevalence of TTV (%)	
ASIA	China	Jin peng et al. (2015)	Chronic Hep B	100.0	
			Chronic Hep C	97.5	
	India	Magu et al. (2015)	Hep A	77.4	
			Hep B	87.6	
			Hep C	77.0	
			Hep Non B,non C	92.8	
			Irshad et al. (2008)	Liver diseases	26.7
	Iran	Izadi et al. (2016)	Chronic Hep B	6.7	
			Chronic Hep C	13.3	
		Koohi et al. (2012)	Chronic Hep C	92.0	
		Mousavi-Nasab et al. (2013)	Chronic Hep B	50.8	
			Chronic Hep C	66.5	
	Pakistan	Hussain et al. (2012)	Hep B	89.7	
			Hep C	90.0	
Thailand	Urwijitaroon et al. (2007)	Hep B	25.0		
		Hep C	29.0		
EUROPE	Czech republic	Salakova et al. (2004)	Hep C	89.2	
			Hep non A-E	75.0	
	Hungary	Takacs et al. (2003)	Hep of unknown origin	50.4	
MIDDLE EAST	Qatar	Abuodeh et al. (2015)	Hep B	90.8	
			Hep C	84.9	
	Saudi Arabia	El-taher et al. (2015)	Haemodialysis patients	42.9	
	Turkey	Erensoy et al. (2002)	Haemodialysis patients	75	
			Fulminant Hep	80	
		Kalkan et al. (2005)	Chronic Hep B	31.3	
		Chronic Hep C	53.1		
NORTH and SOUTH AMERICA	Brazil	Masau et al. (2012)	Haemodialysis patients	68.8	
			Agatha et al. (2016)	Hep C related chronic liver diseases	45.0
			Devalle and Niel. (2004)	Hep B	54.0
	Uruguay	Cancela et al. (2016)	Hepatitis (Hep B, Hep C, Hep with unknown etiology)	79.0	

The most common viral hepatitis diseases observed to have a higher rate of TTV infection are Hepatitis B and Hepatitis C. On top of that, the number of patients diagnosed with hepatitis B and C infections worldwide including Malaysia are higher compared to other types of viral hepatitis (Raihan, 2016). Nonetheless, the possibilities of TTV to infect a person suffering from another type of hepatitis such as Hepatitis A and non-A to non-E hepatitis have been shown by a few studies.

It was reported that, studies in India and Saudi Arabia have shown the presence of TTV viremia in patients with hepatitis A, non B and non C hepatitis as well as non A-E hepatitis which is in concordance with the first finding of TTV in 1997 by Nishizawa et al (Al-Mozaini et al., 2006; Asim et al., 2010; Magu et al., 2015). Hence, the findings conclude that TTV infection is not mainly focused or related with Hepatitis B and C, but almost all type of hepatitis has the risk to be co-infected with TTV which might give a clear picture in proving the correlation between TTV infection and hepatitis.

The detection of TTV infection among hepatitis patients are not restricted to any specific phylogenetic groups of TTV. However, the most prominent TTV group detected is genogroup 3 and followed by genogroup 5 as shown by PCR analyses (Abuodeh et al., 2015). In contrast, genogroup 2 is the least isolated. Several studies have shown that multiple infections of TTV from different isolates in a single person seems to be common in nature (Ninomiya et al., 2008; Cancela et al., 2016;). The virulence and role of every isolate of TTV so far remain unclear and require further perceptible (Peng et al., 2015). Apart from the information regarding TTV virulence, the knowledge on the molecular structure of every isolate should be deeply investigated and recorded because it is crucially important as it influences the actual pathogenesis of TTV and for early detection of any possible mutation occurred that can potentially cause an outbreak in the near future. The bar chart below (Figure 5) shows a comparison of similar findings from two different studies on the prevalence of TTV among five main phylogenetic groups.

Prevalence comparison of TTV between the phylogenetic groups

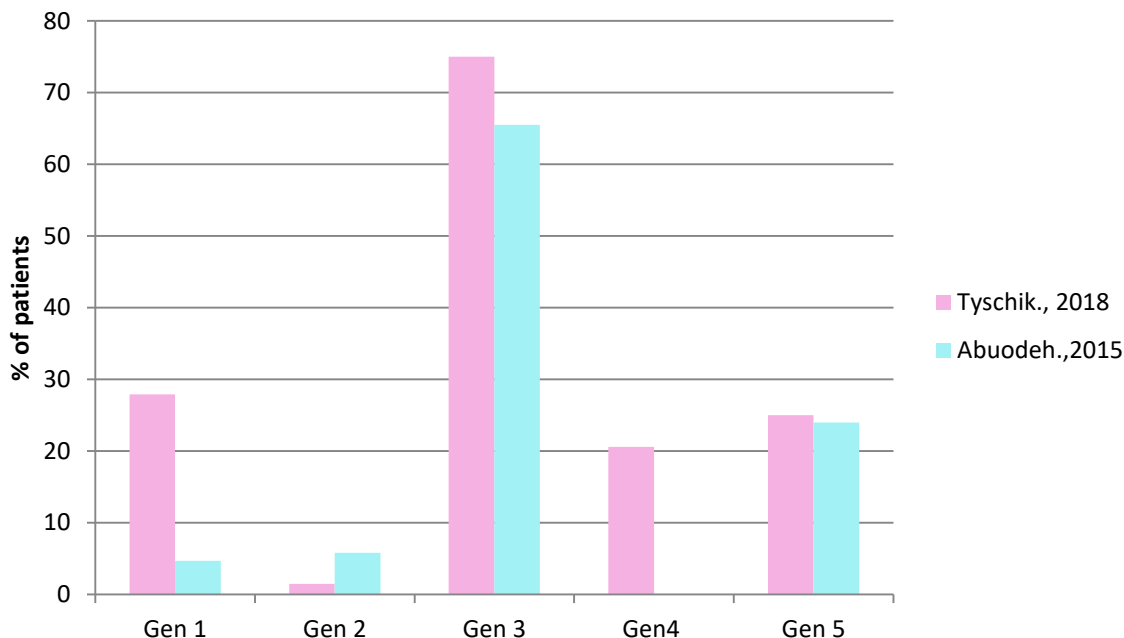


Figure 5: The bar chart of the data which retrieved from Abuodeh et al., 2015 and Tyschik et al., 2018 study.

4.0 Mortality rates of TTV infection among hepatitis patients

There are several studies conducted to compare the prognosis of TTV-infected hepatitis patients with non TTV-infected hepatitis patients (Tanaka et al., 1999; Tuveri et al., 2000; Desai et al., 2005). The study revealed that the mortality rates of hepatitis patients with co-infection of TTV are higher with 75% (Desai et al., 2005) and even 100% (Tanaka et al., 1999) compared to hepatitis without the co-infection of TTV with only 28.57% and 50% respectively.

It was established that the liver cells are highly susceptible to TTV infection. Hence, the virus is capable and likely to cause direct cell damage in the liver when there is an occurrence of unrestricted viral replication which leads to portentous infection and unlimited spreading of the virus in the liver (Desai et al., 2005).

Other than that, TTV infection was shown to be associated with the occurrence of hepatocellular carcinoma (HCC) and not dependent on liver cirrhosis which are the risk factor of HCC as well as the severity of biochemical and histological parameters of liver damage (Ikeda et al., 1999; Rosa et al., 2017). Hence, the findings are in agreement with the previous study in 2002 which showed high TTV loads were the significant independent factors related to the development of HCC among Japanese chronic hepatitis C patients (Tokita et al., 2002). Nonetheless, the finding does not relinquish a clear picture on the actual pathogenesis of TTV in development of hepatitis which potentially leads to hepatocellular carcinoma due to fewer cases of single TTV infection could be a reference in order to observe the implications of TTV mono-infection (Desai et al., 2005).

5.0 Prevalence of TTV in non-hepatitis diseases

TTV infection is not solely found in hepatitis or liver diseases but is also detected in other non-related liver diseases as well. The rate of TTV infection in diseases related to other systems such as respiratory, genetic and cancerous is proved to be significantly high in many studies (Pifferi et al., 2005; Alavi et al., 2011; Costa et al., 2012; Cimponeriu et al., 2013). The number of studies conducted to observe any association of TTV pathogenesis with any non-hepatic diseases are increased by the year. The trend shows diseases with blood related diseases are the common diseases believed to have a relation with TTV pathogenicity (Figure 6). It is because the most common transmission of TTV is through parenteral transmission (Jafari et al., 2012).

5.1 Blood-related diseases

Thalassemia is one of the genetic disorders which involved multiple blood transfusion due to haemolytic anaemia conditions. Therefore, a few studies have been conducted and hypothesised that thalassemia patients might have high exposure to transfusion-transmitted infection caused by viral or bacterial during blood transfusion process (Vimolket et al., 1999; Hu et al., 2005; Alavi et al., 2012; Jalali et al., 2017).

A study in Iran showed the rate of TTV infection is positively correlated with the increasing age of a thalassaemic individual. This is due to the older the age, the more frequent blood transfusion they experienced (Zabetian et al., 2016). However, the findings could not verify blood transfusion is the only route of TTV transmission. The statement is proven with the presence of TTV infection among patients with no history of blood transfusion (Hassuna et al., 2017; Wei et al., 2015). Even though the rate of TTV infection among cardiovascular, tumour and gastroenteritis patients is low, it is suggested TTV can be transmitted not only via blood and injection but also by other routes (Okamoto et al., 2001; Zabetian et al., 2016)

Number of TTV study conducted from 2001 to 2017

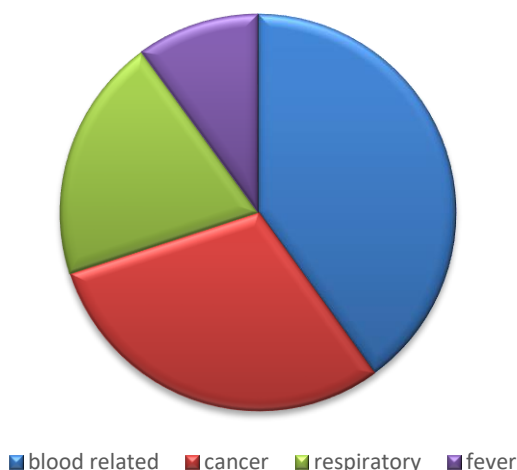


Figure 6: The pie chart above displayed the number of studies conducted among different type of non-hepatic disorder to observe the prevalence of TTV infection from 2001 to 2017. The chart was constructed by using Microsoft Excel version 2010.

5.2 Fever

In Switzerland, an incident involving two boys, age of 9 months and 42 months old showed a clinical presentation of febrile with unknown etiology of viral infection and were later detected with TTV viral load by using Next Gene Sequencing (NGS) (Cordey et al., 2018). The boy who was 42 months old died at day 8 of hospitalization and later discovered that the boy had been infected with HIV and a few other viral infections as well as TTV. Even though the clinical manifestations of the kids are moderate, the prognosis is quite bad. It could be one of the evidences that TTV could serve as a co-virus with other types of an infectious agent or TTV isolates with rearranged genetic components that contribute to fatal disease pathogenesis.

5.3 Respiratory

In another study on the prevalence of TTV, lung tissues of idiopathic pulmonary fibrosis (IPF) patients were detected with replicative intermediate forms of TTV and act as an evidence for the presence of TTV multiplication sites in the lung. Consequently, the prognosis on TTV-infected IPF patients is found to be severe compared to non TTV-infected IPF patients with below 3 years of survival rate (Bando et al., 2001). Other than that, high TTV loads were also detected in nasal swab secretion of severe respiratory diseases patients such as bronchopneumonia when compared with acute respiratory diseases (ARD) (Maggi et al., 2003). On top of that, asthmatic diseases which is a common acute respiratory disease especially among children had been detected in the nasal swab of infected children with high TTV infection compared to non-asthmatic children (Pifferi et al., 2005).

5.4 Cancer

Top of the lists of severe and fatal causing diseases which has high mortality rates throughout the world population is none other than cancer. Therefore, with the special feature of TTV which is ubiquitous, it is believed that TTV can be present in normal and neoplastic tissue. Numerous studies are focused on the prevalence of TTV in normal tissue with related diseases.

One study in Germany has demonstrated the existence of TTV is beyond the prediction. There were various types of human cancer specimens tested and results showed more than 50% of the specimens were positive with TTV. However, the findings cannot be concluded as a causal relationship between those two above mentioned as the positive result of TTV infection might be due to the occurrence of tissue inflammation or by the support from rapid division of cancer cells (Villiers et al., 2007; Chu et al., 2011; Cimponeriu et al., 2013).

Moreover, the prevalence of TTV in the cancerous cell is still a discussion whether it is one of the features in neoplastic pathology as it was detected to be tenfold higher in PMBCs patients compared to healthy subjects. This might be due to the function as a reservoir for TTV genome thus influenced the chronicity of the infection and transmission (Zhong et al., 2001; Villiers et al., 2002) or it is just a common event in patients with any severe diseases. Therefore, further study should be conducted to ascertain the specific target sites of cancerous tissue or any focused on normal tissues that might have potential in specific influence or enhance the replication of TTV genome (Spandole et al., 2015).

6.0 Prevalence of TTV in healthy individuals

It was estimated that 50% of 220 mammalian viruses known to infect humans are non-pathogenic (Parker, 2016). One of the viruses is TTV and is known as a new commensal virus which infected worldwide human population regardless of age, ethnicity and gender. Numerous studies on TTV infection in healthy subjects are conducted and the prevalence of TTV is high in patients with a history of blood transfusion. On the definition of 'healthy subjects', few studies have been conducted to screen the availability of TTV infection in blood donor, which fit with the definition of healthy. Similarly, the infection rate is high. In addition, a study in Brazil manifested the infection rate of TTV in blood donor is higher compared to haemodialysis patients (Massaú et al., 2012). Meanwhile, studies in other countries on the prevalence of TTV infection in healthy individuals is equally high when compared with severe-diseases patients (Urwijitaroon et al., 2007; Hussain et al., 2012; Magu et al., 2015; Peng et al., 2015).

6.1 Possible route of TTV transmission among healthy individuals

There are a few possible routes of TTV transmission that influence the rate of TTV infection among healthy populations. One of them is by blood or blood products via parenteral transmissions such as injection, hemodialysis and blood transfusion (Jafari et al., 2012; Massaú et al., 2012). Parenteral transmission is a common route experienced by everyone in life such as medical examination, blood donation and so on. Nonetheless, this transmission could not be concluded as the only route of TTV infection as it does not correlate well with the ubiquity of TTV (Spandole et al., 2015).

Apart from that, another main route which is believed to be the reason for high TTV infection among the healthy population is through saliva and breath (Spandole et al., 2015). It was shown that the titre of TTV antibody found in human saliva is much higher than in serum. The findings prove that saliva is one of the efficient media for virus transmission including TTV.

Besides, circulating air could act as intermediate for TTV transmission. It was demonstrated in the previous study which showed the presence of TTV DNA in the breath of infected individuals (Chikasue et al., 2012) as well as in pharyngeal mucus (Chung et al., 2007). Hence, it is suggested that the common dissemination paths of TTV are in the respiratory tract and salivary droplets. Besides, other body fluids that were positively detected with TTV DNA and suggested as one of the media for TTV transmission are semen and cervical specimens through sexual transmission (Saláková, Němeček, & Tachezy, 2009).

Detection of TTV DNA in the early stage of life gives an idea of how TTV can be transmitted. There were few studies conducted in observing TTV prevalence among healthy infants in order to determine the efficiency of TTV transmission through umbilical cord blood and amniotic fluid or known as transplacental transmission. However, there was a study in 2017 which showed that there was no detection of TTV in the cord blood samples of TTV-infected pregnant mother (Tyschik, Shcherbakova, Ibragimov, & Rebrikov, 2017). Thus, they concluded that the vertical maternal-fetal transmission of TTV is not via transplacental transmission as the findings are inconsistent and remain unclear.

Therefore, TTV transmission is suggested to be via mother-to-child postnatal transmission such as breastfeed or from the environment. On the other hand, breast milk was detected for the presence of TTV DNA by 23.3–67.3% and it was believed to be one of the major TTV exposure for babies (Iso et al., 2001). Similarly, a study in 2018 proved that the TTV prevalence among the first year of life is increasing with age (Tyschik et al., 2018). Hence, the exposure of TTV infection among healthy individuals can be seen to start at the early stage of life but the risk of being infected is depending on the individual’s immune status.

7.0 Comparison of TTV prevalence in hepatitis, non-hepatitis patients and healthy individuals

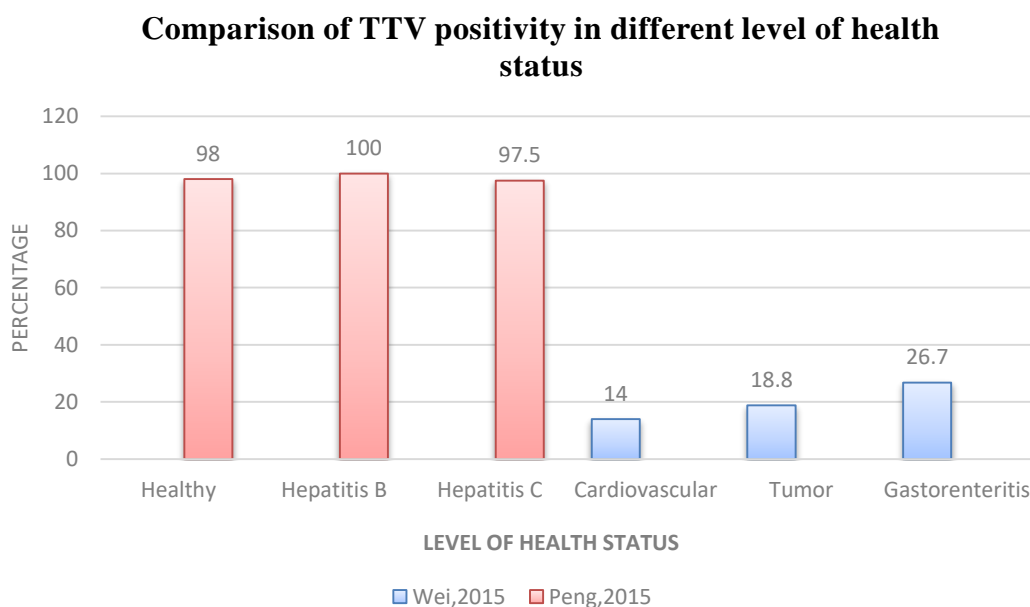


Figure 7: The above bar graph shows the comparison of TTV positive percentage in a few different levels of health status. The data retrieved from the article by Wei et al and Peng et al, both in 2015 study.

The prevalence of TTV pattern has been observed for 20 years since it was first discovered. The above bar chart (**Figure 7**) represents two of TTV studies conducted in Asia continent. Both studies by Wei et al and Peng et al had shown the differences in term of percentage for TTV infection among five different health background. In summary, the highest percentage of TTV infection is among Hepatitis B patients while the lowest is in cardiovascular diseases (CVD) patients. This result is in agreement with other studies which reported more than 70% TTV positivity (Koohi et al., 2012; Hussain et al., 2012b; Magu et al., 2015; Izadi et al., 2016) among hepatitis patients. The low percentage of TTV infection in non-hepatic diseases have been postulated to have an association with less exposure to blood transfusion. The percentage of TTV infection among nonhepatic diseases were proven in few studies (Berkhouse & Gray, 2017; Hettmann et al., 2016; Kalkan et al., 2005) even though the majority of the percentage are not more than 50%. In contrast, the percentage of TTV infection detected among healthy individuals were surprisingly high (Hussain et al., 2012a; Koohi et al., 2012; Costa et al., 2015; Peng et al., 2015; Hsiao et al., 2016). The actual affiliation of TTV infection among healthy individuals remains controversial due to lack of information gathered from the sample subject on the history of the parenteral exposure. Thus, the presence of TTV genome in non-hepatic disease as well as in healthy individuals neglect the hypothesis for the direct association of TTV infection with the development of hepatitis diseases.

8.0 Conclusion

TTV infection is an interesting event to be explored as every single person have the risk to be infected regardless of their health status. It can be hypothesised that an individual's immune status might have some affiliation with the risk TTV infection. Yet, the actual mechanism of pathogenicity of TTV might have potential in altering and evading immune system remains unknown. The prevalence study of TTV infection across the population in every continent have been extensively studied with the best method selected. Therefore, further study on the pathogenicity of TTV is needed to scrutinize deeper into the pathogenesis of TTV. The virus might have an association either directly as a silent infector or fatal co-infector which influence the prognosis and development of any severe diseases.

9.0 Acknowledgements

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10.0 Disclosures

The authors declare no conflict of interest.

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