JOURNAL OF PURE AND APPLIED MICROBIOLOGY, October 2012. Vol. **6**(Spl. Edn.), p. 09-17

Prevalence of Torque Teno Virus (TTV) Infection in Liver Disease Patients

Shafquat Jahan[1]*, Meher Rizvi[1], Md. Azam[1], M.R.Ajmal[2],Indu Shukla[1] and Abida Malik[1]

[1]Department of Microbiology,Jawaharlal Nehru Medical College, Aligarh Muslim University, Aligarh, India.
[2]Department of Medicine, Jawaharlal Nehru Medical College, Aligarh Muslim University, Aligarh, India.

(Received: 04 March 2012; accepted: 10 June 2012)

To investigate the prevalence of Torque Teno virus (TTV) in patients with acute and chronic liver disease, present at a tertiary care hospital in north India. The present study was conducted on 135 patients over a period of one and half year. Detailed clinical history was elicited such as fever, anorexia, nausea, vomiting, abdominal discomfort, high coloured urine, weight loss, any past H/O jaundice, alcohol intake , blood transfusion and H/O pregnancy. MELD (Model for End Stage Liver Disease) scoring was done to evaluate the extent of liver damage.DNA extraction was done from patient's sera by chloroform-ethanol technique. Nested PCR was performed using predesigned primers (by fermentas) . TTV DNA bands were seen on agarose gel electrophoresis. 20 cases (15%) were positive for the TTV DNA. Of which 12 had acute viral hepatitis(AVH),while 6 had chronic viral hepatitis (CVH) and 2 of them had Cirrhosis. Mean levels were AST 49.5 IU/L, ALT 65.75 IU/L , ALP 40.38 KAU, Total Bilirubin 6.95 mg%, and INR 2.32 respectively. The mean of MELD Score was 18.30 ranging from 9 to 36. MELD Score above 20 were found in 6 patients of acute liver disease, and 2 patients of chronic liver disease. TTV prevalence in liver disease is significant though the real clinical impact is still unclear.

Key words: TTV Virus, Liver disease, Prevalence, Clinical.

Hepatitis A-E viruses alone account for more than 80% of viral hepatitis(Simons *et al*; 1995).TTV virus is newly discovered DNA virus classified within a new genus Anellovirus, closely related to Circoviridae family. First described in 1997 by Japanese workers, who detected part of its genome whilst, in an attempt to identify additional agents of human hepatitis, in sequential blood samples of patients with cryptogenic post transfusion hepatitis. Other similar viruses are SENV,SANBAN,YONBAN,PMV,KAV but are classified as distinct genotypes of TTV (Takahashi k *et al*; 2000,Tanaka Y et al; 2001,Kojima H *et al*; 2003).

TTV virus is a small ,non-enveloped, icosahedral, single-stranded, circular-DNA virus (Nishizawa T;1997).TTV virus particle has a density of 1.31- 1.35g/µl and is approximately spherical,30-32nm in diameter and devoid of external lipid (Okamoto *et al*; 1998,Mushanwar *et al*; 1999,Itoh *et al*; 2000).DNA of TTV virus is approximately 3800nm long (Erkev *et al*., 1999, Kamahora et al; 2000,Muljono *et al*., 2001,Okamoto *et al* and Mayurmi *et al* ; 2001, Hino *et al*., 2002).

TTV DNA has been detected in many healthy persons and the diversity in the strains of the virus has been reported(Okamoto H *et al*;1999). TTV DNA was detected in 12% of healthy

* To whom all correspondence should be addressed.
Tel.: +91-9358438730, 9557977647;
E-mail: Shafquat.aamir@gmail.com

blood donors, although the serological prevalence of TTV infection in healthy blood donors was lower than that in patients with fulminant or chronic cryptogenic liver diseases (Okamoto HT *et al*;1998). TTV infection was also investigated (Hino K *et al*, Kao JH et al, Forns X *et al* ;1999, Martinez NM *et al*;2000) in patients on maintenance hemodialysis (HD), as they are assumed to be at risk of blood-borne virus infections such as hepatitis C virus (HCV), because of the repeated blood transfusion and the high frequency of exposure to invasive techniques (Hardy NM *et al*, Dentico P *et al*;1992).

In the few years since its discovery, TTV has been intensively studied to assess its molecular properties and whether it causes liver disease. This has led to recognition that the viremia is an extremely frequent occurrence in apparently healthy individuals worldwide, a feature so unusual among viruses that it has even been proposed that TTV might be a commensal virus. To the best of our knowledge, this concept had never been put forward in virology, although it is common wisdom for many other microorganisms (P. Simmonds *et al*;1999). Knowledge on TTV is growing fast, but many fundamental aspects remain to be elucidated.

MATERIAL AND METHODS

Selection of cases

Subjects

The present study was done on liver disease patients in the Department of Microbiology, Jawaharlal Nehru Medical College and hospital, AMU, Aligarh. The patients recruited in our present study were enrolled after taking informed consent from them. The subjects were either admitted in Medicine In Patients Department or were attending Medicine Out Patients Department and Gastroenterology clinic, of Department of Medicine, J. N. Medical college and Hospital, AMU, Aligarh and were recruited if they fulfilled the criteria for inclusion.

Ethical clearance

This study was conducted after obtaining permission from institutional ethics committee of J.N. Medical College and the procedure followed in the study was in accordance with institutional guidelines and predesigned preformed .

Study group

Over fifteen hundred patients presenting with the sign and symptoms of liver disease were evaluated on the basis of various investigations such as liver function test (AST ,ALT ,T. Billirubin and ALP), Creatinine , PT/INR , MELD Score ,ultrasound , CT scan , G .I. Endoscopy etc. Detailed clinical history was elicited from all the patients .The presence of TTV Virus in patients was correlated with their clinical finding and MELD Score.

Sample collection and transportation

10 ml venous blood was drawn from the patients and the control, taking all aseptic precautions. Blood sample were collected in RNAase and DNAase free containers and aliquoted in plain tubes without anticoagulant . Serum was separated from blood sample by centrifugation at 3000 rpm for 15 minutes and aliquoted in SV4 vial at -20°C till further testing .

All the patients were screened for HAV, HBV, HCV,HEV, HIV infection by using enzyme immunoassay kits of high sensitivity and specificity. Anti- HAV IgM for HAV were detected by using ELISA Kits from DRG International Inc., USA. HBsAg for HBV were detected by using ELISA Kits from SD Bio standard diagnostic , India. Anti HCV for HCV were detected by using ELISA Kits from J. Mitra &Co. Pvt. Ltd., India .HEV IgM for HEV were detected by using ELISA Kits from M.B.S.S.R.L .Medical Biological Service, Milano. Anti HIV1/2 IgG for HIV were detected by using ELISA Kits from Span Diagnostic Ltd., India.

DNA Extraction for TTV VIRUS using Phenol chloroform isoamylalcohol method (Saiki *et al;* 1988)

To 300 µl of autoclaved double distilled water add 200 µl of sample to the eppendorf and mix well .To this equal amount of Tris saturated phenol was added & mix properly .This was incubated at 65°C in water bath for 2 hrs. After incubation the eppendorf was centrifuged at 15000 rpm for 20 min. To this 250 µl of Tris and 250 µl of CIA was added and mixed thoroughly and centrifuged at 20,000 rpm for 10 min ,equal amount of CIA was added and mixed and centrifuged at 12,000 rpm for 10 min. The aqueous phase was transferred to the fresh eppendorf and 1/3rd volume of 7.5 M ammonium acetate and 1.5 volume of chilled absolute ethanol was added. The sample

were kept at -70°C overnight. Spin at 12,000 rpm for 20 min. The pellet were washed in 300 µl of 70 % ethanol, and centrifuged at 12,000 rpm for 10 min. The pellets were air dried and dissolved in 25 ml of sterile double distilled DNA ase free water and store this extracted DNA at -20°C.

Methodology for amplification of TTV virus

Nested PCR was employed for detection of both TTV (295 bp) Virus. Sera from HBV and HCV positive as well as negative patients were tested for TTV . Nested PCR involves sequential use of two primer sets.

Primer Design

16S rRNA genes were the targets for amplification of *Torque Teno Virus* specific nucleic acid for PCR .Primers were ordered from Fermentas, Life Sciences, USA.

TTV Primers

TTV- DNA (conserved region of ORF2) was detected by nested PCR

First set of primers were

NS1 (sense) 5' GGGTGCCGAAGGTGAGTTTAC-3' (175-195),NS2 (anti-sense) 5'-GCGGGGCACGAAGCACAGAAG-3' (474-494),

Second set of primers were

NS3 (sense) 5'-AGTTTACACACCGAAGTCAAG- 3' (189-209) and NS4 (antisense) 5' AGCACAGAAGCAAGATGATTA-3' (463-483)as described by Biagini et al, 1999 (Accession No. AB008394).

Amplification of TTV Virus

TTV Outer (by using first set of primers)

Primer (Forward) NS1(1µl), Primer(Reverse)NS2(1µl), DNA Template(5µl), Nuclease free water (3µl), Master Mix (10µl), Total reaction mixture was of 20µl.

Cycling condition for TTV Outer

The first round PCR was carried out for 40 cycles, each cycles consisting of denaturation at 94°C for 15 seconds, primer annealing at 55°C for 45 seconds and extension at 72°C for 45 seconds, followed by an additional extension at 72°C for 7 minutes.

TTV Inner (by using second set of primers)

Primer(Forward) NS1(1µl),Primer (Reverse) NS2(1µl),PCR Product (2µl),Nuclease free water (3µl),Master Mix (10µl)

Cycling conditions for TTV Inner: The second round PCR was carried out for 40 cycles, each cycles consisting of denaturation at 94°C for 15 seconds, primer annealing at 50°C for 45 seconds and extension at 72°C for 45 seconds, followed by an additional extension at 72°C for 7 minutes.

After amplification, end product was run on Agarose gel by Electrophoresis

Statistical analysis

Statistical analysis was performed with the IBM SPSS Statistics 19. Results were expressed as means ± standard deviation or as percentages. Means were compared between groups by using the *t –test*, and frequency distributions were compared by using the chi- square test.

RESULTS

TTV was assumed to be one of the possible agents causing non A-G hepatitis. Its characterization and significance in causing hepatitis became more interesting with the findings by Okamoto *et al* (1998a), showing level of TTV-DNA in liver tissue to be 10-100 times higher than those in serum.

Table 1. Clinical symptoms in patients positive for TTV in liver disease groups.(n=20)

TTV + ve Disease groups	Fever n(%)	Anorexia n(%)	Nausea/ Vomiting n(%)	Abdominal discomfort n(%)	Weight loss n(%)	High coloured urine n(%)
AVH(n=12)	10(83.3)	4(33.3)	4(33.3)	3(25.0)	8(66.7)	6(50.0)
CVH(n=5)	5(100)	1(20.0)	3(60.0)	4(80.0)	4(80.0)	2(40.0)
HE/FHF(n=1)	1(100)	0	0	1(100)	1(100)	1(100)
Cirrhosis(n=2)	1(50.0)	0	0	2(100)	1(50.0)	0
Total	17(85.0)	5(25.0)	7(35.0)	10(50.0)	14(70.0)	9(45.0)

The present prospective study was conducted in the Department of Microbiology, Jawaharlal Nehru Medical College, AMU, Aligarh, from January 2010 to June 2011. A total of 135 patients suspected of liver disease were recruited in the study to determine the prevalence of TTV was also determined. Co-infection with other viruses such as HAV, HBV, HCV, HEV were also

Table 2 . Clinical profile of patients positive for TTV in Liver disease groups.(n=20)

TTV + ve Disease groups	Ascitis n(%)	Icterus n(%)	Splenomegaly n(%)	JVP n(%)	Pedal oedema n(%)
AVH (n=12)	0	11(91.7)	1(8.33)	0	0
CVH (n=5)	4(80.0)	4(80.0)	3(60.0)	1(20.0)	0
HE/FHF (n=1)	1(100)	1(100)	1(100)	1(100)	0
Cirrhosis(n=2)	1(50.0)	2(100)	0	1(100)	1(100)
Total	6(30)	18(90)	5(25)	3(15)	1(5)

Table 3. Distribution of AST levels (IU/L) in patients positive for TTV in Liver disease groups.(n=20)

TTV + ve Disease groups	Normal (2-20) n (%)	Mild (20-40) n (%)	Moderate (40-60) n (%)	Severe (>60) n (%)
AVH (n=12)	4(33.33)	4(33.33)	0	4(33.33)
CVH (n=5)	3(60)	1(33.33)	1(33.33)	
HE/FHF (n=1)		0	0	1(100)
Cirrhosis(n=2)	1(50)	1(50)	0	
Total	8(40)	6(30)	1(5)	5(25)

Table 4. Distribution of ALT (IU/L) in patients positive for TTV in Liver disease groups.(n=20)

Disease groups	Normal (2-15) n(%)	Mild (15-30) n(%)	Moderate (30-45) n(%)	Severe (>45) n(%)
AVH (n=12)	3(25)	5(41.66)	0	4(33.33)
CVH (n=5)	4(80)	0	1(20)	0
HE/FHF (n=1)	0	0	0	1(100)
Cirrhosis (n=2)	1(50)	1(50)	0	0
Total	8(40)	6(30)	1 (5)	5(25)

Table 5. Relationship between TTV & INR levels in different liver disease groups.(n=20)

TTV +ve disease groups	Normal (0.9-1.3) n(%)	Mildly deranged (1.3-2.3) n(%)	Moderately deranged (2.3-3.3) n(%)	highly deranged (>3.3)n (%)
AVH (n=12)	2(16.66)	9(75)	0	1(8.33)
CVH (n=5)	3(60)	1	1(10)	0
HE/FHF (n=1)	0	0	1	0
Cirrhosis(n=2)	1	1(50)	0	0
Total	6(30)	11(78.57)	2(14.28)	1(7.14)

determined. Thirty healthy age and sex matched controls were included in this study.

The mean age of distribution was 33.90±18.15 years in cases and 40.20±6.42 years in controls. Majority (66.66%) had AVH, 26.66% had CVH, 6.66% had FHF and . However in the study done by Irshad M *et al*; 2008 , 25% had AVH, 22% had CVH, 26% had Cirrhosis, 10% had FHF and 18% had HCC.

The age group of the patients studied was 0-90 years. There were 90 patients with acute viral hepatitis (AVH, age range: 7-74 years), 18 patients with chronic viral hepatitis (CVH, age range: 7-70 years), 17 patients with liver cirrhosis (age range: 12-85 years), 9 patients with fulminant hepatic failure (FHF, age range: 11-60 years), 1 patients with hepatocellular carcinoma of 50 years of age, however in the study done by Irshad M *et al*; 2008 the distribution of liver disease patients in different age groups was as follows; AVH (age range: 21-48 years), CVH (age range: 19-48 years), Cirrhosis (age range: 34-57 years), FHF (age range: 28-46 years), HCC (age range: 24-71 years). There is more wider range of distribution of AVH, CVH, Cirrhosis, FHF and patients in our study as we had taken samples from the paediatric ward also.

Most of the patients in our study were male 100(74.07%) and 35(25.9%) were female i.e male to female ratio is 2.8:1.Bhagyalaxmi A, *et al* in 2007 also reported 2.8:1 male: female ratio in his study. Similar findings were observed during the investigation of hepatitis outbreak in Ahmedabad city during 1975-1976 (the male: female ratio was 2.8:1) Srinivasan MA *et al* ;1976. High incidence of infective hepatitis was reported in Tirupati town in 1981 with male preponderance. Dai CYet al in 2002, done a similar study in Taiwan and found 2.87 male: female ratio which is also in accordance with our study.

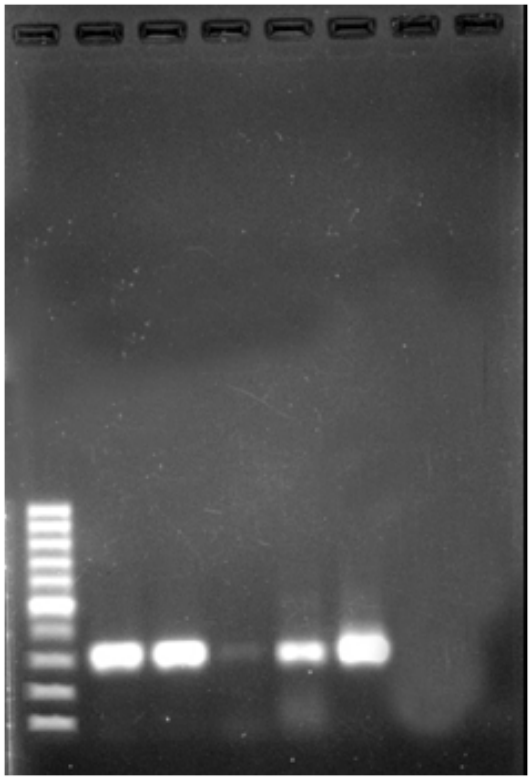

Fig. 1. 2,3,5,6- Showing amplified TTV(295bp) DNA, 1- 100bp DNA ladder,4-negative control

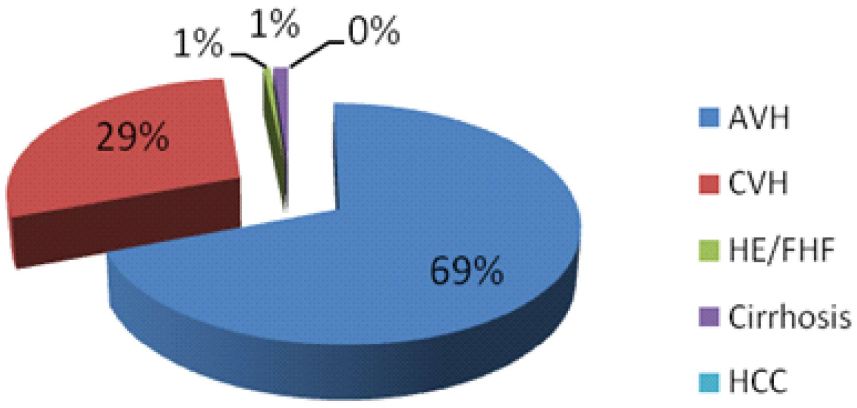

Fig. 2. Prevalence of TTV in different liver disease groups

The various presentation of the patients with clinical suspicion of liver disease were studied in each patient. The main presenting complaint of the patients at the time of admission in the hospital was fever (82.96%), followed by weight loss 53.3%, high colored urine 48.14%, abdominal discomfort 45.18%, anorexia 34.07% and nausea/vomiting 30.37% (Table 1).

On examination, the most common presenting sign in this study was icterus which was appreciated in 77.03% of the patients, 40% patients with ascitis, 53.84% raised JVP and 36.84% pedal oedema (Table 2).

In this study, the prevalence of hepatitis B and hepatitis C was 46% & 2.2% respectively, which is in accordance with the prevalence of hepatitis B & hepatitis C as reported by Kaur (2002) i.e 42.4% & 3.3% respectively. Mohan Lakshmi also reported the same prevalence of hepatitis B (42.5%) in south Indian population in 2011. In an another study done by Saravanan *et al* (2008), among the chronic hepatitis patients with cirrhosis, 59% were HBV infected and 41% were HCV infected, while patients suffered from HCC, 67% were HCV infected and 33% were HBV infected. Some of the studies done by different authors in other parts of India show less incidence of HBV infection than the present study: Tandon (1984) 26%, Dharmadhikari (1990) 21.6%, and Ichhpujani (1991) 8.8%. Whereas ICMR (1983) reported incidence of 62% of hepatitis B virus infection in adults and Sebestian (1990) reported 52% of HBV in acute hepatitis, which are higher than our studies. However the prevalence of hepatitis B was 4.3-5.6% & that of hepatitis C was 1.3-1.9% in USA (2009)(CDC, Division of viral hepatitis).

The prevalence of TTV infections in India has not been well documented. Only a few reports are available (Chattopadhyay *et al*, 2005) that demonstrate the presence of TTV in patients population randomly selected from various studies. In this study, we found the presence of TTV DNA in 15% of patients with different liver diseases shown in Fig. 1. A relatively high prevalence of TTV in liver diseases,in comparison to that in healthy persons (3%) suggests that TTV might play an important role in causation of liver disease. Several studies have revealed high (1.9 to 36%) TTV DNA prevalence in healthy control groups (Yzebe D *et al*; 2002). Earlier preliminary report by Irshad *et al* ; 2006 demonstrated little role of TTV in causation of liver diseases. TTV infection has been found to be common in healthy humans with prevalence which may exceed 90%. Its prevalence in healthy populations in India is lower than those previously reported for Turkish (51.6%), Japanese (92.0%) and Polish (78%) blood donors.

The break-up of TTV prevalence in different liver disease groups in our study was 60% for AVH, 25% for CVH, 1% of Cirrhosis and 0.5% of FHF(Figure -2). TTV dominated in AVH (60%) followed by 25% for CVH. Irshad M (2002) did a study in which the prevalence of TTV in different liver disease groups was 23.0% for AVH, 23.4% for CVH, 34.8% for Cirrhosis and 29.4% FHF. As this study was done in the All India Institute of Medical Science which is an apex institute of India, there were more turnover of severely debilitated patients. Another study done in Taiwan TTV viremia was found to be 46% of acute hepatitis patients.

12(60%) of the TTV positive patients had deranged AST level. 5(25%) of the TTV positive patients had severely deranged AST level, of which 4 and 1 suffered from AVH and HE/FHF respectively. 6(30%) of mildly deranged AST level, maximum 4 suffered from AVH. Mean value of AST was 41.7 ± 39.82. There was no statistically significant difference between the AST levels of TTV positive and TTV negative cases. (Table 3)

12(60%) of the TTV positive patients had deranged ALT level.5(25%) of the TTV positive patients had severely deranged ALT level, of which 4 and 1 suffered from AVH and HE/FHF respectively. 6(30%) of mildly deranged ALT level, maximum 5 suffered from AVH. Mean value of ALT was 44.05±48.8. There was statistically significant difference($p<0.05$) between the ALT levels of TTV positive and TTV negative cases. Relationship between TTV & INR levels in different liver disease groups shown in table 5.Majority 14(70%) of the patients had deranged INR level of which 11(55%) suffered from AVH.

9(75%) of the patients with AVH, had mildly deranged INR level while 4 had normal INR level. 2(10%) had moderately deranged INR level and 1(50%) of which had CVH and the other had HE/FHF.Mean value of INR was 1.84 ± 1.38. There was no statistically significant difference between the INR level of TTV positive & negative cases.

DISCUSSION

In the few years since its discovery, TTV has been intensively studied to assess its molecular properties and whether it causes liver disease.

TTV has been considered to be one of the possible agents causing non A-G hepatitis. Its characterization and significance in causing hepatitis becomes more relevant with the findings by Okamoto *et al* (1998a), showing level of TTV-DNA in liver tissue to be 10-100 times higher than those in serum. Recognition that the viremia is a frequent occurrence in apparently healthy individuals worldwide presented a conundrum on its possible role in liver disease on the one hand and it being a mere commensal on the other (Simmonds *et al*., 1999).

The prevalence of TTV infections in India has not been well documented. Only a few reports are available (Chattopadhyay *et al*,2005) that demonstrate the presence of TTV in patients population randomly selected from various studies. In this study, we found the presence of TTV DNA in 15% of patients with different liver diseases. A relatively high prevalence of TTV in liver diseases, in comparison to that in healthy persons (3%) suggests that TTV might play an important role in causation of liver disease. Several studies have revealed high (1.9 to 36%) TTV DNA prevalence in healthy control groups (Yzebe D *et al*; 2002).

In our study, TTV dominated in AVH (60%) followed by 25% for CVH. Irshad M (2002) reported 23.0% prevalence in AVH cases, 23.4% in CVH, 34.8% in cirrhosis and 29.4% FHF. As this study was done in the All India Institute of Medical Science which is an apex institute of India, there were more turnover of severely debilitated patients. Another study done in Taiwan TTV viremia was found to be 46% of acute hepatitis patients.

There was statistically significant difference ($p<0.05$) between the ALT levels of TTV positive and TTV negative cases. The mean ALT level in our study was 44.05 ± 20.84 which was almost same as reported by Chattopadhyay (2005) 41.6 ± 19.17 and in an another study done by C. Y. DAI (2002) mean ALT was found to be 37.0 ± 17.3. In our study we found a significant difference between the ALT levels of TTV positive (44.05 ± 48.8) and negative (52 ± 43.62) cases and $p<0.05$. On the basis of present studies we can conclude that TTV might be responsible for the causation of deranged ALT level.

A relationship between TTV & INR levels in different liver disease groups was observed. Majority 14(70%) of the patients had deranged INR level of which 11(55%) suffered from AVH. No association was observed between TTV infection and AST levels.

A preliminary report by Irshad *et al.,* 2006 demonstrated little role of TTV in causation of liver diseases. TTV infection has been found to be common in healthy humans with prevalence which may exceed 90%. Its prevalence in healthy populations in India is lower than those previously reported for Turkish (51.6%), Japanese (92.0%) and Polish (78%) blood donors.

In this study, the prevalence of hepatitis B and hepatitis C was 46% & 2.2% respectively, which is in accordance with the prevalence of hepatitis B & hepatitis C as reported by Kaur R in 2002 i.e 42.4% & 3.3% respectively. T. Mohan Lakshmi also reported the same prevalence of hepatitis B (42.5%) in south Indian population in 2011. In an another study done by Saravanan *et al* (2008), among the chronic hepatitis patients with cirrhosis, 59% were HBV infected and 41% were HCV infected. Our results are in agreement with other studies that reported TTV DNA rates between 15 % and 36 % (Lai *et al*., 2002, Naoumov *et al*, Tanaka.,1998). M. Irshad in 2006 had conducted a study on the co-infection of TTV virus with other hepatitis viruses, and he found 34% co-infection with HBV.

REFERENCES

1. Simons JN, Leary TP, Dawson GJ, Pilot-Matias TJ, Muerhoff AS, Schlauder GG, *et al*. Isolation of novel virus-like sequences associated with human hepatitis. *Nat Med* 1995; **1**:564-9.
2. Takahashi K, Iwasa Y, Hijikata M, *et al*. Identification of a new human DNA virus (TTV-like mini virus, TLMV) intermediately related to TT virus and chicken anaemia virus. *Arch Virol.* 2000;**145**: 979-993.
3. Tanaka Y, Primi D, Wang RY, *et al*. Genomic and molecular evolutionary analysis of a newly identified infectious agent (SEN virus) and its relationship to the TT virus family. *J Infect Dis* 2001; **183**: 359-67.

4. Kojima H, Kaita KD, Zhang M, *et al*. Genomic analysis of a recently identified virus (SEN virus) and genotypes D and H by polymerase chain reaction. *Antiviral Res.* 2003; **60**: 27-33.
5. Nishizawa T , Okamoto H, Konishi K, Yoshizawa H, Miyakawa M, Mayumi M .A novel DNA virus (TTV) associated with elevated transaminase levels in posttransfusion hepatitis of unknown etiology. *Biochem Biophys Res Commun* 1997; **241**: 92-97.
6. Mushahwar, I. K., J. C. Erker, A. S. Muerhoff, T. P. Leary, J. N. Simons, L. G. Birkenmeyer, M. L. Chalmers, T. J. Pilot-Matias, and S. M. Desai, Molecular and biophysical characterization of TT virus: evidence for a new virus family infecting humans. *Proc. Natl. Acad. Sci*. USA 1999; **96**: 3177-3182.
7. Okamoto H, Nishizawa T, Kato N, Ukita M, Ikeda H, Iizuka H, *et al*. Molecular cloning and characterization of a novel DNA virus (TTV) associated with post-transfusion hepatitis of unknown etiology. *Hepatol Res* 1998; **10** : 1-16.
8. Itoh, K., M. Takahashi, M. Ukita, T. Nishizawa, and H. Okamoto., Influence of primers on the detection of TT virus DNA by polymerase chain reaction. *J. Infect. Dis***.** 1999; **180**:1750–1751.
9. Erker, J. C., T. P. Leary, S. M. Desai, M. L. Chalmers, and I. K. Mushahwar., Analyses of TT virus full-length genomic sequences. *J. Gen. Virol.* 1999; **80**: 1743-1750.
10. Muljono .D .H ,Nishizawa . T , *et al*., Molecular epidemiology of TT virus (TTV) and characterization of two novel TTV genotypes in Indonesia. *Arch Virol*, 2001; **146**: 1249-66.
11. Okamoto, H., T. Nishizawa, N. *et al.,* Heterogenous distribution of TTV viruses in distinct genotypes in multiple tissues from infected humans. *Virology*, 2001; **288**: 358-68.
12. Hino S, TTV , a new human virus with single stranded circular DNA genome.*Rev Med Virol,* 2002; **12**: 151-8.
13. Hino K, Okuda M, Ishiko H, Okita K: Detection of TT virus in hemodialysis patients. *Nippon Rinsho* 1999, **57**: 11413-6.
14. Okamoto H, Nishizawa T, Kato N, Ukita M, Ikeda H, Iizuka H, *et al*. Molecular cloning and characterization of a novel DNA virus (TTV) associated with post-transfusion hepatitis of unknown etiology. *Hepatol Res* 1998; **10**: 1-16.
15. Okamoto H, Nishizawa T, Ukita M, *et al*. The entire nucleotide sequence of a TT virus isolate from the United States (TUS01): comparison with reported isolates and phylogenetic analysis. *Virology* 1999; **259**: 437-48.
16. Kao JH, Chen W, Hsiang SC, Chen PJ, Lai MY, Chen DS. Prevalence and implication of TT virus infection: minimal role in patients with non-A-E hepatitis in Taiwan. *J Med Virol* 1999; **59**: 307-312.
17. Forns, X., P. Hegerich, A. Darnell, S. U. Emerson, R. H. Purcell, and J. Bukh. High prevalence of TT virus (TTV) infection in patients on maintenance hemodialysis: frequent mixed infections with different genotypes and lack of evidence of associated liver disease. *J. Med. Virol.* 1999; **59**: 313–317.
18. Martinez NM, García F, García-Valdecasas J, Bernal C, García F, López I, Alvarez M, Piédrola G, Maroto MC: Prevalence and viral persistence of TT virus in patients on hemodialysis. *Eur J Clin Microbiol Infect Dis* 2000, **19**:878-80.
19. Hardy NM, Sandroni S, Danielson S, Wilson WJ: Antibody to hepatitis C virus increases with time on hemodialysis. *Clin Nephrol* 1992, **38**: 44-8.
20. Demographic features of sporadic acute hepatitis as determined by Dentico P, Buongiorno R, Volpe A, Carlone A, Carbone M, Manno C: Prevalence and incidence of hepatitis virus (HCV) in hemodialysis patients: study and risk factors. *Clin Nephrol* 1992.
21. P. Simmonds, L. E. Prescott, C. Logue, F. Davidson, A. E. Thomas, and C. A. Ludlam, *Letter, J. Infect. Dis.* 1999; **180**:1748–1749.
22. Irshad M, Singh Shiwani, Irshad Khushboo , Agarwal .S. K , Joshi .Y.K . Torque teno virus: Its prevalence and isotypes in North India, *World J Gastroenterol.* 2008; **14**(39):
23. Bhagyalaxmi A, Gadhvi M, Bhavsar BS, Epidemiological Investigation of an Outbreak of Infectious Hepatitis in Dakor Town, *Indian Journal of Community Medicine,* 2007; **32**(4).
24. Dai CY, Chuang WL, Chang WY. Co-infection of SENV-D among hepatitis C patients treated with combination therapy with high dose interferon-alfa and ribavirin. *World J Gastroenterol*. 2005; **11**: 4241-4245.
25. Srinivasan MA, Banerjee K, Pandya PG, Kotak RR, Pandya PM, Desai NJ, *et al*. Epidemiological investigations of an outbreak of infectious hepatitis in Ahmedabad city during 1975-76. *Indian J Med Res* 1978; **67**:197-206.
26. T.Mohana Lakshmia*, A.Vaithilingamb, A.Franklina, E Prabhakar Reddy The prevalence of serological markers of viruses causing acute hepatitis in south indian population. *Int J Biol Med Res*. 2011; **2**(4): 925-928.
27. Kaur R, Gur R, Berry N, Kar P. Etiology of endemic viral hepatitis in urban North India *Southeast Asian J Trop Med Public Health*. 2002; **33**(4): 845-8.
28. Tandon BN, Joshi YK, Tandon M. Acute liver

failure. Experience with 145 patients. *J Clin Gastroenterol* 1986; **8**: 664-668.

29. Ichhpujani R L , Riley I W, Duggal L, Kumari S, Gupta P S , Sehgal S. Ikeda H, Takasu M, Inoue K, Okamoto H, Miyakawa Y, Mayumi M. Infection with an unenveloped DNA virus (TTV) in patients with acute or chronic liver disease of unknown etiology and in those positive for hepatitis C virus RNA. *J Hepatol* 1999; **30**: 205-12.
30. Dharmadhikari C A, Kulkarni R D, Kulkarni V A ,Udgaonkar U S , Pawar S Singh S, Mohanty A, Joshi YK, Deka D, Mohanty S, Panda KS. Mother to child transmission of Hepatitis E virus. *Indian J Pediatr* 2003; **30**: 37-9.
31. Chattopadhyay S, Das BC, Gupta RK, Kar P. Presence of TT virus infection in chronic hepatitis patients from a hospital in New Delhi, India. *Indian J Med Res* 2005; **122**: 29-33.
32. Irshad M, Sharma Y, Dhar I, Singh J, Joshi YK. Transfusion transmitted virus in association with hepatitis A-E viral infections in various forms of liver diseases in India. *World J Gastroenterol* 2006; **12**: 2432-2436
33. Yzebe D, Xueref S, Baratin D, Bouletreau A, Fabry J, Vanhems P. TT virus. A review of the literature. *Panminerva Med* 2002; **44**: 167-77.
34. Irshad M, Chaudhuri PS, Joshi YK. Superoxide dismutase and total anti-oxidant levels in various forms of liver diseases. *Hepatol Res* 2002; **23**: 178-184.
35. Takashi K, Hoshino H, Ohta Y, Yoshida N, Mishiro S. Very 21. high prevalence of TT virus (TTV) infection in general population of Japan revealed by a new set of PCR primers. *Hepatol* 1998; **12**: 233-9.
36. Desai MM, Pal RB, Banker DD. Molecular epidemiology and 27. clinical implications of TT virus (TTV) infection in Indian subjects. *J Clin Gastroenterol* 2005; **39**: 422-9.