African Journal of Microbiology Research Vol. 6(32), pp. 6230-6234, 23 August, 2012
Available online at http://www.academicjournals.org/AJMR
DOI: 10.5897/AJMR12.119
ISSN 1996-0808 ©2012 Academic Journals

Full Length Research Paper

Study the prevalence of TT virus infection in South Iranian volunteer blood donors

Azinfar Sara[1], Kavoos Solhjoo[1], Abdol Reza Sotudeh Jahromi[1] and Ramin Yaghobi[2]*

[1]Jahrom University of Medical Sciences, Medicine School, Department of Microbiology, Jahrom, Iran.
[2]Shiraz Transplant Research Center-Shiraz University of Medical Sciences-Shiraz- Iran.

Accepted 8 March, 2012

Exposure to blood transfusion was associated with increase in the risk of transfusion transmitted (TT) viral infection and this led to the hypothesis that TT virus may be related to undefined post-transfusion hepatitis. Therefore in this study the prevalence of TT virus DNA in volunteer blood donors in relation to Alanine transaminase (ALT) and aspartate aminotransferase (AST) levels were evaluated. In a cross sectional study the blood samples were randomly collected from 499 volunteer blood donors. The genome of TT virus infection was evaluated in studied populations by an in-house semi-nested polymerase chain reaction (PCR) protocol. Some possible risk factors of TT virus infection including: age, gender, and AST and ALT levels were collected from volunteer blood donors. TT viral DNA was detected in the serum of 66 of 499 (13.4%) volunteer blood donors. The AST and ALT had significantly higher levels in volunteer blood donors with TT viremia in comparing with uninfected population. The adolescence (p=0.219) and gender (p=0.874) were not significantly correlated with increasing the prevalence of TT viremia. The moderate prevalence of TT viremia was diagnosed in volunteer blood donors in this part of Iran for the first time. Also define a significant association between TT virus infection and elevation of liver enzymes emphasize on the importance of this viral infection in undefined hepatic disorders that should be confirmed in completed studies.

Key words: Transfusion transmitted (TT) virus, alanine transaminase (ALT), aspartate aminotransferase (AST), volunteer blood donors.

INTRODUCTION

Transfusion transmitted (TT) virus originated through a search focused on viral causes of undefined post-transfusion hepatitis, idiopathic chronic liver disease and fulminant hepatic failure (Simons et al., 1995; Linnen et al., 1996; Nishizawa et al., 1997; Okamoto et al., 1998). TT virus previously classified under the Circovirus genus, get its new name: TorqueTenoVirus and placed under the Anellovirus genus belong to the Circoviridae family (Masuko et al., 1996; Prescott et al., 1998; Matsumoto et al., 1999). TT virus DNA detectable in liver tissues of infected patients in high titres than serum suggesting that TT virus is hepatotropic and can be found in a proportion of patients with different non-A-G hepatitis (Okamoto et al., 1998; Lopez-Alcorocho et al., 2000). The majority of TT virus infected patients had no biochemical or histological evidence of significant liver damage (Erensoy et al., 2002). TT virus infection was present in 1% of blood donors, 18% of patients with a history of exposure to blood products, and 4% of patients without parenteral risk factors. Prior exposure to blood transfusion was associated with increasing risk of TT virus infection (Charlton et al., 1998). TT virus prevalence in patient with various acute and chronic liver diseases was increased compared to that of blood donors from the same geographic localization (Okamoto et al., 1998; Charlton et al., 1998; Hafez et al., 2007; Suresh et al., 1999; Naumov et al., 1998). The TT viral DNA has been detected at comparable prevalence rates in the blood of healthy persons and patients and this led to the hypothesis that

*Corresponding author. E.mail: rayaviro@yahoo.com. Tel: +98-711-6476331. Fax: +98-711-6476331. Zip Code: 7193711351.

Table 1. The primer sequences of TT virus semi nested PCR protocol.

Primer	Sequence
Forward simple PCR primer (NG059)	5´ACA GAC AGA GGA GAA GGC AAC ATG 3´
Forward semi nested PCR primer (NG061)	5´GGC AAC ATG TTA TGG ATA GAC TGG 3´
Reverse primer (NG063)	5´CTG GCA TTT TAC CAT TTC CAA AGT T 3´

TT virus might be essentially non-pathogenic in nature (Pistello et al., 2001). The majority of individuals who become TTV-DNA-positive after blood transfusion usually have normal ALT and do not develop chronic hepatitis, although TT viremia frequently persists for several years (Irshad et al., 2008). TT virus infection has been reported from other different geographical areas of the world, including: Asia (Okamoto et al., 1998b; Tanaka et al., 1998; Orii et al., 1999), Europe (Hohne et al., 1998; Naoumov et al., 1998; Simmonds et al., 1998), Middle and South Africa (Prescott et al., 1998), North America (Charlton et al., 1998), and South America (Niel et al., 1999) and (Nakano et al ., 1999; Castro et al., 2007). Therefore in this study the occurrence of TT virus genome in volunteer blood donors in relation to alanine aminotransferase (ALT) and aspargine aminotransferase (AST) levels were evaluated.

MATERIALS AND METHODS

Population and samples

In this cross sectional study the EDTA-treated blood samples were randomly collected from 499 volunteer blood donors who admitted in the Jahrom blood transfusion center and related plasma samples were stored at -20°C till analyzing protocols were enrolled. Some possible risk factors of TT virus infection including: age, gender, and AST and ALT levels were collected from each studied population.

Biochemical indices

The levels of ALT and AST were evaluated in studied volunteer blood donors by related analyzing kits (Pars Azmoon-Iran) according to manufacturer instruction. The normal levels of ALT and AST that evaluated by these tests are 40U/Lit.

Molecular analysis of TT virus infection

DNA extraction protocol

The DNA genome of TT virus was extracted from all EDTA-treated blood samples of studied volunteer blood donors by DNP^{TM} Kit (CinnaGen-Iran) according to manufacturer instruction.

Semi nested PCR protocol

The presentation of TT virus-DNA genome was analyzed by an in-house semi nested-PCR protocol. The primer sequences which are used in the first simple PCR protocol were NG059 NG063 that amplifies a 286 bp fragment of TT virus genome located in the N22 on open reading frame 1 (ORF1) of the TTV genome (Table 1). In the simple polymerase chain reaction (PCR) protocol, the total volume of PCR master mix was containing: 5 µL of template DNA, 10 pmol/µL of primers, 0.25 mMol of dNTPs, 1U of Taq DNA polymerase, Tris-HCL 10 mM, KCl 30 mM, 1.5 mMol of $MgCl_2$, and 13 µL of distilled water. The primer sequences which are used in the second semi nested PCR protocol were NG061 and NG063 that amplify a 271 bp fragment of the N22 located on open reading frame 1 (ORF1) of the TTV genome (Table 1, Figure 1). The 5 µL of simple PCR product was used in second round of semi nested-PCR step with the same condition as simple PCR mix. The total volume per reaction in the two rounds of simple and nested PCR protocols was 20 µL.

The thermocycling condition of simple PCR step was initiated with a first round at 94°C for 10 min followed by a second round of 35 cycles at 94°C for 30 s, 60°C for 45 s, and 72°C for 45 s, and finalized with extension at 72°C for 7 min. The semi nested PCR protocol was initiated with a first round at 94°C for 10 min followed by a second round of 30 cycles at 94°C for 30 s, 59°C for 45 s, and 72°C for 45 s, and finalized with extension at 72°C for 7 min.

Statistical analysis

The significant relationships of molecular prevalence of TT virus infection in volunteer blood donors with possible studied risk factors were analyzed by use of parametric and non parametric analyses with SPSS for Windows (version 15, Chicago, IL, USA). A level of statistical significance was accepted as $p \leq 0.05$.

RESULTS

The 467 of 499 (93.6%) volunteer blood donors were male and 32 of 499 (6.4%) of them were female. The age distribution of studied population was between: 17-68 years old with mean of 35.8±9.76. The widespread blood born viral infective markers including: HBSAg, HCVAb, HIVAb, and VDRL (PRP), were all negative in studied blood samples.

TT viremia

TT virus genome was detected in the serum of 66 of 499(13.4%) volunteer blood donors by semi-nested PCR protocol. The 433 of 499 (86.6%) volunteer blood donors were not shown TT viremia in their related serum samples (Table 2).

TT viremia and risk factors

The age ($p=0.219$) was not significantly correlated with

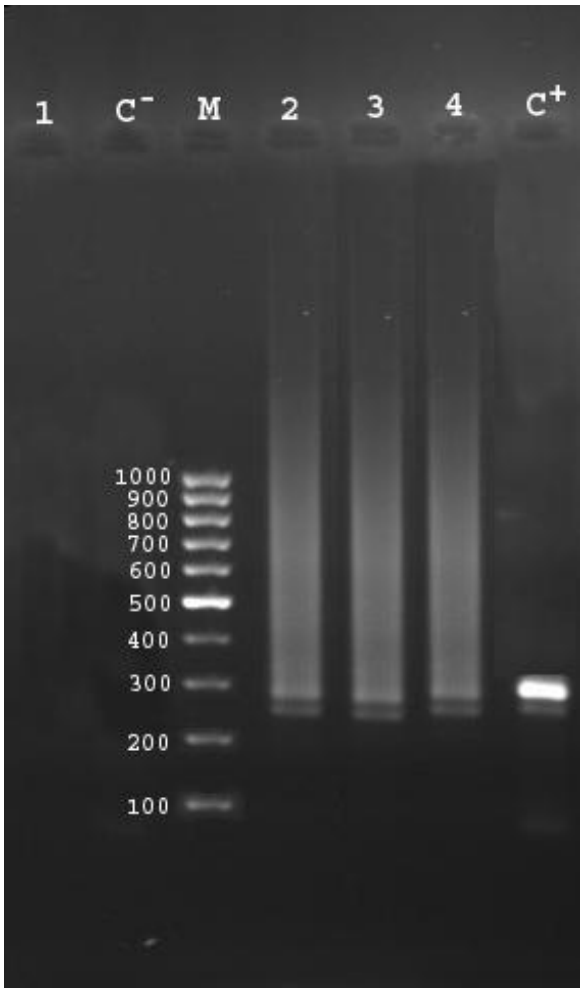

Figure 1. Electrophoresis of semi-nested PCR products in healthy blood donors. C+= Positive control, C- = Negative control, 1= Negative sample, 2, 3, 4= Positive samples, M= 100 bp DNA Ladder.

Table 2. Frequency of TT virus DNA in the serum of volunteer blood donors.

Percent	Number	TT virus DNA
13.40	66	Positive
86.60	433	Negative
100	499	Total number

increasing the prevalence of TT viremia. On the other hand, 94% of studied blood donors were male and rest of them were female. Therefore calculation of significant association between sex distribution and infectivity with TT virus was not possible. Also the adolescence was not significantly correlated with abnormal increasing of the AST (p=0.532) and ALT (p=0.784) levels. The sex was not significantly correlated with abnormal increasing of the AST (p=0.218) and ALT (p=0.417) levels.

TT viremia and AST/ALT Levels

The AST level was significantly higher level in volunteer blood donors with TT viremia in comparing with uninfected population (Table 3). The ALT level was significantly higher level in volunteer blood donors with TT viemia in comparing with uninfected population (Table 3).

DISCUSSION

Efforts focused on identification of TT viral infection have been made in the last 3 decades about reducing the risk of transmitting viral infections through donation of blood and blood derivates (Pisani et al., 2000; Vrielink et al., 1998). This exploration which is an example of how many unidentified viruses may detected in blood donations, led to the hope that TT virus may has role in presentation of significant proportion of idiopathic hepatitis (Pisani et al., 2000). Therefore the prevalence of TT viremia in volunteer blood donors of Jahrom in south of Iran was evaluated in relation to liver enzymes. In this study TT viremia was found in the 13.4% of volunteer blood donors. These results are in the line or controvert with the results of other earlier reports presented from different geographical regions of Iran. In Ahwaz (located in southern of Iran) 23.7% of volunteer blood donors were infected by TT virus. But similar to this study the HBSAg, HCVAb, and HIVAb were not found in any of studied populations (Jalalifar et al., 2007; Zandieh et al., 2005). In Tabriz (located in North West of Iran) TT viremia was found in 65% of blood donors TT viremia was found in 65% of blood donors (Bouzari et al., 2009). In Isfahan (located in center of Iran) 105 of 132 of blood donors were infected by TT virus DNA (Bouzari et al., 2007). Diagnosis of different prevalence of TT viremia in volunteer blood donors reported from different geographical parts of Iran is related to different populations, sample size, and/or molecular diagnostic protocols with variable sensitivity and specificity (Hafez et al., 2007). World-wide distribution of TT viremia in volunteer blood donors reported with a range between: 1.9-64% (Okamoto et al., 1998; Simmonds et al., 1998; Niel et al., 1999; Desai et al., 1999). TT viral infection in blood donors of other countries with different geographical regions are reported as follow: In Italy and French ranging from 18.6 to 22% (Seemayer et al., 2001; Masia et al., 2001; Katsouliou et al., 2001). In US ranging from 1-12.8% and in UK ranging from 1.9-10% (Simmonds et al., 1998; Naoumov et al., 1998; Charlton et al., 1998), in Japan ranging from 12-26% (Yamada et al., 1998), in the north of China 31% and in the south of China 22% (Chuan et al., 1999), in Korea 8.2% (Mee et al., 2003), in Germany ranging from 3.2-13% (Wolff et al., 2000; Seemayer et al., 2001), in Egypt ranging from 20 -29.4% in Egypt (Gad et al., 2000), in Thailand 36% (Tanaka et al., 1998), in South American 10-62% (Niel et.

Table 3. The levels of AST/ALT and TT virus Infection.

Biochemical indices (U/Lit)	TT virus DNA		p value
	Positive (U/Lit)	Negative (U/Lit)	
ALT Level	60.01± 32.3	21.7± 23.1	0.001
AST Level	55.3 ± 32.2	20.9 ± 18.8	0.001

al., 1999). Also TT virus infection reported from other different geographical areas of the world (Okamoto et al., 1998b; Tanaka et al., 1998; Orii et al., 1999; Hohne et al., 1998).

In this study the ALT and AST liver enzymes increased significantly in blood donors with TT viremia. Similarly, earlier studies reported the association between elevated levels of ALT and AST with infectivity of TT virus in volunteer blood donors (Bouzari et al., 2009; Ukita et al., 1999). But other Iranian reports and also some reports from other countries could not find an association between elevation of liver enzymes with TT viremia (Zhong et al., 2001; Bouzari et al., 2009; Seemayer et al., 2001).

Conclusion

The moderate prevalence of TT viremia was diagnosed in volunteer blood donors of this part of Iran for the first time. Also define a significant association between TT virus infection and elevation of liver enzymes emphasize on the importance of this viral infection in undefined hepatic disorders that should be confirmed in completed studies.

REFERENCES

Bouzari M, Mokhtarzadeh A (2009). Frequency of TTV and its role in HBV and HCV infected blood donors in East Azarbayejan province of Iran. S.J.I.B.T.O., 5(4): 247-255.

Bouzari M, Shaykh Baygloo N, Zandieh T (2007). Prevalence of TT virus in general population of Isfahan. Hakim. Res. J. 9(4): 52-58.

Castro Amarante M, Kashima S, Tadeu Covas D (2007). TT virus (TTV) genotyping in blood donors and multiple transfused patients in Brazil. Virus Genes, 35: 503–509.

Charlton M, Adjei P, Poterucha J, Zein N, Moure B, Therneau T, Krom R, Wiesner R (1998). TT-virus infection in North American blood donors, patients with fulminant hepatic failure, and cryptogenic cirrhosis. Hepatology, 28(3): 839–842.

Chuan HE, Nomura F, Yukimasa N, Itoga S, Yamada-osaki M, Sumazaki R , Nakai T (1999). Transfusion-transmitted virus infection in China: Prevalence in blood donors and in patients with liver diseases. J. Gastroenterol. Hepatol., 14: 899–903.

Cleavinger PJ, Persing DH, Li H, Moore SB, Charlton MR, Sievers C, Therneau TM, Zein NN (2000). Prevalence of TT virus infection in blood donors with elevated alt in the absence of known hepatitis Markers. Am. J. Gastroenterol., 95(3): 772-776.

Desai SM, Muerhoff AS, Leary TP, Erker JC, Simons JN, Chalmers ML, Birkenmeyer LG, Pilot-Matias TJ, Mushahwar IK (1999). Prevalence of TT virus infection in US blood donors and populations at risk for acquiring parenterally transmitted viruses. J. Infect. Dis., 179: 1242-1244.

Erensoy S, Sayıner A.A, Turkoglu S, Canatan D, Akarca U.S, Sertoz R, Ozacar T, Batur Y, Badur S, Bilgiç A (2002). TT Virus Infection and Genotype Distribution in Blood Donors and a Group of Patients from Turkey. Infect. 30(5): 299-302.

Gad A, Tanaka E, Orii K, Kafumi T, Serwah AE, El-Sherif A,Nooman Z, Kiyosawa K (2000). Clinical significance of TT virus infection in patients with chronic liver disease and volunteer blood donors in Egypt. J. Med. Virol. 60: 177-181.

Hafez MM, Shaarawy SM, Hassan AA, Salim RF, Abd El Salam FM, Ali AE (2007). Prevalence of transfusion transmitted virus (TTV) genotypes among HCC patients in Qaluobia governorate. Virol. J. 4(135): 1-6.

Hohne M, Berg T, Muller AR, Schreier E (1998). Detection of sequences of TT virus, a novel DNA virus, in German patients. J. Gen. Virol. 79: 2761–2764.

Irshad M, Singh Sh, Irshad Kh, Kumar AS, Kumar JY (2008). Torque teno virus: Its prevalence and isotypes in North India. World J. Gastroenterol., 14(39): 6044-6051.

Jalali FMA, Zandieh T, Imam SJ, Galehdari H, Pourfathollah AA, Baba Ahmadi M (2007). TTV prevalence in Ahwaz blood donors by using semi-nested PCR. S.J.I.B.T.O., 3(5): 389-395.

Katsouliou A, Paraskevis D, Anastassopoulou CG, Chryssou SE, Sypsa V, Boletis J, Malliori M, Tassopoulos NC, Hatzakis A (2001) Prevalence and genotypic distribution of TT virus in Athens, Greece. J. Med. Virol., 65: 423-429.

Linnen J, Wages J Jr, Zhang-Keck ZY, Fry KE, Krawczynski KZ, Alter H, Koonin E, Gallagher M, Alter M, Hadziyannis S, Karayiannis P, Fung K, Nakatsuji Y, Shih JWK, Young L, Piatak M Jr, Hoover C, Fernandez J, Chen S, Zou JC, Morris T, Hyams KC, Ismay S, Lifson JD, Hess G, Foung SKH, Thomas H, Bradley D (1996). Margolis H, Kim JP. Molecular cloning and disease association of hepatitis G virus: a transfusion-transmissible agent. Science, 271: 505-508.

Lopez-Alcorocho JM, Mariscal LF, de Lucas S, Rodriguez-Inigo E, Casqueiro M, Castillo I, Bartolomé J, Herrero M, Manzano ML, Pardo M, Carreno V (2000). Presence of TTV DNA in serum, liver and peripheral blood mononuclear cells from patients with chronic hepatitis. J. Viral. Hepat., 7: 440–447.

Masia G, Ingianni A, Demelia L, Faa G, Manconi PE, Pilleri G, Ciancio A, Rizzetto M, Coppola RC (2001). TT virus infection in Italy: prevalence and genotypes in healthy subjects, viral liver diseases and asymptomatic infections by parenterally transmitted viruses. J. Viral. Hepat., 8: 384-390.

Masuko K, Mitsui T, Iwano K, Yamazaki C, Okuda K, Meguro T, Murayama N, Inoue T, Tsuda F, Okamoto H, Miyakawa Y, Mayumi M (1996). Infection with hepatitis GB virus C in patients on maintenance haemodialysis. N. Engl. J. Med., 334: 1485-1490.

Matsumoto A, Yeo AET, Shih JWK, Tanaka E, Kiyosawa K, Alter HJ (1999). Transfusion-associated TT virus infection and its relationship to liver disease. Hepatol. 30: 283-288.

Mee JJ, Jong HS, Soon PS, Young CL, Dong WR (2003). TT virus and hepatitis G virus infections in Korean blood donors and patients with chronic liver disease. World J. Gastroenterol., 9(4): 741-744.

Mushahwar K (1999). Prevalence of TT Virus Infection in US Blood Donors and Populations at Risk for Acquiring Parenterally Transmitted Viruses. J. Infect. Dis., 179: 1242–1244.

Nakano T, Park Y, Mizokami M, Choi J, Orito E, Ohno T, Kato T, Kondo Y, Tanaka Y, Kato H, Kato T , Kim B (1999). TT virus infection among blood donors and patients with non-B, non-C liver diseases in Korea. J. Hepatol., 30: 389-393.

Naoumov NV, Pertova EP, Thomas MG, Williams R (1998). Presence

of a newly described human virus (TTV) in patients with liver disease. Lancet., 352: 195–197.
Niel C, de Oliveira JM, Ross RS, Gomes SA, Roggendorf M, Viazov S (1999). High prevalence of TT virus infection in Brazilian blood donors. J. Med. Virol. 57: 259–263.
Nishizawa T, Okamoto H, Konishi K, Yoshizawa H, Miyakawa Y, Mayumi M (1997). A novel DNA virus (TTV) associated with elevated transaminase levels in posttransfusion hepatitis of unknown etiology. Biochem. Biophys. Res. Commun. 241: 92-7.
Okamoto H, Nishizawa T, Kato N, Ukita M, Ikeda H, Iizuka H, Miyakawa Y, Mayumi M (1998). Molecular cloning and characterization of a novel DNA virus (TTV) associated with post transfusion hepatitis of unknown etiology. Hepatol. Res. 10: 1-16.
Orii K, Tanaka E, Umemura T, Rokuhara A, Iijima A, Yoshisawa K, Imai H, Kiyosawa K(1999). Prevalence and disease association of TT virus infection in Japanese patients with viral hepatitis. Hepatol. Res. 14: 161–170.
Pisani G, Antigoni IV, Bisso G, Wirz M, Iudicone P, Miceli M, G Entlli G (2000). Prevalence of TT viral DNA in Italian blood donors with and without elevated serum ALT levels: molecular characterization of viral DNA isolates .Haematol. 85: 181-185.
Pistello M, Morrica A, Maggi F, Vatteroni ML, Freer G, Fornai C, Casula F, Marchi S, Ciccorossi P, Rovero P, Bendinelli M (2001) . TT virus levels in the plasma of infected individuals with different hepatic and extrahepatic pathology. J. Med. Virol. 63: 189-195.
Pourshams A, Azimi K, Kiani L, Sarrafi M, Farhadi LM, Malekzadeh R (2004).TT Virus Infection Among Iranian Blood Donors: Its Prevalence and Relationship to Serum Alanine Aminotransferase (ALT) Level. Govaresh. 9(2): 106-109.
Seemayer CA, Viazov S, Neidhart M, Brühlmann P, Michel BA, Gay RE, Roggendorf M, Gay S (2001). Prevalence of TTV DNA and GBV-C RNA in patients with systemic sclerosis, rheumatoid arthritis, and osteoarthritis does not differ from that in healthy blood donors. Ann. Rheum. Dis. 60: 806-809.
Simons JN, Leary TP, Dawson GJ, Pilot- Matias TJ, Muerhoff AS, Schlauder GG, Desai SM, Mushahwar IK (1995). Isolation of novel virus-like sequences associated with human hepatitis. Nat. Med. 1: 564 -570.
Simmonds P, Davidson F, Lycett C (1998). Detection of a novel DNA virus (TTV) in blood donors and blood products. Lancet., 352: 191-195.
Tanaka H, Okamoto H, Luengrojanakul P, Chainuvati T, Tsuda F, Tanaka T, Miyakawa Y, Mayumi M (1998). Infection with an unenveloped DNA virus (TTV) associated with post-transfusionnon-A to G hepatitis in hepatitis patients and healthy blood donors in Thailand. J. Med. Virol. 56: 234–238.
Ukita M, Okamoto H, Kato N, (1999). Excretion into bile of a novel unenveloped DNA virus (TT virus) associated with acute and chronic non-A-G hepatitis. J. Infect. Dis. 179: 1245-8.
Wolff C, Diekmann A, Boomgaarden M, Korner MM, Kleesiek K (2000). Viremia and excretion of TT virus in immunosuppressed heart transplant recipients and in immunocompetent individuals. Transplant. 69: 351-356.
Yamada OM, Sumazaki R, Noguchi E (1998). Transfusion- transmitted virus. Lancet., 352: 1309–1310 (Correspondence).
Zandieh T, Babaahmadi B, Pourfathollah A, Galedari H, Emam J, Jalalifar MA (2005).Transfusion Transmitted virus (TTV) infection in Thalassemic Patients. Iran. J. Publ. Health, 34(4): 24-28.