

BRIEF COMMUNICATION

NEW PREVALENCE ESTIMATE OF TT VIRUS (TTV) INFECTION IN LOW- AND HIGH-RISK POPULATION FROM SÃO PAULO, BRAZIL

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SUMMARY

The prevalence of TT virus (TTV) infection was investigated by Polymerase Chain Reaction (PCR) in low- (blood donors and healthy children/adolescents) and high-risk (hemophiliacs) groups from São Paulo, Brazil. Primers based on the untranslated region (UTR) of the viral genome proved to be much more ubiquitous, leading to much higher frequencies for both groups ($\geq 81\%$) than the earlier N22-PCR directed to the open reading frame 1 (blood donors, 5.5%, and hemophiliacs, 42.3%). The UTR-PCR also revealed an interesting profile for healthy children/adolescents: very high prevalence at the early years and significant decrease in male teenagers. The N22-PCR, in turn, demonstrated higher frequency in hemophiliacs treated with fresh blood products (58%), than in those treated with virus-inactivated clotting factors (9.4%) and blood donors (5.5%).

KEYWORDS: TT virus; Emergent viruses; Parenteral transfusion; Blood bank

The novel unenveloped single-stranded DNA virus - TTV - has a high infectious capacity by both parenteral and non-parenteral route and, yet unclearly, has been associated with liver disease of unknown etiology^{5-7,10}.

Early studies in Japan have been performed by PCR amplification based on the N22 region within the open reading frame 1 of the viral genome, and TTV infection was found in approximately 50% of non A-G fulminant or chronic hepatitis patients compared to 12% of blood donors⁵.

In contrast, when PCR primers were deduced from the untranslated region (UTR), a much higher frequency (92%) was found in healthy Japanese individuals¹¹. In Brazil, early studies were based on the N22 region^{3,8} and, more recently, TTV genotyping was based on the UTR in a population of healthy subjects⁴.

Our blood center at São Paulo is the largest of Latin America (*c.a.* 300,000 donations/year), and the present work is part of a monitoring program to look for new blood-transmissible agents, which may be hazardous for recipients. Therefore, soon after TTV was first identified, we began an investigation on TTV infection in our blood donors and hemophiliac population, and included children/adolescents without history of blood transfusion or liver disease. TTV DNA was detected by PCR amplification directed to the N22 region⁵ and UTR¹¹.

The overall prevalence of TTV infection based on the UTR-PCR for the blood donors and hemophiliacs was considerably higher than the frequencies obtained for the N22 region, $p < 0.02$ and $p < 0.05$, respectively (Table 1). This discrepancy may reflect the higher variability in the region complementary to the primers employed for the N22-region amplification, what would not be surprising in view of the high genetic variability described for TTV^{1,6}. However, one could argue that the N22-PCR reaction is not as sensitive as the UTR-based one. This question was well assessed by OKAMOTO *et al.*, by means of a titration experiment with two cloned TTV DNAs, in which the two sets of primers (N22 and UTR) could, equally, detect nearly a single copy of the target DNA⁶. Furthermore, another possible reason for the diverse figures obtained for both UTR- and N22- based PCR reactions may be the variation in viral titers among TTV strains in mixed infections, as previously reported^{4,7}.

The difference observed between the N22 estimates for blood donors and hemophiliacs ($p < 0.02$; Table 1) can be explained by the high parenteral exposure of the latter group. Furthermore, among hemophiliacs who had been treated with fresh blood products, the frequency of the N22-specific TTV was remarkably higher (58.0%; $p < 0.001$) than those treated with virus-inactivated clotting factor (9.4%), those who were not treated (11.1%) and blood donors (5.5%).

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Table 1
Frequency of TTV infection by N22- and UTR-PCR in different Brazilian groups

Group (age in years)	N22	UTR-A	
	Positive/total (%)	Positive/total (%)	Positive/total (%)
Blood donors* (16-61)	22/401 (5.5) ^a	168/197 (85.3) ^c	
Hemophiliacs [#] (0.6-81)	55/130 (42.3) ^b	127 /130 (97.7) ^d	
Children/adolescent [‡] (2-17)	not done	112/138 (81.2)	
		Male	Female
	(2-4)	12/13 (92.3)	8/8 (100)
	(5 - 9)	17/20 (85.0)	21/24 (87.5)
	(10 - 14)	18/21(85.7)	20/26 (76.9)
	(15 - 17)	7/15 (46.7) ^a	9/11 (88.8)

a vs b and a vs c $p < 0.02$; b vs d $p < 0.05$; [‡] there was a significant decrease between the 15 - 17 year-old group and the younger-male groups ($p < 0.05$). There was no significant difference between the remaining group combinations. *Serum samples collected from consecutive blood donors attending the FPS/HSP, from July to August, 1997. [#]Sera from hemophiliac patients attending the Hemophiliac centers of FPS/HSP and the "Hospital das Clínicas" of the University of São Paulo, from August, 1997, to August, 2000. [‡]Sera from a previous cross-sectional study to determine the prevalence of viral hepatitis in the city of São Paulo²; samples collected at random from February to May, 1996. Statistical significance ($\geq 95\%$) was analyzed by the Fisher's exact test.

Hence, it is possible that the acclaimed causal association between TTV and some illness-related group is, at least in part, due to parenteral exposure. Yet, there may be particular pathogenic variant(s) worth searching for.

There was no significant difference between the TTV frequencies observed for the three groups based on the UTR-PCR. Furthermore, the distribution of TTV infection according to age within the studied groups showed no significant difference, except for the children, which displayed an interesting profile (Table 1). Prevalence was remarkably high in children aging 2 to 4 years (95%) - decreasing continuously thereafter and reaching a lower prevalence (47%; $p < 0.02$) in 15 to 17 year-old male gender adolescents. The design and data of the present study allow no explanation for this yet unknown phenomenon, that is contrary to other report⁹; this discrepancy may be due to differences in origin and size of the populations on both studies.

Our findings reinforce that TTV infection is widespread among Brazilians, as so is for Japanese populations¹¹. Moreover, the high prevalence of the UTR-specific isolates found for both high- and low-risk groups indicates that the parenteral route of transmission may not play a major role for this infection and, as a rule, TTV is benign - requiring no systematic screening in blood banks.

RESUMO

Nova estimativa da prevalência da infecção pelo vírus "TT" (TTV) em populações de baixo e alto risco de São Paulo, Brasil

A prevalência da infecção pelo vírus "TT" (TTV) foi investigada pela técnica da Reação da Polimerase em Cadeia (PCR) em grupos considerados de baixo risco (doadores de sangue e crianças/adolescentes

saudáveis) e de alto risco de exposição parenteral (hemofílicos); todos provenientes da cidade de São Paulo. Oligonucleotídeos empregados como *primers*, homólogos à região não traduzível (UTR) do genoma viral, mostraram-se muito mais universais, revelando frequências muito mais altas em ambos os grupos ($\geq 81\%$) do que os *primers* anteriormente utilizados, baseados na região genômica traduzível "N22" (doadores de sangue, 5,5%, e hemofílicos, 42,3%). O "PCR-UTR" também revelou um perfil interessante em crianças/adolescentes saudáveis: alta prevalência nos primeiros anos de vida e queda significativa em meninos adolescentes. O "PCR-N22", por sua vez, apresentou alta frequência em hemofílicos que receberam derivados de sangue fresco (58%) relativa àqueles que foram tratados com fatores de coagulação submetidos à inativação viral (9,4%) e doadores de sangue (5,5%).

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