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HEPATITIS G VIRUS;
REPLICATION SITES DETECTION IN DIFFERENT HUMAN TISSUES

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ABSTRACT... nasir-lhr@yahoo.com The total forty five samples were tested for the presence of hepatitis G virus (HGV) positive (genomic) and negative (replicative) strand by RT-PCR. These samples were divided into three groups containing 15 each. Group I contained blood samples only, group II had blood samples along bone marrow samples of the same patients and group III contained different tissue samples collected from autopsy cases. In group I no sample was found out to be HGV positive while in group II 6.66 % samples were HGV positive and replicative strand was detected only in bone marrow tissue. In group III, 13.33 % samples were found to be HGV positive and in two positive samples of group III, replicative strand was detected in blood, bone marrow and spleen tissue. These results suggested that blood, bone marrow and spleen are the major replication sites of hepatitis G virus.

INTRODUCTION

Hepatitis G Virus (HGV)/GB virus C is a newly described virus associated with hepatitis in humans. Its pathogenesis and site of replication in humans is still controversial. It was first discovered in 1996[1]. HGV is a positive, single stranded RNA virus that shares about 25-40% homology with HCV[7]. HGV genome organization was found to be similar to that of HCV with a single open reading frame as well as 5' and 3' untranslated regions[8]. In addition the analysis of the predicted amino acid sequences indicated the presence of structural and non-structural proteins as well as number of putative proteolytic cleavage sites in a relative position found in HCV. So it can be assumed that HGV replicates through negative strand RNA and hence its presence can be regarded as a direct evidence of viral replication[11].

The role of HGV as a hepatitis virus also remains controversial[13] however some early reports suggested an association between HGV and fulminant hepatitis[16] but

others have not confirmed this association[12].

The 5' terminal region of HGV containing elements necessary for the regulation of viral gene expression is not known. The data from some studies shed light on the mechanism of viral replication[9] but the site of replication is still not found clearly[14]. The major obstacle in the study of HGV replication site is the lack of availability of multiple tissue samples from the infected individual[15]. Therefore recent study was planned to find out the site of replication of HGV in multiple tissue samples from Lahore and other areas of the country.

MATERIALS & METHODS

Grouping of samples

The samples were collected from Lahore, Faisalabad and other areas of the country. These samples were categorized under three groups containing 15 samples each. Group I contained blood samples alone. Group II contained blood samples as well as bone marrow samples from the same patients while group III contained different tissue samples (bone marrow, spleen, liver, brain, blood and intestine) from autopsy cases.

- **RNA extraction**

RNA was extracted from tissue samples and blood by using Gentra isolation kit (purescript) protocol. For RNA isolation from blood mononuclear cells, 0.3 ml of the blood sample was used while 0.025 g of tissue was used for RNA isolation purposes.

- **RT-PCR**

RT-PCR (Reverse Transcriptase Polymerase Chain Reaction) was carried out by using M-MLV (Molony Murine Leukemia virus) reverse transcriptase enzyme first in cDNA synthesis and then set of inner as well as outer sense and antisense primers to amplify desired cDNA sequence. The total volume of reaction mixture for cDNA synthesis was 0.019 ml and contains (FSB 1X (First strand buffer), dNTPs 0.001M, DTT 0.002 M, Outer sense primer 10 pM (Pico mole), M-MLV reverse transcriptase 200 units and extracted RNA 0.03 g). The remaining total volume was adjusted with distilled water. This mixture for different samples was incubated at 37 ^{0}C for fifty minutes and then at 94 ^{0}C for 4 minutes for inactivation of reverse transcriptase enzyme.

The synthesized cDNA was then subjected to first round PCR for (+) strand detection. The first round PCR mixture contained (PCR buffer 0.8 X, $MgCl_2$ 0.0024 M, dNTPs 2(10^{-6}) M, Outer sense and antisense primer 10 pM each, Taq polymerase 1 unit and cDNA 0.004 ml). The remaining volume was adjusted up to 0.025 ml with distilled water. This PCR mixture was subjected to different denaturation, annealing and polymerization conditions and most optimized condition was found to be 95 ^{0}C for 4 minutes, 62 ^{0}C for 1.5 minutes and then 35 cycles of (94 ^{0}C for 50 seconds, 62 ^{0}C for 50 seconds, 72 ^{0}C for 45 seconds) followed by 72 ^{0}C for 12 minutes. At the end cool the samples to 4 ^{0}C so that further enzyme activity can be prevented. The product size was 307 bp.

The first round PCR product was then subjected to second round PCR (nested PCR). The second round PCR mixture also contained the same components in same concentrations as in first round PCR mixture except here first round PCR product volume used was 2 µl. In nested PCR similar temperature conditions were provided as for first round PCR. The product size of this round was 202 bp.

The RT-PCR for (-) strand (replicative strand) detection was carried out only for those samples in which (+) strand was already detected. The cDNA reaction mixture contained same components with concentrations as already mentioned for (+) strand detection but only difference is that here instead of outer antisense primer, outer sense primer was used. Remaining conditions and components of reaction mixtures of first and second round PCR were same as for (+) strand detection.

- **AGAROSE GEL ELECTROPHORESIS**

The PCR product was analyzed by agarose gel electrophoresis. Agarose gel of 2 % concentration of agarose was used while for visualization of RT-PCR product ethidium bromide 0.01 ml (from 10 mg/ ml stock solution) was added in 100 ml agarose gel.

RESULTS & DISCUSSION

The molecular analysis of different human tissues was carried out for the detection of HGV replicative (-) strand by RT-PCR (Table I). The samples were categorized in three groups viz. I, II, III containing 15 samples each. The blood samples were isolated from group I while from group II bone marrow samples were also isolated along blood samples. These two groups consisted of live patients. However, in group III persons (autopsy cases) different tissue samples were isolated viz. blood, bone marrow, spleen, liver, brain and intestine. The different human tissues were selected because there exist still controversy regarding the site of replication of this HGV[14,10].

Table I. Analysis of different tissue samples for the presence of genomic and replicative strand of hepatitis G virus by RT-PCR.

Group	Total No.of Samples tested	HGV +ve samples	HGV +ve samples %	Case No.	Replicative (-) strand detection					
					Blood mono nuclear cells	Bone marrow	Spleen	Liver	Brain	Intestine
I	15	-	-	-	-	ND	ND	ND	ND	ND
II	15	1	6.66	-	-	0	ND	ND	ND	ND
III	15	2	13.33	I	0	0	0	-	-	-
				II	0	0	0	0	0	-

The most earlier workers suggested that this virus replication site is bone marrow, liver or blood[4,5,6] but recent study suggested that brain and spleen may also be possible sites of replication in addition to bone marrow, blood and liver (Fig. 1). So this HGV is not essentially a hepatocyte as reported earlier.

The replicative (-) strand detection was carried out only in those samples in which (+) strand was already detected by RT-PCR (Fig. 1) because HGV is a positive stranded RNA virus which replicate via (-) strand synthesis[8]. For replicative strand detection outer sense primer instead of outer anti sense primer was used because if (-) strand is present in that tissue, only then cDNA can be synthesized in the presence of M-MLV reverse transcriptase enzyme other wise in case of (+) strand the primer is not complementary to it and hence no cDNA can be made resulting in no amplification in RT-PCR.

The samples were collected from different cities of Punjab and obtained data indicated that this virus is gradually spreading in population possibly due to practice of folk medicines and blood transfusions. This observation is in agreement with Bowden & Dawson et al[2,3]. The blood transfusion is perhaps the major cause because the blood is not screened for HGV in the country. So, the recent study not only describes the possible site of replication but also indicates the possible reasons of this virus spread in the country.

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Life Is a Tragedy for Those Who Feel, and a Comedy for Those Who Think.

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