Research article

Polymorphism within the 5'UTR genomic region of hepatitis G virus (HGV) isolated from beta-thalassemia population in Iraq

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ABSTRACT

Introduction and Aim: Beta-thalassemia patients develop chronic infections due to the hepatitis G virus (HGV), due to frequent blood transfusions. This study aimed to isolate these viruses from β-thalassemia Iraqi patients and investigate into the 5'UTR genomic region of the virus to investigate the prevalent genotypes in this region.

Materials and Methods: The study included 154 beta-thalassemia patients. Blood samples were collected from each individual participating in this study. Genomic RNA was isolated and subjected to cDNA synthesis. The 5′ untranslated region (5′ UTR) of the DNA was amplified by polymerase chain reaction using specific HGV primers and sent for sequencing. The sequences were genotyped using bioinformatics tools.

Results: The results showed hepatitis G virus infection to be prevalent in 18.2% of the beta-thalassemia patients. Sequencing and alignment of the HGV 5'UTR sequences showed several nucleotide variations. A phylogenetic analysis revealed the following HGV genotypes to infect beta-thalassemia patients genotype 4(58.3%), genotype 2b (33.3%) and genotype 1b (8.3%).

Conclusion: Genotyping of the 5'UTR region of the HGV gene showed the genotypes 1b, 2b and 4 to be prevalent among beta-thalassemia patients in Iraq.

Keywords: Beta-thalassemia; polymorphism; Hepatitis G virus; 5'UTR; genotypes.

INTRODUCTION

Thalassemia is a disease condition where changes in the hemoglobin gene (HBB) lead to a quantitative change in the amount of hemoglobin produced in the body. Thalassemia is an autosomal recessive condition and an increasing global health problem (1). Different types of thalassemia may be distinguished by the globin chain (s) that is under-produced. Types α, β, δβ, δ, and γδ β- thalassemia have been identified as the most common forms. β-thalassemia causes severe anemia and therefore they are reliant on blood transfusions as a necessary treatment option (2, 3). Transfusion-transmitted infections, particularly hepatitis G virus (HGV) infection, are the major problems associated with blood transfusion in thalassemic patients (4, 5). The HGV is an enveloped, positive-sense-stranded RNA virus belonging to the *Flaviviridae* family (6). The HGV genome size is approximately 9.4 kb in length with its RNA organization consisting of 2 structural and 5 non-structural genes located between the genomic 5' UTR region and the 3' UTR end (6, 7). HGV is closely related to the Hepatitis C virus (HCV) and can be transmitted via blood transfusion, intravenous drug use, hemodialysis, and vertical transmission (6). HGV is widespread across the globe with isolates from various regions of the world reported to differ genetically from one another (8). Based on genetic heterogeneity, the virus has been classified into several genotypes and subtypes. Epidemiological studies have revealed genotype 1 to be found in Japan, USA and West Africa (9), genotype 2 (subtypes 2a and 2b) in the United States, Europe and East Africa (10), genotype 3 in Asia (11, 12), genotype 4 in Australia, Myanmar and Vietnam (13, 14), genotype 5 in Japan and South America (9, 15) and genotype 6 in Indonesia (16).

Although HGV is highly prevalent in Iraq, there has been no reports on the circulating genotypes in this region. Hence, the purpose of this study was to investigate the prevalence of HGV infection in transfusion-dependent β-thalassemia patients in Iraq, as well as look into the genotype distribution among these Iraqi thalassemia patients.

MATERIALS AND METHODS

Sample collection

Patients with β-thalassemia were recruited from the Ibn AL-Baladi Hospital, Maternity and Children's Hospital and the Al-Karama Teaching Hospital, Baghdad, conducted between February and May 2014. The study included 154 participants (87 males and 67 females) aged between 18-50 years requiring frequent blood transfusions. The patients underwent routine testing to determine whether or not they were infected with HGV.

Ethical approval

Participants were informed of the study and a written consent was obtained. The study was approved by the Ethics Committee at the College of Medicine at Al-Mustansiriyah University, the Ethics Committee at the Al-Karama teaching hospital under the Iraqi Ministry of Health, and the Ethics Committee at Baghdad's Ibn Al-Baladi Hospital Maternity and Children's Hospital. Blood (5ml) was drawn by venipuncture from each participant and stored at -20 C^o for further analysis.

RNA extraction, cDNA synthesis and Polymerase Chain Reaction (PCR)

Total RNA was extracted from whole blood using the RNA isolation QIAamp kit (QIAGEN, USA), in accordance to the manufacturer's instructions. The RNA was converted into cDNA with GOScript Reverse transcriptase system (Promega, USA). Viral cDNA in plasma sample was detected using oligonucleotide primers deduced from highly conserved 5'UTR region of genome of HGV. The reaction solution was used as templates for polymerase chain reaction (PCR). HGV 5'UTR regions were amplified using specific primers as follow: HGV-Forward (G1) 5´CGGCCAAAAGG TGGATGGATG-3´and HGV-Reverse (G2): 5´CGAG GAGCCTGAGGTGGGG3´ which amplified a product size of 185bp (17). The PCR thermocycling were applied in medicine area (18, 19). However the PCR assay was performed with Hot start PCR using a Labnet Thermo cycler (USA). conditions were as follows (40 cycles): initial denaturation at 95°C for 1 min, 55°C for 45 s for annealing and 72°C for 1 min for extensions, and final extension for 4 min. at 72°C. The amplified products were resolved by 2% agarose gel for electrophoresis, stained with ethidium bromide and bands developed visualized under UV light. Bands obtained were excised, purified, checked for concentration and outsourced (NICEM, South Korea) for sequencing.

Sequence analysis

The nucleotide sequences obtained were subjected to a BLAST (Basic Local Alignment Search Tool) search using the BLAST program (http://www.ncbi.nlm.nih.gov/BLAST.cgi) available at the National Center for Biotechnology Information (NCBI). Thirteen representing sequences pertaining to the 5'UTR regions of the HGV genotypes available in the GenBank database were downloaded (Table 1) and used in comparison of the sequences. Multiple sequence alignment of the sequences was carried out using the Multalin program (20). Phylogenetic analysis of the sequences was carried out by the Neighbor joining method using the MegaX software (21).

RESULTS

In this study, PCR assay of the 5'UTR region showed only 28 among the 154 participants to be positive for the hepatitis G virus. Among these 12 isolates (A03, B03, C03, D03, E03, F03, G03, H03, A04, B04, C04, and D04) were further studied for their variation in the 5'UTR region. Thirteen representative HGV 5'UTR sequences were downloaded from the GenBank database (Table 1) and used in comparison of sequences obtained in the present study.

Table 1: Geographical origins, GenBank accession no, genotypic data of HGV sequences used in this study

GenBank accession no.	Country of origin	Genotype	Reference
AB003291	Japan	1a	(12)
U363801	West Africa	1b	(13)
U59540.1	USA	1b	(15)
U44402	USA	2a	(14)
U45966	USA	2a	(14)
NC_001710	USA	2a	(14)
U63715	East Africa	2b	(13)
AY196904	USA	2b	(16)
U94695	China	3	(17)
D90601.1	Japan	3	(17)
U91722.1	Thailand	4	-
AF177591.1	Australia	4	(19)
AB003292.1	Japan	5	(12)

An alignment of 134 bp of the HGV 5'UTR sequences revealed several of the nucleotides within this region to be highly conserved. The nucleotide variation in each of the sequences in reference to the HGV isolate NC_001710 is shown in Fig.1.

Based on the sequences, a phylogenetic tree was constructed to determine the relationship between known genotypes of HGV to that generated in this study. The results showed that of the 12 isolates in this study clustered into 3 genotypes (Fig.2). Among them, the majority (7 out of 12) belonged to genotype cluster 4, followed by 4 isolates belonging to genotype 2b and 1 isolate to Type 1b (Fig.2). Further, it was observed that 5 of the isolates (C03, D03, F03, H03 and C04) were identical to the Australian isolate (acc.no. AF177591.1). Similarly, the Type 2b (A03, E03, A04 and B04) and Type 1b (B03) isolates were seen to be

grouped to USA isolates AY196904 and U59540 respectively (Fig. 2).

DISCUSSION

This study revealed 2 important facts. First, although being comparatively highly conserved, the HGV 5'-UTR sequence is still heterogeneous. Second, phylogenetic analysis revealed that the isolates clearly separated into three distinct branches, indicating the existence of three distinct genotypes. The majority of pestiviruses and flaviviruses, which HGV is linked to, have rather well-conserved 5'-UTR sequences. This is explained by the fact that this area is crucial for viral protein translation and replication. This chromosomal area is nonetheless heterogeneous, despite these limitations. It is interesting to note that certain isolates have constant nucleotide alterations, indicating that they are phylogenetically close to one another (14). The phylogenetic trees of the reported isolates supported this deduction. The isolates, which came from seven nations on three continents (Africa, America, and Asia), formed three separate phylogenetic branches. These three primary clusters were discovered using three separate molecular phylogenetic analysis techniques.

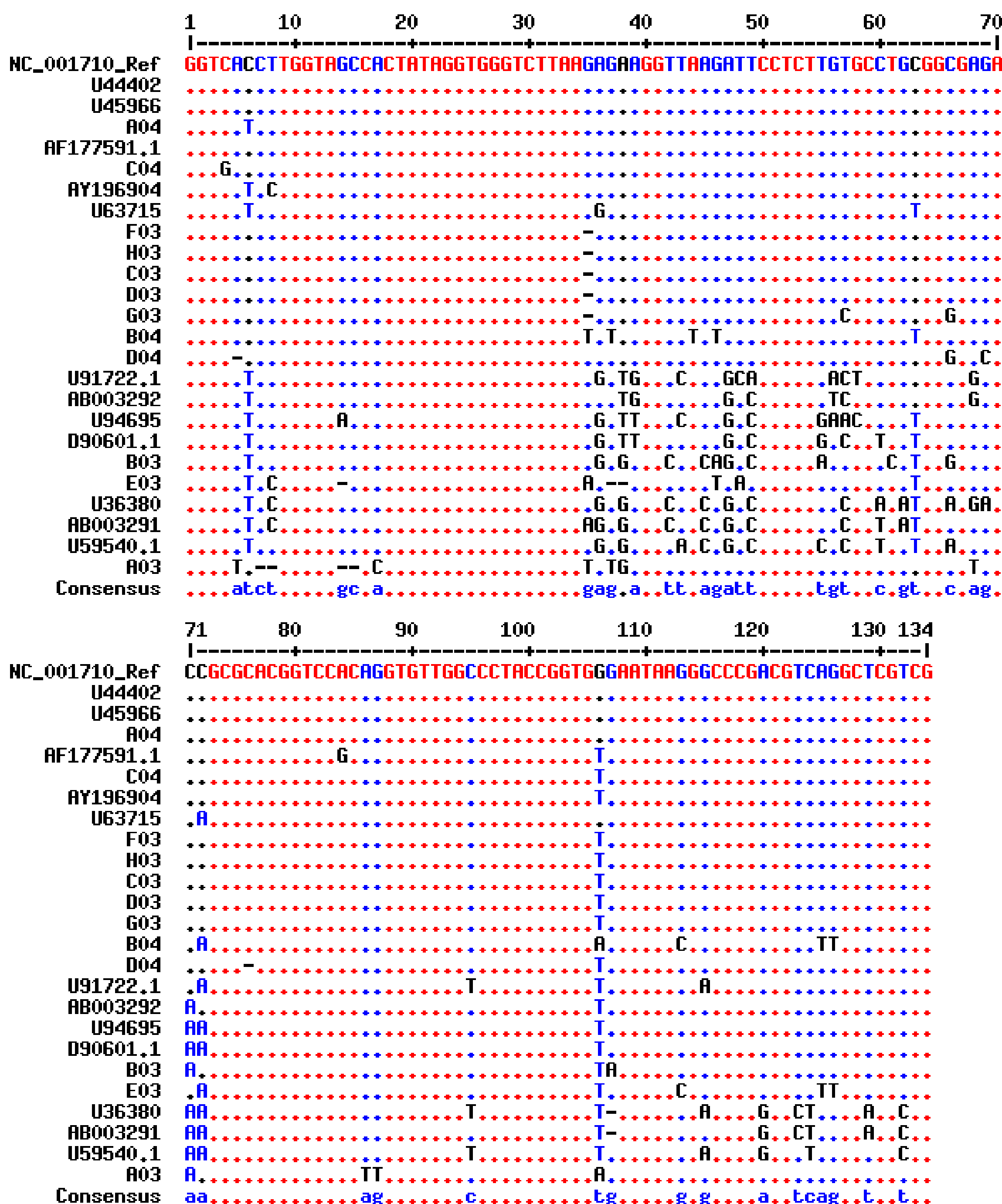

Fig. 1. Alignment of HGV 5’UTR sequences generated in this study as well those obtained from GenBank database. Dots indicate nucleotides identical to the HGV reference sequence (HGV, NC_001710).

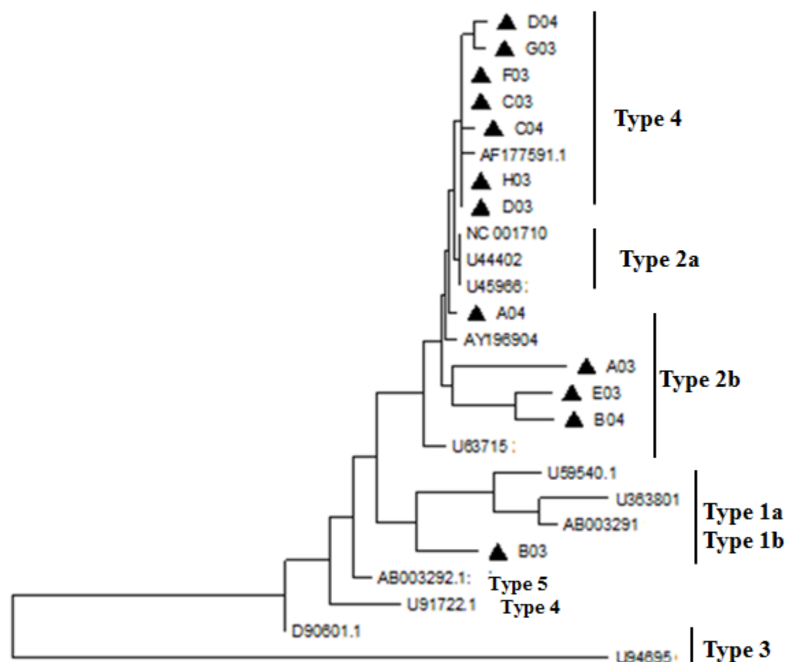

Fig. 2. The phylogenetic tree generated for the HGV 5' UTR sequences in this study. ▲ Indicates the HGV 5'UTR sequences generated in this study.

The analysis revealed that Type 4 HGV were more prevalent. Of the total of 12 HGV isolates collected in our study, seven were type 4, four were type 2b, and one were type 1b (Fig. 2). In the current study, no type 3 or 5 isolates were found. As variants of a virus may differ in traits including their patterns of pathogenicity, virulence, responsiveness to therapy and serologic reactivity, it is vital to investigate the diversity among the sequences of different isolates. Although HGV/RNAs have been found in both patients with liver disease and healthy people, it is unclear how HGV infection contributes to liver disorders. Although it seems that these viruses are frequently infected repeatedly, they do not seem to be linked to severe liver damage (22).

Our study demonstrated the presence of several nucleotide variations in each of the sequences of the 5' UTR region of the HGV genome. This is due to the fact that the majority of mutations result in polymorphism in viral isolates and, as a result, increase the virulence and pathogenicity of the virus that is responsible for infections. It was found that the blood samples of patients who participated in the current study included a total of 41 unique mutations (23). Results also revealed similarities to the frequencies observed previously for the community of Iraqi patients, suggesting that the most common mutations detected in this study among 274 chromosomes from transfusion-dependent β-thalassemia patients and carriers may have several potential sources (24).

Sequence analysis of the (HGV) gene in the populations of the studied sample revealed that among some common mutations, there is predominant mutation (25). Hepatomegaly, short height, and low hemoglobin levels were some of the uncommon and hematological symptoms that this ailment developed as. Another odd symptom that this condition manifested as was hyperpigmentation of the palms and soles of the feet due to hemochromatosis (26). Variations in the polymorphism observed in this study may be attributable to factors such as patient size and clinical characteristics, HGV cDNA detection techniques (particularly primer choice), treatment duration, infection control measures utilized in the treatment unit, and geographical location (27).

CONCLUSION

The present study reports the distribution of HGV genotypes among β-thalassemia patients in Iraq. The results showed that the circulating genotypes to be 1b, 2b, and 4 among these patients.

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CONFLICT OF INTEREST

There is no conflict of interest related to this article.

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