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Prevalence and Impact of GBV-C among HIV-1 Infected Patients under HAART in Addis Ababa, Ethiopia

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Abstract Hepatitis G virus or GB virus C (GBV-C) is a virus in the *Flaviviridae* family that does not cause any disease, but from various epidemiological observations was found to improve the clinical outcomes of HIV infection and treatment. It has the same mode of transmission with HIV. In Ethiopia, there has not been any study made on the prevalence or genotyping of GBV-C in circulation. Therefore no information is available. To fill this gap prevalence study was conducted on HIV patients under treatment. Eighty one serum samples were collected from patients on follow up study at the Addis Ababa Regional Laboratories and Research Institutes. RNA was extracted, reversed transcribed, and amplified by Real Time polymerase chain reaction (PCR), using primers for 5- untranslated region (5-UTR) of the GBV-C. Results showed 7 (8.6%) out of the 81 of HIV patients to be coinfected with GBV-C. Comparison of the mean CD4 count of the coinfected subject was found to be significantly different from the GBV-C negative patients at ($P < 0.05$). Further studies on clinical, immunological and genotypic analysis on larger samples are under investigation.

Keywords: *GB Virus C, HIV, HAART, PCR, HGV*

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1. Introduction

Acquired Immunodeficiency Syndrome (AIDS) is a long-term progressive disease that is caused by Human Immunodeficiency Virus (HIV). Today, this disease is a major health challenges worldwide. Approximately 37 million people are currently living with HIV of which 24 million suffer from accumulated AIDS-related deaths and 2.6 million are new infections [1]. In these pandemic developing countries, especially the people of sub-Saharan Africa are the hardest hit [2]. The pathogenesis of HIV infection is a result of chronic infection of T-cells. The proliferation provides niches for replication of HIV, increase in viral load and further invasion and depletion of the CD4 T-cells [3].

At present, there are drugs that increase the survival of infected individuals by suppression HIV replication. The standard treatment, in order to reduce the chance of the virus developing resistance it is given as a combination of at least three drugs: nucleoside reverse transcriptase inhibitors (NRTIs), nonnucleoside reverse transcriptase inhibitors (NNRTIs), protease inhibitors (PIs), fusion Inhibitor (FIs), co-receptor Inhibitors (CRIs), and integrase Inhibitor (INIs) [4].

These drugs have made a profound decrease in HIV associated morbidity and mortality [5]. Despite all efforts to limit its distribution it is still in circulation worldwide including the developed countries. That is because people fear to use the drug because of drug resistant strains, undesirable side effects from the long term use, and the price of the drug. It is restricted from use to pregnant and breast feeding women, children less than 5 years of age, and people co-infected with TB or severe Hepatitis B which makes treatment always incomplete. In sub-Sahahran Africa, only 37% of people living with HIV were receiving treatment in 2013 [6,7]. Furthermore, discontinuing the treatment even for weeks will cause high increase of the viral load [8].

The presence of the above problems made the development of new drugs a compulsory step but remains a major area of challenge. To this end many approaches are being tested. Of these, the use of new pharmacologic agents and microorganisms like Newcastle Disease Virus [9] and Adenovirus [10].

Here we propose the latter strategy of protection i.e. HGV/ GBV-C super infection as a novel therapeutic approach against HIV.

These viruses were identified by two independent groups in the mid of the 90s from patient with liver disease. Genome analysis showed that the two viruses shared 96% of the amino acid sequence and 86% of the nucleotide sequences and this suggest that they are different isolates of the same virus, and the term GBV-C is the commonly used since they are not associated with any disease [11,12,13,14].

GBV-C is an RNA virus in the family Flaviviridae, and it has been shown to play a role in modulating the effects of HIV in humans. Its positive impact was first observed

in 1998 when the GBV-C co infected HIV patients were found with lower viral load than mono-infected individuals [15]. Later, two large independent studies with long follow up showed that GBV-C is also associated with longer survival rate and improved clinical conditions [16,17]. Other studies have also shown that the protections include high CD4+ counts, low viral load, higher survival rate, good response to HAART and late progression to AIDS [18-24]. It does this, by competing for the receptor and co-receptor molecules used by HIV. Likewise, it also enhances the outcomes of HAART by reducing the activation of CD4+ and CD8+ T-cells that would otherwise serve HIV replication and increase the viral load in circulation [25,26]. With increase in GBV-C, the levels of HIV decreases in inverse proportion whereas, it is directly proportional to the increase of GBV-C RNA fragments. The optimal treatment effects of HAART are obtained in GBV-C/HIV coinfected patients under treatment [27].

However, the above-mentioned effects are not seen in all GBV-C viruses found everywhere. There are multiple GBV-C genotypes and subtype in an individual that may have different impacts on HIV disease [28,29]. Few studies which focused on the importance of the genotypes' influence in HIV disease progression, Muerhoff *et al.* have found two genotypes with minor differences labeled as 2a and 2b, that could produce opposite effects on CD4 count and disease progression [30,31]. Several studies followed showed that various GBV-C genotypes reflect differences as their sensitivity to interferon (IFN), cell tropism and their ability to persist in culture [32,33].

So far there are seven different genotypes and subtypes located in different geographical region, genotype 1 predominant in West Africa; genotype 2 in Europe and USA; genotype 3 in Asia; genotype 4 in the Southeast Asian countries; genotype 5 in South Africa; genotype 6 in Indonesia and genotype 7 isolated in China recently [34-40].

Therefore, it is possible that GBV-C genotype could at least partially account for the variable influence of GBV-C infection on HIV disease progression.

In east Africa and Ethiopia the situation is not known, there are no reports on the prevalence or the genotype of GBV-C among HIV patients under HAART or on the degree of protection of GBV-C. Forty seven % of the total population in Ethiopia is aged between 15 to 49 years and the highest HIV prevalence occurs in the age group 15-24 and those between 15 to 49 years account for 90 % of all infections [41].

Taking all these facts this study aims to learn the prevalence and the impact of GBV-C virus, and explain the modulation of the immune system it may provide for further access to new therapeutic strategies of HIV infection.

This is part of a wider study that targets to find the phenotype and genotypes of GBV-C in Ethiopia with the hope of isolating and characterizing the strains with a potential for use in the treatment of HIV infections.

2. Material & Methods

2.1. Study Design and Settings

This retrospective cross-sectional study was conducted in Addis Ababa University, Ethiopia, on frozen serum samples collected from HIV patients on follow-up and voluntary counseling and test (VCT) collected from the following hospitals and health centers in Addis Ababa city: Zawditu Memorial Hospital, AIDS Health Care Foundation, Kotebe and Kirkos Health Center.

2.2. Samples and Data Collection

The study population comprised a total of 81 HIV patients' samples under HAART that were sent to the health research laboratory in Addis Ababa for follow up studies. Following collections, the serum samples were divided into 2 microtubes and transported in an icebox to the Immunology and Molecular laboratories at the department of Microbial Cellular & Molecular biology, Addis Ababa University. Demographic data, including age, gender, medical history and health status were collected from the patients cards and archives from the hospital or clinic of the origin.

2.3. CD4 Cell Count and HIV Viral Loads

CD4 cells count and plasma HIV RNA load were performed in the health research laboratory in Addis Ababa.

2.4. RNA Extraction

For the preparation of total RNA from human sera, we used the Ribo Virus kit (Sacace Biotechnologies, Italy) according to the manufacturer's instructions. In brief, 150 µl of serum was incubated with 600 ml of lysis buffer containing guanidine thiocyanate at 70°C for 5min. After the addition of 600 ml of ethanol, the precipitated RNA was applied onto a silica-based spin column for purification and was finally eluted with 50 ml of Rnase-free H2O.

2.5. RT-PCR & Amplification

One-step reverse transcription of the RNA and Real Time amplification of the cDNA was been performed by using (V2-FRT, Sacace, Italy), which includes all the components to generate RT-PCR amplified products from GBV-C/HGV RNA. Briefly, the total reaction volume was 25 ul, containing 10 ul of extracted RNA and 15 ul of the master mix which was prepared based on manufacturer's instructions. cDNA synthesis was performed at 50°C for 15min, followed by inactivation of the reverse transcriptase enzyme at 95°C for 5 min. The two PCR rounds are the same. The amplification was done in 5 cycles for the first round (95°C for 5 s, 60°C for 20 s, 72°C for 15 s) and 40 cycles for the second rounds of the PCR (95°C for 5 s, 60°C for 30 s, 72°C for 40 s with a final extension at 72°C for 15 s). The fluorescence detection and analysis of the PCR product was performed during the final extension by using the FAM, JOE/HEX channels of the Mx3000P instrument. Negative control of amplification and positive control of amplification were maintained as provided by the kit. Internal control which serves as amplification control was used to identify possible reaction inhibition.

2.6. Ethical Consideration

Ethical clearance was obtained from the Ethical Review committee of the College of Natural Science, Addis

Ababa University and Ministry of Science and Technology.

2.7. Statistical Analysis

Student t *test* was used to determine the differences of age, WHO stage, CD4+ and Viral Load, Chi-square tests were used to compare categorical variables such as gender and HAART treatment. P value < 0.05 was considered statistically significant.

3. Results

A total of 81 HIV patients samples under HAART were included in this study, from those 37 patients (45.7 %) were male and 44 patients (54.3 %) were female. The majority of the participants 31 (38 %) were aged between 31 – 40 years. Forty eight (48 %) percent of the study subjects were married and most of them were illiterate.

The overall prevalence of GBV-C RNA in this current study was 7 patients(8.6%) out of the 81 samples of the study. GBV-C incidence was relatively higher in the age group of 31 – 40 years and in female, but not statistically significant ($p > 0.5$). Similarly higher prevalence was seen among illiterate, primary school and married participants but not statistically significant (Table 1).

GBV-C positive patients displayed markedly lower HIV viral load (54 vs. 14.676 Copies/ ml) (Table 2). Moreover, five of the GBV-C positive participants are categorized in the first WHO clinical stage and two in the second stages based on the patient's symptoms which ranges from night sweat, acute diarrhea and cough while GBV-C negative patients suffer from more sever diseases.

The CD4 cell counts, HIV viral loads and WHO clinical stages were evaluated in GBV-C positive and GBV-C negative participants Table2. Comparing the CD4+ means between the GBV-C positive and GBV-C negative groups in HIV positive patients showed significant difference. Furthermore, positive and negative GBV-C patients were sub divided into small groups based on age, sex and date of starting ART. The number of CD4+ cells over time increase more rapidly in GBV-C positive patients compared to GBV-C negative patients as shown in (Figure 1).

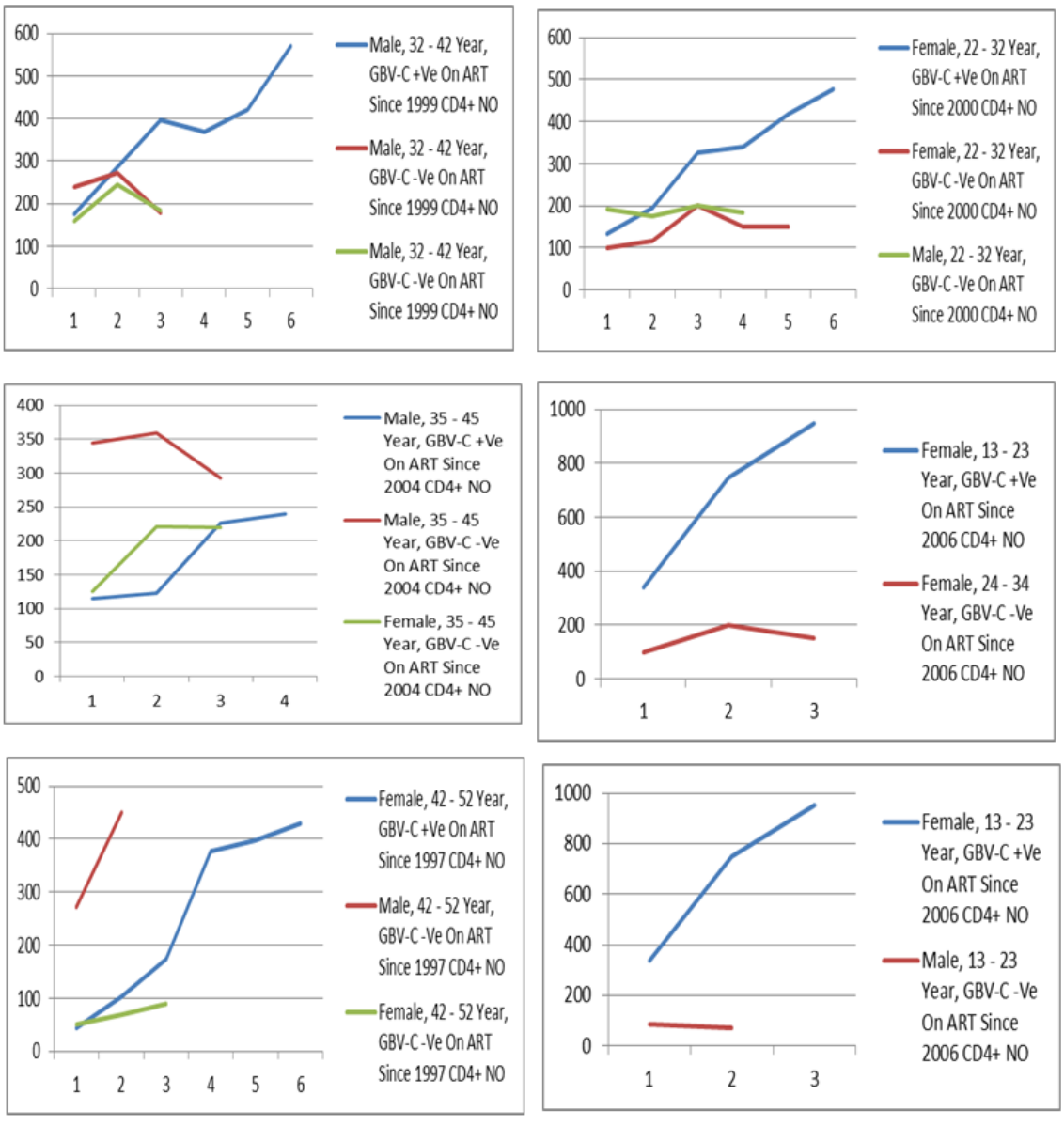

Figure 1. Comparison of CD4+ count between GBV-C positive and negatives

Table 1. Prevalence of GBV-C by socio-demographic variables among HIV patient under HAART

Variables	GBV-C RNA		
Age group	Positive no (%)	Negative no (%)	Total no (%)
11 – 20	1 (17)	5 (83)	6 (8)
21 – 30	1 (7)	13 (93)	14(17)
31 – 40	3 (10)	28 (90)	31 (38)
41 – 50	1 (5)	19 (95)	20 (25)
51 – 60	1 (10)	9 (90)	10 (12)
Sex			
Male	3 (8.1)	34 (91.9)	37 (45.7)
Female	4 (9)	40 (91)	44 (54.3)
Marital status			
Married	5 (10.4)	43 (89.6)	48 (59)
Single	2 (6)	31 (94)	33 (41)
Education			
Illiterate	2 (8)	22 (92)	24 (30)
Primary school	2 (9)	20 (91)	22 (27)
Secondary school	1 (6)	17 (94)	18 (22)
Diploma and above	2 (12)	15 (88)	17 (21)

Table 2. Immunologic, virologic, and clinical outcomes

Characteristics	GBV-C RNA	
	Positive	Negative
Clinical Status		
WHO Clinical Stage 1	5	9
WHO Clinical Stage 2	2	22
WHO Clinical Stage 3	0	28
WHO Clinical Stage 4	0	18
CD4 (mean)	411 cells/mm3	200 cells/mm3
Viral Load (mean)	54 Copies/ ml	14.676 Copies/ ml

4. Discussion

GBV-C/HGV is a member of the Flaviviridae family and it is closely related to the Hepatitis C Virus (HCV). This virus does not cause any disease in human, but claimed from epidemiological observations to improve the clinical outcome of HIV infections and treatments in different countries. Further investigations on GBV-C viruses that examined their effects on HIV infection have revealed that they are not harmful and, were found to be beneficial to HIV coinfected persons [42,43,44]. Co-infection rate with GBV-C is frequent in patients with HIV infections due to similar routes of transmission [45,46,47].

This is the first study determining the frequency and impact of GBV-C infection among HIV patients under HAART. The study was carried out in patients from different hospitals and AIDS centers in Addis Ababa.

The GBV-C RNA was detected among 7 (8.6 %) patients out of 81 in this retrospective cross-sectional study.

Our data was slightly less from other reports that show GBV-C prevalence of 14–45% among HIV patients [48]. Higher rate of GBV-C among HIV individuals (51%) were reported in Scotland [51].

This controversy may be due to the number of patients and the laboratory systems_in the detection of the virus RNA and the antibody which was one of our limitations. The detection of the viral RNA with reverse transcription polymerase chain reaction [49], indicated_active infection, however the clearance of the virus is associated with the development of antibodies against envelope glycoprotein (E2) which are detected by immunoassays [50]. It has been reported that 30 up to 65% of HIV infected patients have antibodies against the virus E2 enveloped protein which indicates prior GBV-C infection [16,50]. The route of transmission is another important factor in the incidence of GBV-C. The virus is more prevalent among homosexuals rather than heterosexuals individuals [51].

GBV-C incidence was relatively higher in the age group of 31 – 40 years and in female, but the difference was not statistically significant ($p > 0.5$). Similarly higher prevalence was seen among illiterate, primary school and married participants but also not statistically significant.

The mean of the CD4 count and viral load was significantly higher among GBV-C positive patients in comparison with the negative patients, which is in agreement with previous reports investigating influence of GBV-C on CD4+ and HIV viral load [16,18,23].

To assess the effect of GBV-C on the response to HAART among HIV patients, we followed up the fluctuation in the number of CD4+ over year's number. We classified the GBV-C positive and negative according to the date of starting HAART, age group and gender as shown in Figure 1. Our data shows a high increase in the CD+ number among all the GBV-C positive patients compared to GBV-C negative patents that show decline in the CD4+ number or slow improvement.

Moreover, five of the GBV-C positive participants are categorized in the first WHO clinical stage of the HIV patients and two remaining in the second stages. However, this classification is based on the patient's symptom which ranges from night sweating, acute diarrhea and cough while the GBV-C negative patients suffer from more sever diseases.

Our study was a preliminary survey to evaluate the incidence of GBV-C infection among HIV patients in

Ethiopia. Furthermore, there is no information regarding the predominant genotypes of GBV-C in Ethiopia which might have a different effect on the disease progression as reported in various other geographical regions.

Ongoing attempts are undertaken on GBV-C, especially in the context of other kinds of co-infections such as HCV and HBV on HIV patients.

Finally, better understanding of GBV-C influence on HIV infection is important to identify novel approaches to HIV therapy to slow the progression of HIV infection, without treatment problems such as drug resistance and toxicity.

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