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Prevalence of Human Pegivirus (HPgV) Among HIV/AIDS Patients in Aminu Kano Teaching Hospital, Kano – Nigeria

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Abstract

Human Pegivirus (HPgV) also known as hepatitis G virus is a positive sense, single-stranded RNA virus. Association between HPgV and diseases remains uncertain, however, studies have shown that co-infection of HPgV and HIV has positive impact on HIV/AIDS patient by slowing HIV disease progression. This study was conducted to find the prevalence of HPgV, its co-infection with HBV, HCV and the relationship of HPgV infection with HIV progression among HIV/AIDS patients attending Aminu Kano Teaching Hospital (AKTH) Kano, Nigeria. Four hundred (400) blood samples of HIV infected individuals on HAART were tested for HPgV and HIV viral load. Reverse transcription real-time polymerase chain reaction (RT-rtPCR) was used to detect HPgV RNA and HIV viral load while the socio-demographic and risk factors for infections were recorded using structured questionnaire. Data was analyzed using SPSS version 20.0. The prevalence of HPgV RNA was 11.5% (n=46), the frequency of HPgV infection was higher in peoples of age range 41-50years 15 (32.6%) and the prevalence increase with age from 0-50 and decrease in adult >50 years. Higher prevalence was observed among male participants 27 (58.7%), and those with multiple sexual partners 36 (78.3%). There was no significant difference in HIV viral load between those with HPgV positive and HPgV negative (p=0.297). the coinfection rate of HPgV and HBsAg was found to be 5.5%, HPgV and HCVAb was 4.3% and 2.3% coinfection rate for HPgV/HCVAb/HBSAg. Further community-based survey is therefore needed in HPgV infected individual to assess the role of HPgV in

HIV progression which may open new strategies for the development of HPgV based biotherapy for management and control of HIV/AIDS.

Keywords: Human Pegivirus/HGV, Human Immunodeficiency virus, Aminu Kano Teaching Hospital, Kano

Introduction

Human Pegivirus (HPgV) also known as Hepatitis G virus or GB virus C is a human lymphotropic virus. It is a linear, icosahedral, monopartite, enveloped, single stranded positive sense (ssRNA) virus belonging to the Flaviviridae family, a member of the Pegivirus genus and Hepatitis G virus (HGV) type specie (Nasidi and Rogo, 2015). Molecular and phylogenetic tree analysis of nucleotides and amino acids sequences of HPgV, HBV, HCV and HIV suggests relationships among them; however, closer amino acid sequence relationship exists between HPgV and HCV. The presence of HPgV RNA in serum indicates active HPgV infection whereas detection of antibodies to the HPgV envelope glycoprotein E2 is associated with prior infection. Unlike HCV, simultaneous detection of viral RNA and antibodies to E2 or other viral proteins is not common (Williams, 2004). In majority of HPgV infection, viremia is cleared within 2 years of infection (Tanaka *et al.*, 2004) and the RNA level can reach up to 10^2 - 10^7 copies per milliliter of serum in immune-competent individuals, 10^4 - 10^7 copies/ml in immune-suppressed individuals and 10^8 copies/ml in HIV infected individuals (Ohto *et al.*, 2000). The mutation rate of the HPgV genome has been estimated at 10^{-2} to 10^{-3}

substitutions/site/year (Romano *et al.*, 2008). HPgV is mainly transmitted through exposure to blood or blood products, sexual contact, and less frequently mother-to-child transmission (Stapleton *et al.*, 2004) HPgV has worldwide distribution; higher prevalence was observed among individuals with the risk of blood and blood product exposures, those on haemodialysis, and intravenous drug users (Bukh *et al.*, 2004). HPgV has been classified into six genotypes and many subtypes with distinct geographical distributions (Fenget *al* 2011). Co-infection of HPgV and HIV is common because of similarity in mode of transmission. HPgV infection has not been proved to be associated with any disease. However, studies have shown that its infection leads to improve clinical outcomes in HIV infected individuals (Bharatai and Stapleton, 2012; Carolynne *et al.*, 2012; Bassit *et al.*, 2002). In vitro studies also confirm these clinical observations and demonstrate an anti-HIV replication effect of HPgV (Bharatai and Stapleton, 2012). Numerous studies had been carried out on HPgV in different countries in Africa, and the world in general. However, there is paucity of published studies undertaken in Nigeria even though HPgV infection has worldwide distribution. In addition, a lot of discrepancies on the prevalence of HPgV between different countries were observed, and therefore the need to know what the situation is in Nigeria. Also, the information on the association of HPgV and response to HAART is equivocal and has never been confirmed in a Nigerian context. It is therefore important to determine HPgV prevalence the magnitude of its co-infection, influence of co-infection on HAART as well as the risk factors/socio-demographic factors for infection. The result which may trigger more research on HPgV co-infection with HIV and in other high risk groups. It will also give an insight of the degree of HPgV interaction with HIV and help to create awareness among health workers on the prevalence rate among HIV positive individuals. It will also serve as a guide or template for further studies on its occurrence among both low and high risk groups in the community.

Materials and Methods

Study design and Study area

The study is a hospital based descriptive cross-sectional study. It study was conducted at Aminu Kano Teaching Hospital (AKTH), a tertiary health institutions in Kano state, located along Zaria road in Tarauni Local Government Kano state. It runs an anti-retroviral disease clinic which caters for more than 25,000 HIV/AIDS patients enrolled in the hospital.

Ethical consideration

The study was conducted according to ethical standards for human studies after obtaining ethical clearance from AKTH ethical review committees with approval number AKTH/MAC/SUB/12A/P-3/VI/1984. Participants were appropriately informed about the merit, confidentiality and safety of being included in the research. The samples taken were used for the stated study only.

Methodology

Four hundred (400) blood specimens were collected from individuals living with HIV/AIDS attending AKTH, by the use of aseptic Vacutainer set technique as described by Cheesbrough (2002). The participants included 250 females and 150 males with age range between 1-71 years. Simple random sampling technique was used to obtain the study sample size, a close ended structured questionnaire was used to obtain the participants socio-demographic data, and associated risk factors for HPgV co-infection with HIV. Sample collection was done at Anti-retroviral disease (ARD) clinic AKTH, HPgV RNA was isolated and detected by qualitative real time polymerase chain reaction technique (RT-PCR) using Rotor gene 6000, HBV and HCV test procedures were carried out according to the standard rapid immunochromatography technique. HGV RNA was extracted from serum samples using Ribo-prep nucleic acid extraction kit by Amplisens, Russia according to the procedure described in the user manual, Molecular detection of HPgV RNA was performed using commercially prepared real-time reverse-transcription polymerase chain reaction (PCR) kit Amplisens, Russia, according to the procedure described in the user manual. Rapid test (Strip type by CTK Biotech) kits were

used to test all the samples for HBV and HCV according to manufacturer's instructions.

Statistical Analysis

The data generated was analyzed using the software Statistical Package for Social Sciences (SPSS) version 20 (2011, IBM Corporation, U.S.A.) for calculation of percentages, categorical variables. Anova (univariate) test was used to assess the level of variation and significance of differences among groups. In all cases $p \leq 0.05$ at 95% level of confidence was considered significant in all statistical comparisons.

Results

The data generated shows that 46 (11.5%) tested positive for HPgV while 354 (88.5%) tested negative for HPgV, 68 (17%) were positive for HBsAg, 28(7%) were positive HCV Ab. HPgV and HCV Ab coinfection constituted 17 (4.3%%) while 22 (5.5%) were positive for HPgV and HBsAg. Nine (2.3%) of the participants tested positive for all three infections (HPgV, HCV Ab, and HBsAg) (Table 1). The mean HIV viral load for HPgV negative and positive are 930.59, 348.24 and standard deviation are 377.68 and 280.3 respectively. The analysis of data to

determine the relationship between HPgV presence and HIV viral load showed no significant difference ($p=0.297$). Highest prevalence was observed among group with age range 41-50 of which 70 (17.5%) were tested and 15 (32.6%) were positive. This was followed by the group with age range 31-40 of which 142 (35.5%) were tested and 12 (26.1%) were positive (Table 2). Based on gender, higher prevalence of HPgV was observed among the male participants with 27 (58.7%) while 19 (41.3%) of the female participants were positive for HPgV RNA (Table 3). HPgV distribution based on marital status is shown in table 4. Positive married participants were 6 (13%), 7 (15.2%) were single, 6 (13%) were separated or divorced while 3 (6.6%) were widowed. Out of the 46 HPgV positive participants, 36 (78.3%) had 2 or more sexual partners, 9 (19.6%) had single sexual partners whereas only 1 (2.2%) had no history of sexual intercourse. Based on blood/blood, products usage, 37 (80.4%) had no history of blood/blood product usage while 9(19.6%) had used blood/blood products before. On the basis of parenteral drug usage/invasive medical procedures, 39 (84.8%) of the HPgV positive were rare parenteral drug users, 6 (13%) were frequent drug users while 4 (8.7%) had undergone surgery or dialysis or both (Table 5)

Table 1: HPgV prevalence as single and co-infection with HBsAg and HCV Ab among the participants.

| Infection | Prevalence |
|---------------------------------|------------|
| HPGV alone | 46 (11.5%) |
| HPGV/HBsAg/ HCV Ab co-infection | 9 (2.3%) |
| Co-infection | |
| HPGV/HBsAg | 22 (5.5%) |
| HPGV/HCV Ab | 17 (4.3%) |

Table 2: HPgV prevalence in relation to the age of the patients

| Characteristics | Description | HPgV negative cases | HPgV positive cases | Frequency/Total number tested | p-value |
|-----------------|--------------|---------------------|---------------------|-------------------------------|---------|
| Age | 0-10 | 13(3.7%) | 1 (2.2%) | 14(3.5%) | 0.197 |
| | 11-20 | 31(8.8%) | 1 (2.2%) | 32 (8%) | |
| | 21-30 | 57(16.1%) | 9 (19.6%) | 66 (16.5%) | |
| | 31-40 | 130(36.7) | 12 (26.1%) | 142 (35.5%) | |
| | 41-50 | 55(15.5%) | 15 (32.6%) | 70 (17.5%) | |
| | 51-60 | 47(13.3%) | 5 (10.9%) | 52 (13%) | |
| | >61 | 21(5.9%) | 3 (6.5%) | 24 (6%) | |
| | Total | 354 (100%) | 46 (100%) | 400 (100%) | |

Table 3: HPgV prevalence in relation to the gender of the patients

| Characteristics | Description | HPgV negative cases | HPgV positive cases | Frequency/Total number tested | p-value |
|-----------------|--------------|---------------------|---------------------|-------------------------------|---------|
| Gender | Male | 123(34.7%) | 27(58.7%) | 150(37.5%) | 0.712 |
| | Female | 231 (65.3%) | 19(41.3) | 250(62.5%) | |
| | Total | 354 (100%) | 46 (100%) | 400(100%) | |

Table 4: HPgV prevalence in relation to the marital status of the participants

| Characteristics | Description | HPgV negative cases | HPgV positive cases | Frequency/Total number tested | p-value |
|-----------------|--------------------|---------------------|---------------------|-------------------------------|---------|
| Marital status | Married | 188 (53.1%) | 28 (60.9%) | 216 (54%) | 0.488 |
| | Single | 73 (20.6%) | 7 (15.2%) | 80 (20%) | |
| | Separated/Divorced | 62 (17.5%) | 8 (17.4%) | 70 (17.5%) | |
| | Widowed | 31 (8.8%) | 3 (6.5%) | 34 (8.5%) | |
| | Total | 354 (100%) | 46(100%) | 400(100%) | |

Table 5: HPgV prevalence by risk factor distribution among the respondents

| Risk factors | HPgVnegative cases | HPgV positive | Frequency/Total number tested | P-Value |
|----------------------------------------------------|--------------------|------------------|-------------------------------|---------|
| Number of sexual partners | | | | |
| 0 | 29 (8.2%) | 1 (2.2%) | 30 (7.5%) | |
| 1 | 93 (26.3%) | 9 (19.6%) | 102 (25.5%) | 0.263 |
| 2 and above | 232 (65.5%) | 36 (78.3%) | 268 (67%) | |
| Total | 354 (100%) | 46 (100%) | 400 (100%) | |
| History of blood/blood product usage | | | | |
| Yes | 67(18.9%) | 9 (19.6%) | 76 (19%) | 0.263 |
| No | 287 (81.1%) | 37 (80.4%) | 324 (81%) | |
| Total | 354 (100%) | 46 (100%) | 400 (100%) | |
| Parenteral drug usage/ invasive medical procedures | | | | |
| Frequent | 45 (12.7%) | 3 (6.5%) | 48 (12%) | |
| Rare | 269 (76%) | 39 (84.8%) | 308 (77%) | 0.336 |
| Surgery/Dialysis | 40 (11.3%) | 4 (8.7%) | 44 (11%) | |
| Total | 354 (100%) | 46 (100%) | 400 (100%) | |

Discussion

In the present study, prevalence of HPgV and response to HAART among 400 patients living with HIV/AIDS attending AKTH ARD clinic was evaluated. The positive detection rate (prevalence) of HPgV-RNA among the study population was 46 (11.5%). Our observed prevalence is higher than the prevalence rate of 0.8% reported by Sarki (2015) among blood donors in Kano. Higher prevalence reported in this study compared to that of Sarki (2015) may be due to the fact that HIV patients are relatively high-risk group while blood donors are low risk group for HPgV infection. Furthermore, the higher sensitivity of the molecular techniques used in this study compared with the phenotypic method used by Sarki (2015) could explain the difference. The prevalence rate of 11.5% reported in this study agrees with prevalence rate in patients with HIV reported in various countries 11.3%-15.5%, 13.5%, 14%-22%, 16.7% and 21% for Iran, Argentina, Spain, Ghana and Brazil respectively (Ramezani *et al.*,

2013). Relatively higher prevalence of 30.8% was reported in Botswana by Kombo *et al.* (2017). The high prevalence observed may be due to the high prevalence of HIV (high risk group) in the country. Furthermore Alcalde *et al.* (2010) reported that HPgV prevalence worldwide is between 17%-41% among HIV positive individual. This is in contrast to the 11.5% reported in this study. The discrepancy observed may be due to reduction in the incidence rate of HIV worldwide with time associated with increased global awareness on HIV and other sexually transmitted diseases coupled with the use of protective devices and better HIV/AIDS management and control. These differences may also be explained by epidemiological variations, including variation in rate of blood transfusions, sexual behavior and a variable adherence to universal precautions (Kombo *et al.*, 2017). The data generated indicate that high prevalence was found among married participants and low among those with no history of sexual intercourse that fall within

the age range 0-10, which means sexual transmission is a significant route of transmission in the study area. Mother to child transmission is suspected in only one participant of age 2 years who was positive for HPgV, even though other route of transmission cannot be overlooked. The high prevalence observed among married participants and people with multiple sexual partners when compared with those with history of blood and blood product usage and frequent parenteral drug users, showed that sexual transmission is a more efficient transmission route in the study area. The observation in this study agrees with the postulation that sexual transmission constituted important risk behavior for transmission of blood borne viruses (Bien, 2013). Our finding is however at variance with the report of Zhijiang *et al.* (2017) who reported the frequency of HPgV infection in injectable/parenteral drug users as significantly higher than in heterosexuals. This discrepancy may be due to low number of injectable drug users amongst the study group.

On the basis of parenteral drug usage/invasive medical procedures high prevalence of HPgV positive was observed among rare parenteral drug users, while low prevalence was observed among the frequent drug users and people who had undergone surgery or dialysis or both. This suggests a low rate of transmission parenterally and through invasive medical procedure.

Moreover, the result showed that the rate of HPgV infection was significantly higher in subjects between the ages of 41-50 years. The frequency of infection in subjects (between age 0-50) is higher and then decline as the age progress (50 and above). Similar trends have been observed in other previous studies (Zhijiang *et al.*, 2017; Da mota *et al.*, 2016). This can be attributed to individual in this age group being more sexually active, and are prone to higher incidence of blood/product, and parenteral drug use than older patients (Zhijiang *et al.*, 2017; Bharatai and Stapleton 2012). Another possible explanation for the infection trend is that E2 antibody against HPgV re-infection is found to be more prevalent in older people (Bien, 2013) though, E2 antibody was not determined in this study.

Previous data on HPgV reported from 13 Sub-Saharan African countries shows that the majority reported prevalence data and/or analysis was on HPgV diversity without exploring the clinical significance of its coinfection (Singh *et al.*, 2017). In this study, the relationship between HPgV infection and HIV viral load was analyzed. Analysis of the data generated shows that there is no significant difference between the mean viral load of HPgV positive and HPgV negative subject ($p=0.297$). This indicates that HPgV has no relationship with HIV viral load of patients in the study group. Our finding agrees with a study by Kombo *et al.* (2017) that shows that HPgV RNA exhibit no relationship with HIV viral load but shows significant difference with CD4 count. Several Scientists (Bharatai and Stapleton 2012; Bassit *et al.*, 2002) reported that HPgV infection has been found to lower the progression of HIV into AIDS and slows down its morbidity and mortality. We could not draw a definite conclusion on relationship between HPgV RNA and HIV viral load among HIV patients on HAART from the present study because HPgV viral load, HPgV diversity and other immunological markers were not studied. Therefore, further studies are needed to establish the relationship between HPgV RNA and HIV viral load and CD4 count. As HPgV presence has no impact on studied subject viral load, this implies that HPgV presence does not have any relationship with HIV viral load among the study participant. The implication of this finding is that HPgV presence does not have any effect on studied subject's response to HAART.

Conclusion

The present study provided an insight into the prevalence of HPgV and its relationship in HIV coinfecting patients on HAART. The prevalence of HPgV among subject living with HIV attending HAARTclinics in AKTH is 11.5%. The most important route of transmission observed in the current study area is sexual transmission. These data suggest that HPgV infection is common among subjects living with HIV in Kano and has no significant impact on HIV viral load as observed in this study. Establishing the mode of action and the relationship between HPgV and HIV in co-

infected individuals may open new strategies for the development of HPgV based biotherapy for management and control of HIV/AIDS.

Recommendations

- I. Further community-based surveys in the general population and among the high-risk groups, is recommended to establish the prevalence and the socio-demographic risk factors associated with HPgV infection in the study area.
- ii. Other clinical, virologic and immunologic factors should be determined in HPgV infected individual to assess and establish their role in HIV progression.
- iii. The clinical and pathological significance of this infection needs better evaluation, in patients infected with other hepatitis viruses, haemodialysis patients and patients with fulminant hepatitis
- iv. Molecular epidemiology of HPgV is recommended to reveal the specific strains and genotype circulating in the area

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