

Seroprevalence of Human Herpesvirus Type 8 (HHV-8) Among People Living with HIV (PLHIV) Attending Babura General Hospital, Jigawa State.

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ABSTRACT

Human Herpesvirus type 8 is among the frequent opportunistic infections among individuals infected with human immunodeficiency virus (HIV) and may result in severe morbidity and mortality among this group of patients. It is known to be the causative agent of Kaposi's sarcoma (KS), as well as other malignancies such as primary effusion lymphoma and multicentric Castleman's disease. It is one of seven currently known human cancer viruses, or oncovirus. This study investigates the prevalence of HHV8 antigen among PLWHA as well as its correlations with the patients CD4 counts and Viral loads. This cross-sectional study involved 182 blood sample collected from HIV Seropositive individuals attending antiretroviral therapy clinic (ART), Babura General Hospital, Jigawa State, Nigeria, these Sample were analysed for HHV-8 antigen using Enzyme linked immunosorbent assay and CD4 cell Count. socio-demographic information such as age, gender, marital status was obtained from patient folder. Of the 182-subject studied, 6(3.3%) were tested positive for HHV-8 Antigen. All subjects (100%) who were HHV8 Positive have low CD4 Count and high HIV Viral loads. There was statistically significant difference between HHV8 and respondents CD4 Count ($p = 0.001$) and also respondents' compliance to Clinic visit ($p = 0.000$). However, no association observed between HHV8 and respondents Gender and their Age. This study shows that individuals with higher CD4+ counts has zero prevalence of HHV8 infection and hence have low risk of developing complication from the virus, it also indicates that HHV8 was higher among HIV patient with lower CD4 Counts and high HIV Viral load.

Keywords: HHV8, HIV/AIDS.

1. INTRODUCTION

Human herpesvirus 8 (HHV-8) is one of the member of the gamma- herpesvirus family. Also known as Kaposi's sarcoma-associated herpesvirus (KSHV), it is known to be the causative agent of Kaposi's sarcoma (KS), as well as other malignancies such as primary effusion lymphoma and multicentric Castleman's disease [16], [42], [13].

It has a diameter of 140 nm and a genome of between approximately 165 kb [12], and 170 kb [31], double stranded linear DNA and icosahedral capsid envelope [35]. It is covered by a tegument containing protein and closed during budding of the cell. The membrane is derived from outer envelope of lipid membrane from various host and specific virus glycoprotein [28].

It is also estimated to be 1% to 5% in the general U.S. population¹, compared with 10% to 20% in certain Mediterranean countries and 30% to 80% in parts of sub-Saharan Africa. (Aids Info, 2018)

2. MATERIALS AND METHODS

The study was carried out in Babura General Hospital, Jigawa State. Babura is a local Government Area in the north of Jigawa state, Nigeria. Its headquarters are in the town of Babura. The current District Head who doubles as the Sarkin Bai of Ringim, Ringim Emirate Council, Alhaji Hadi Mustapha (Councilor and King Maker) has been on the throne since 1993. it's located on latitude 12.774⁰N, longitude 9.0157⁰E and covers an Area of 383sqm with a population of 208,101 at the 2006 Census (NPC, 2006). The area is mostly inhabited by Fulanis.

2.1 Study Population

The study was carried out among HIV Seropositive patients receiving treatment at Anti-retroviral therapy Clinic Babura General Hospital Jigawa State. It consist of HIV infected

Subjects (ART-Naïve and Experienced) both sexes of different age groups attending General Hospital Babura.

2.2 Study Design

The study design was a descriptive cross sectional and hospital-based study.

2.3 Sample Size Determination

The minimum sample size was thus determined using Fishers Formula [6].

$$n = \frac{z^2 pq}{d^2}$$

Where n= Minimum sample size required.

z =Percentage point on a normal distribution curve equivalent to 95% confidence interval=1.96.

p = Prevalence of HHV-8 Antibodies among HIV Infected Patients conducted

In Benin City of Edo State.

=87% = 0.87

q =Complementary probability to p , $q=1-p$

d =Degree of precision = 0.05

Substituting these values into the formula,

$$n = \frac{(1.96)^2 \times 0.87 \times (1 - 0.87)}{(0.05)^2}$$

Therefore, the minimum sample size = 173.

To increase the precision and to allow for nonresponse the sample size was raised by 10%.

Therefore, the sample size was rounded up to $173 + 17 = 190$.

2.4 Sampling Technique

A total number of HIV seropositive Patients receiving treatment was collected from the Patients register in ART clinic Babura General Hospital, systematic sampling method was used to select the required sample size from a list of all the patients in the register.

First Subject for the study was obtained using simple random selection of patients among the study population.

2.5 Sample Preparation for ELISA

Patients Serums was obtained from their blood sample by collecting the whole blood aseptically in a covered test tube. After collection of the whole blood, the blood was allowed to clot by leaving it undisturbed at room temperature for 15–30 minutes. The clot was removed by Centrifuging at 1,000–2,000 \times g for 10 minutes in a refrigerated centrifuge. The resulting Supernatant is designated Patients serum, which was stored at -20°C until assay [33].

2.6 Assay Procedure for ELISA

Using Pipette, fifty microliter of negative and positive controls were added to the negative and positive control wells respectively.

In a sample wells, forty micro litre (40ul) of sample dilution buffer and ten micro litre (10ul) of sample were added. Sample were loaded onto the bottom without touching the well wall and mix well with gentle shaking. After 30 minutes of incubation at room temperature and sealed with closure plate membrane, it was diluted 30 times for 96T and washed 5 times

In addition, fifty micro litre (50ul) of HRP – Conjugate reagent was added to each well except the blank control well, it was incubated for 30 minutes and washed.

Fifty micro litre (50ul) of chromogen solution A and B was added to each well, mix with gentle shaking and incubated at 37⁰C for 15 minutes, light was avoided during colouring.

Lastly, fifty microliter (50ul) of stop solution was added to each well to terminate the reaction and colour in the wells was observed to change from blue to yellow.

Absorbance was read at 450nm wavelength using microplate reader and optical density (OD) value of the blank well was set at zero.

2.7 Interpretation of Results

Test effectiveness: the average value of positive control ≥ 1.00 ; the average value of negative control ≤ 0.10 . The critical value (CUT OFF) calculation: critical value = the average value of negative control + 0.15 Negative judgement: if the OD value < CUT OFF, the sample is HHV8 negative. Positive judgement: if the OD value \geq CUT OFF, the sample is HHV8 positive.

2.8 CD4 Counts ®(Cyflow) for HIV Positive Subjects

Procedure

Five millilitres (5mls) of blood was collected into an EDTA container and was mix well with the anticoagulant.

Twenty micro litre (20ul) of antibody were added to twenty micro litre (20ul) of blood sample. Incubation of the mixture was done in dark for 15minutes followed by Eighty millilitre (80mls) of buffer solution added to the mixture and the tube containing the mixture were plugged into flow cytometry, lastly, analysis was done in 60 seconds and the CD4 cells counts obtained

2.9 Data Analysis

Data was analyzed both manually and by the use of SPSS statistical software version 20 and was presented in the form of tables and Charts drawn using Microsoft word and Microsoft excel. The prevalence of HHV8 was expressed in simple proportions and percentages for the study groups. A statistical test of significance Chi square (X^2) was applied to determine whether there is statistically significant association between HHV-8 and HIV infection and between various variables. A P – value of 0.05 or less is considered significant.

2.10 Ethical Considerations

Approval of the authorities of the institution was sought for and obtained before commencing the study. Informed consent of individual respondents was also sought for and obtained.

3. RESULTS

In this study, we examined and tested the sera of 190 HIV infected patients Mean age was 34.38 ± 8.6 with a range of 14 – 60years. Male were 56(30.8%) while Female were 126(69.2%). 6people (3.3%) were tested Positive for HHV8 antigen, among HHV8 Positive individual, one (1) is male (0.55%) and

5 were Female (2.75%). The sex composition in this study is that of 70 Females for 100 males giving a male to female ratio of 1: 2.3, as in,

Table 4.1. All of the respondents with positive HHV8 Antigenaemia have low CD4 Count.

In respondents with CD4 0 – 200: 6(11.5%) were HHV8 Positive, 46(88.5%) were HHV8 Negative. Respondents with CD4 Counts 201 – 499 and > 500 were all found to be 100% HHV8 Negative. Table 4.4 shows Those with positive HHV8 antigenaemia were found to be within the range of CD4 Count 0 – 200 and those with low CD4 Count were found to have high HIV Viral load. All HIV/HHV8 Co – infected patients have poor compliance to clinic visit. Good and Fair Compliance were seen in HHV8 negative respondents while Poor compliance was seen in 100% of HHV8 Positive respondents, no significant difference observed between HHV8 and respondents' gender (Fisher exact $p = 0.6689$), age group (Fisher exact $P = 0.779$) and their Marital Status (Fisher exact $p = 0.467$).

4. DISCUSSION

The sample consisted of 182 Patients from ART Clinic Babura General Hospital with age range 14 – 60years and mean age of 34.38 ± 8.6 years. Majority of the respondents (70.9%) fall within the age group of 21 - 40. This does not correspond to study by [2] in Edo state and in Jos by [40]. The differences could be due to age distribution pattern of the study areas. Conversely it tall with a study done in Indonesia by [28].

The sex composition in this study is that of 70 Females for 100 males giving a male to female ratio of 1: 2.3. This is not a case in study conducted in Tanzania (2.75:1) by [25] and 1.64:1 In Indonesia by [28]. These differences could be due to age group selection of respondents, environmental factors as well as socio-cultural differences between the various study areas.

Majority of respondents with positive HHV8 were Females This is not a case in study conducted in Tanzania by [25] in which those with positive HHV8 Antigenaemia were mostly Males, the differences in gender could possibly result from more women attend the Clinic as compared to their Males counterpart as they are mostly Vagrant attending ART Clinic elsewhere, this also explains why more women are recruited for the study. No significant difference observed between HHV8 and respondents gender (Fisher exact $p = 0.6689$), age group (Fisher exact $P = 0.779$) and their Marital Status

(Fisher exact $p = 0.467$). This findings exactly confirmed the findings of [28] in Indonesia (age; $p = 0.5$, gender; $p = 0.78$ and Marital status; $p = 0.6$) but not in keeping with that of [13] among Ugandan HIV Populations.

The overall Seroprevalence of HHV8 in HIV positive individuals in this study is found to be 3.3%. This is not in keeping with previous studies 87% by [2]. South- South Nigeria, 70% by [39], in Cameroon, 31.2% by [41] in Xinjiang, China and 14.5% in East Java, Indonesia by [28]. These differences could be due to differences in the nature of the study in which this study was conducted on antigen detection while that of previous study were on anti body detection. Majority of the respondents might have antibody against the HHV8 virus because they might have got the infection at one point or the other in their lifetime and might get cured of the Disease when they are commencing on HAART, leaving only traces of antibody in their system which antigen detection procedure may not pick. 3.3%

of the respondents are those having the actual infection at a time of the study. Conversely the result Obtained was in keeping with that found in United State of America 1 – 5% by [3].

All of the respondents with positive HHV8 Antigenaemia have low CD4 Count, Positive relationship was also identified between them ($p = 0.001$) This findings tally with study conducted in China by [20] ($p = 0.0004$). However, no differences in mean CD4+ cell counts according to HHV8 status were found according to [11].

All HIV/HHV8 Co – infected patients have poor compliance to clinic visit. There is significant difference between HHV8 and compliance to clinic visit and follow- up ($P < 0.05$), $X^2 = 25.163$, Fisher exact $P = 0.000(S)$

Coincidentally, some of the Patients with low CD4 Count <200 were found to have clinical evidence KSHV, Presented with hyper pigmented maculopapular skin lesion involving skin and Oral mucosa which is in keeping with clinical evidence of Kaposi sarcoma lesion, this is in line with finding of [3]. Also, significant linear correlation were observed between respondents sample with positive HHV8 Antigenaemia and their respective CD4 Counts and Viral loads. $r(4) = - 0.704(S)$, this is also in line with study conducted by [20].

5. CONCLUSION

From the results obtained from this study it could be concluded that

HHV8 was higher among HIV patient with lower CD4 Counts and high HIV Viral load. The evaluation of CD4+ counts of the HIV patients shows that individuals with higher CD4+ counts has zero prevalence of HHV8 infection and hence have low risk of developing complication from the virus. In particular, low CD4 Count and high HIV Viral load seems to be the major culprit in developing HHV8 Infection and its complications.

5.1 Recommendation

In view of the findings of this study, the following are recommended:

1. There is need for government and other development partners to support interventional programs specifically targeted toward PLWHA in term of treatment compliance and Clinic visit.
2. Government and development partners should support operational research to determine the possible and effective ways and treatment strategies in HHV8/HIV Co infections and their Complications.
3. Government and development partners should strengthen efforts towards public awareness of HHV8/HIV/AIDS Co infections prevention and control especially using the electronic media, Religious and traditional leaders.

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Table 4.1: All of the respondents with positive HHV8 Antigenaemia have low CD4 Count.

Variables	Observation	No. tested	No. HHV8 Positive/%	P - value
Age in years(mean \pm SD)	34.38 \pm 8.6	182	NA	
Gender	Male Female	56 126	1(1.8%) 5(4.0%)	0.668
Age range (years)	10 – 20	9	0(0%)	
	21 – 30	65	3(4.6%)	
	31 – 40	64	1(1.6%)	0.779
	41 – 50 51 – 60	40 4 182	2(5%) 0(0%) 6(3.3%)	
Marital Status	Married Single Divorced Widow/Widower	20 99 58 5	1(5.0%) 2(2.0%) 3(5.2%) 0(0%)	0.467
	Total	182	6(3.3%)	

Table 4.2: SEROPREVALENCE OF HHV8 ANTIGEN AMONG PLWHA

Group	No. of Subject	Percentage
HIV Mono – infected	176	96.7
HIV/HHV8 Co – infected	6	3.3

PLWHA = people living with HIV/AIDS

Table 4.3: Relationship Between CD4 Count and HHV-8

CD4 Count	HHV8 Positive	HHV8 Negative	Total
0 – 200	6	46	52
201 – 499	0	82	82
>500	0	48	48

$P < 0.05$ $X^2 = 15.511$, $df = 2$, Fisher exact $P = 0.001(S)$

Table 4.4 Relationship Between CD4 Count, HIV Viral Load and HHV8 Positive Samples

HHV8 Positive Sample	CD4 Count	HIV Viral Load
1	200	4895
2	92	10351
3	80	4380
4	199	4450
5	34	509721
6	200	2888

$p > 0.05$ $r(4) = -0.704(S)$

Table 4.5: Relationship between Compliance to Clinic Visit and HHV8 antigenaemia

Clinic Compliance	HHV8 Positive	HHV8 Negative	Total
Good Compliance	0	83	83
Fair Compliance	0	63	63
Poor Compliance	6	30	36

$P < 0.05$ $X^2 = 25.163$, $df = 2$, Fisher exact $P = 0.000(S)$

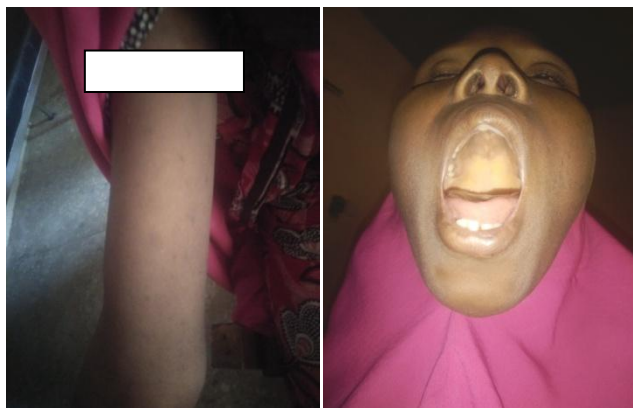


PLATE 1

PLATE 2

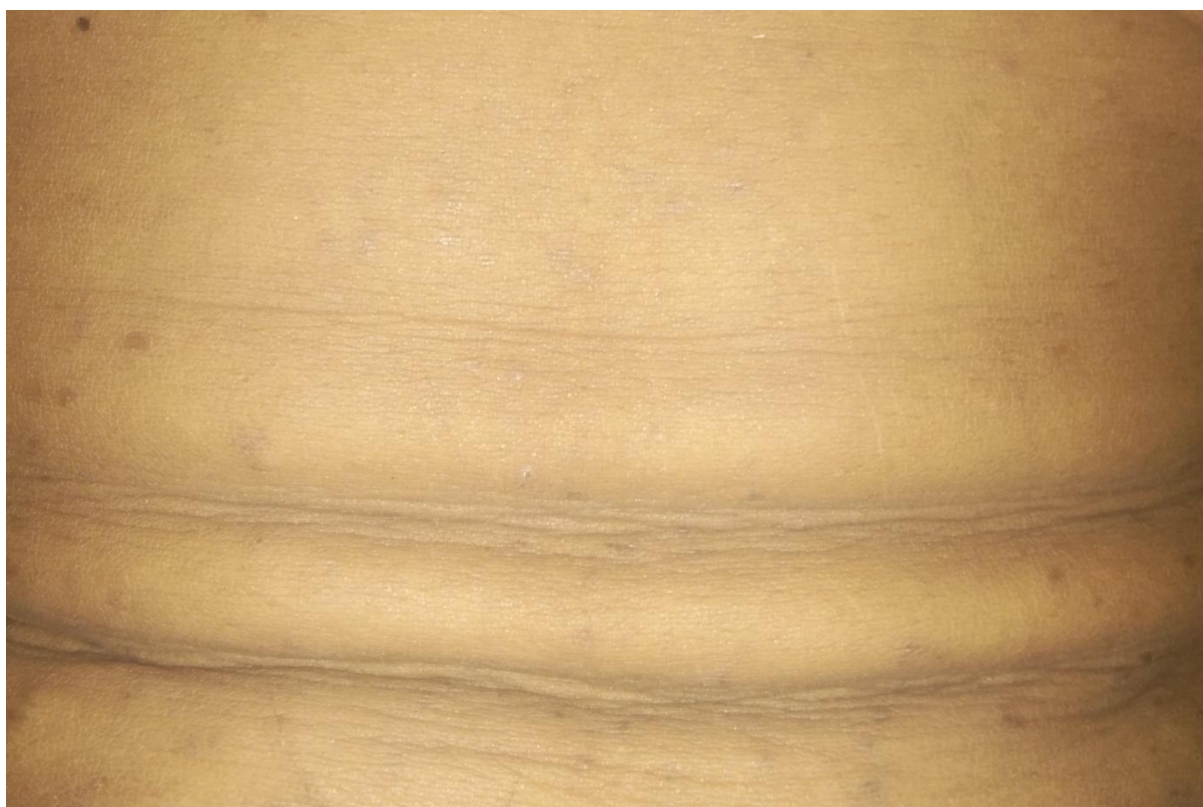


PLATE 3: Hyper pigmented macula popular lesion involving the Abdomen.