

SEROPREVALENCE OF SERUM IgG OF HSV-1 COINFECTED WITH HIV INFECTED PATIENTS WHO ATTENDED OFFA HIGHLY ACTIVE ANTI RETROVIRAL THERAPY (HAART) CLINIC

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ABSTRACT

Purpose: To determine the seroprevalence of IgG of HSV-1 coinfecting HIV patients who attended Offa General Hospital, Highly Active Antiretroviral Therapy Clinic (HAART).

Methods: A cross sectional study used to study the seroprevalence of IgG of HSV-1 coinfecting HIV infected patients that attended Offa Highly Active Antiretroviral Therapy Clinic (HAART). Blood samples were taken from 92 HIV infected patients between January and July 2016. The serum of the patients were examined for HSV-1 IgG with a well calibrated and well validated ELISA machine.

Results: Out of the 92 patient, 98.91% were tested positive to HSV-1 IgG. The age distribution, marital status, sex, occupation, CD4 counts, ART (Antiretroviral therapy) had no significant effect on the results because only one person tested negative to HSV-1 IgG.

Conclusion: HIV infected patients are very susceptible to HSV-1 IgG infection

Recommendation: HIV infected patients should be counseled on the need to comply with antiretroviral drugs and other drugs for

INTRODUCTION

Herpes simplex is a disease which is characterized by the formation of blisters on the skin and mucous membranes as intermittent, lytic replications in epithelia.¹ Herpes simplex virus type 1 (HSV-1) infection commonly occurs in the area of the mouth and nose, and Herpes simplex virus type 2 (HSV-2) infection in the genital area.² Herpes Simplex Virus Type 1 (HSV-1) is a nuclear replicating enveloped virus, usually acquired through direct contact with infected lesions or body fluids (typically saliva). The prevalence of HSV-1 infection increases progressively from childhood, the seroprevalence being inversely related to socioeconomic background.³ Primary HSV-1 infections in children are either asymptomatic or following an incubation period of about 1 week gives rise to mucocutaneous vesicular eruptions. During the primary infection of HSV-1, a strong immune response evolves composed of virus neutralizing antibodies and an antiviral CD4 and CD8 T cell response, which efficiently inhibits virus replication at mucosal sites

and in the nervous system.³ Aside from the activities of CD4 and CD8 T cells as interrupters of virus spread, other powerful inhibitors of this process are neutralizing antibodies. The passive administration of monoclonal antibody (mAbs) against HSV glycoproteins before infection and up to 3 days after infection inhibits the zosteriform spread of the virus.⁴ This indicates that as the virus moves from one compartment to another, i.e., from nerve ending to epithelial cells or vice versa, it becomes a target for neutralizing antibodies.⁵

Acquired immune deficiency syndrome (AIDS) is caused by a chronic infection with the HIV. HIV continues to be a major global public health issue. HIV continues to be a major global public health issue. In 2015, an estimated 36.7 million people were living with HIV (including 1.8 million children) – a global HIV prevalence of 0.8%. The vast majority of this number live in low- and middle- income countries. In the same year, 1.1 million people died of

AIDS-related illnesses. Many HIV-1-infected persons are already infected with HSV-2 at the time of HIV-1 acquisition, and cases of primary HSV-2 infection therefore are relatively uncommon among HIV-1-infected individuals. HIV-1-infected persons, however, also can have frequent or persistent HSV lesions, often with extensive or deep ulcerations, particularly among those with low CD4 counts. Notably, primary genital HSV-2 occurring in an HIV-1-infected person is a marker for ongoing unsafe sexual practices.

The degree of immunosuppression is an important factor in determining the HSV-2 reactivation rate, with a correlation between the mucosal rate of HSV-2 shedding and plasma HIV-1 RNA level and an inverse correlation with CD4⁺ cell counts.⁶ However, HSV-2 is frequently shed even in persons with CD4⁺ cell counts >400 cells/mm³, with substantial variability observed between persons at all CD4⁺ cell counts.⁷

Herpes group viruses are common opportunistic pathogens in HIV-infected individuals. Persistent mucosal ulcers caused by herpes simplex infection are difficult to differentiate from ulcerogenic diseases such as aphthous major and necrotizing stomatitis. Persistent oral pain with non-healing tendency is the characteristic clinical features of intraoral HSV infection.⁸ This infection is a matter of concern for the immuno-compromised patient, as it is associated with increased morbidity and mortality. In immuno-compromised patients, antiviral drug regimens may reduce the morbidity and potential mortality caused especially by the HSV.⁹

Narendra et al.⁸ reported that 23% of oral lesions in HIV are viral in origin, distributed in the lip, buccal mucosa, tongue, and palate. Diagnostic culture for HSV for all oral ulcers should be recommended regardless of their location in immuno-compromised patients. With this background of information, we have planned this study to determine the seroprevalence of HSV-1 IgG in HIV patients.

MATERIALS AND METHOD

This study was carried out at the General hospital, Offa, Kwara state, Nigeria. Ethical approval was taken from the ethical board in Kwara state between January and July 2016.

STUDY POPULATION

Data sources were structured interviewer-administered questionnaire, patients' case notes and

laboratory investigations. Ninety two 92 HIV-infected patients were used for the study. Patients were well informed and gave their consent before they were included in the study. Confidentiality was maintained throughout the study.

SAMPLE COLLECTION

Blood samples were collected from the 92 HIV infected individuals, using EDTA (anticoagulant) bottles. The blood samples were then centrifuged to separate the cells from the serum after which the serum was aspirated into sterile and well-labeled Eppendorf tubes, using Pasteur pipettes. Thereafter, the samples were transferred in cold storage to the laboratory, where they were stored at a temperature of about -20°C until the time of the assay.

SEROLOGICAL ASSAY

For the assay, Herpes Simplex 1 IgG (HSV 1 IgG) ELISA kit (as shown in plate 2), manufactured by Diagnostic Automaton Inc., California was used. This test is aimed at detecting IgG antibodies to HSV 1, using the indirect method for ELISA testing, with the aid of ELISA machine (as shown in plate 1).

TEST PRINCIPLE

Purified HSV antigen is coated on the surface of the microwells. Diluted patient serum is added to the wells and the HSV1 IgG specific antibody, if present, binds to the antigen. All unbound materials are washed away. After adding the enzyme conjugate, it binds to the antibody-antigen complex. Excess enzyme conjugate is washed off and TMB Chromogenic substrate is added. The enzyme conjugate catalytic reaction is stopped at a specific time. The intensity of the colour generated is proportional to the amount of IgG specific antibody in the sample. The results are read by a microwell reader compared in a parallel manner with calibrator and controls.¹⁰

PROCEDURE

- ❖ The reagents and materials were brought to room temperature, gently mixed and carefully labeled.
- ❖ The washing buffer was then prepared by adding 800ml distilled water to 200ml wash concentrate, to give a final volume of 1litre.
- ❖ The desired number of coated strips was placed in the well holder. 1:40 dilutions were prepared by adding 5µl of the samples, negative control, positive control and calibrator to 200µl of absorbent solution.
- ❖ This preparation was then mixed well. For the reagent blank, 100µl absorbent solution was dispensed into well 1A while 100µl of

diluted sera, calibrator and controls was also dispensed into the appropriate wells.

- ❖ The preparation was then mixed gently but thoroughly and air bubbles were removed by tapping the holder.
- ❖ At this stage, the microwell plate was incubated at room temperature for 30 minutes.
- ❖ The liquid mixture was removed from the wells and all wells were washed thoroughly, three times using the washing buffer.
- ❖ 100µl of enzyme conjugate was then added to each well and the plate was again incubated for 30 minute at room temperature.
- ❖ The enzyme conjugate was then removed from the wells by washing the wells three times, with the washing buffer. 100µl of TMB Chromogenic substrate was then added to each well after which the plate was incubated for 15 minutes at room temperature. Finally, 100µl of Stop solution was added to each of the wells to halt reaction.
- ❖ After making sure there were no air bubbles in the wells, the plate was placed in the

microwell reader and the Optical Density value (at 450nm) for each well was recorded.¹¹

CALCULATION OF RESULTS

- ❖ The Optical Density (OD) value for each sample is directly related to the Cut-Off (CO) value of the plate. The CO is given as:
 $CO = OD \times f$
 Where CO = Cut-off
 OD = Optical Density of Calibrator (3.401)
 f = factor value (indicated on the label of the Elisa Machine) (0.45)
 $CO = 3.401 \times 0.45$
 $CO = 1.53$
- ❖ The IgG index of each sample is then determined by dividing the OD values of each sample by the obtained OD value of the Cut-Off (i.e. 1.53 in this case).

2.4.5 INTERPRETATION OF RESULTS

- Negative: HSV 1 M Index less than 0.90 is negative for IgG antibody to HSV 1.
- Equivocal: HSV 1 M Index between 0.91-0.99 is deemed inconclusive.



PLATE 1: The Elisa Machine



Plate 2: Elisa Microtitre Plate

RESULTS

Ninety-two individuals were enrolled for this study with mean age of ranged 2-70years. Most of the patients 55(59.78%) were between ages 31-50years. There were 28(30.43%) males and 64(69.57%) females. Majority 66(71.74%) were traders table 1.

Sixty six individuals with CD4count less or equal to 350 were tested positive while twenty five individuals with CD4count greater than 350 were tested positive. Ninety one subjects on antiretroviral

therapy were tested positive. Eighty eight subjects who had never received blood transfusion were tested positive while three subjects who received blood transfusion were infected. Ninety-one subjects who were tested positive, did not show any clinical manifestation of AIDS. None of the subjects examined took antiherpes drugs but only one of them was tested positive. Positive: HSV 1 M Index of 1.00 or greater is positive for IgG antibody to HSV 1.

Table I: Seroprevalence of HSV-1 IgG in HIV Infected Patient Based on Socio-Demographic Status

FACTORS	STATUS	NUMBER TESTED	NUMBER TESTED POSITIVE	PREVALENCE %
AGE DISTRIBUTION	21 – 30	5	5	100
	31 – 40	8	8	100
	41 – 50	30	30	100
	51 – 60	25	24	96
	61 – 70	14	14	100
	61 – 70	10	10	100
SEX	MALE	28	28	100
	FEMALE	64	63	98.44
MARITAL STATUS	DIVORCED	2	2	100
	MARRIED	66	65	98.48
	SINGLE	22	22	100
	WIDOW AND WIDOWER	2	2	100
	WIDOW AND WIDOWER	2	2	100
OCCUPATION	PROFESSIONAL	7	7	100
	ARTISAN	3	3	100
	CIVIL SERVANT	4	3	75
	STUDENT	1	1	100
	TRADER	66	66	100
	UNIDENTIFIED	7	7	100
	NO OCCUPATION	4	4	100
	NO OCCUPATION	4	4	100

Table II: Seroprevalence of HSV-1 IgG in HIV Infected Patients Based on Cd4 Counts, Art, Blood Transfusion Received, Clinical Manifestation, and Anti-Herpes Drugs

FACTORS	STATUS	NUMBER TESTED	NUMBER TESTED POSITIVE	PREVALENCE %
CD4 COUNTS	≤ 350	67	66	98.51
	>350	25	25	100
ON ART?	NO	1	1	100
	YES	91	90	98.90
	YES	91	90	98.90
HAVE YOU EVER RECEIVED BLOOD TRANSFUSION?	NO	89	88	98.8
	YES	3	3	100
	YES	3	3	100
ANY CLINICAL MANIFESTATION OF AIDS?	NO	92	91	98.91
	YES	0	0	0
	YES	0	0	0
ON ANY ANTI HERPES DRUGS?	NO	92	91	98.91
	YES	0	0%	0
	YES	0	0%	0

4.1. DISCUSSION

Herpes simplex is a disease which is characterized by the formation of blisters on the skin and mucous membranes as intermittent, lytic replications in epithelia.¹ It is prevalent world-wide and normally proceeds asymptomatically. Complications result when internal organs are also affected and necrotize.¹² Herpes simplex virus type 1 (HSV-1) infection commonly occurs in the area of the mouth and nose, and Herpes simplex virus type 2 (HSV-2) infection in the genital area.² People with a large number of sexual partners are at increased risk of acquiring HSV-2 infections.² The presence of pre-existing HSV-1 antibodies may reduce the severity of genital HSV-2 infection.² The seroprevalence increases up to the age of 50 years.¹³

In this study, 91(98.9%) of 92 patients with HIV infection were seropositive to HSV-1 IgG. This finding is similar to the report of 90% seropositivity to HSV-1 in three group of HIV infected individual in eastern India in 2015.¹⁴

It is of interest to note that a 2 year infected child in this study has a high CD4 count of 798 and the child is very healthy. The child probably acquired the infection from the mother.. This result shows that HSV-1 infection is no respecter of age once exposed to the virus. The sexually active age of 21 to 50 years of age also has high prevalence to the disease. Both male and female of old age also showed high prevalence of HSV-1 co infection.

Gender does not affect HSV-1 co infection, both have high prevalence of the virulence (i.e not gender specific). A study done by Pebody *et al.*¹⁵ shows that the typical age at which HSV-1 infection is acquired ranges from 5 to 9 years in Central and Eastern European countries like Bulgaria and Czech Republic, to over 25 years of age in Northern European countries such as Finland, The Netherlands, Germany, and England and Wales. Young adults in Northern European countries are less likely to be infected with HSV-1. European women are more likely to be HSV-1 seropositive than men.¹⁵

The HSV-1 infection shows a high prevalence of 100% among the HIV patients who are traders. Most traders in this setting are low socioeconomic status and not likely to maintain or adhere to their oral hygiene.

Once a person is infected with HIV, the virus begins to attack and destroy the CD4 cells of the person's immune system. HIV uses the machinery of the CD4 cells to multiply (make copies of itself) and spread

throughout the body. This process is called the HIV life cycle.

During regular check-ups, HIV care providers will want to know the CD4 count in order to help keep track of how healthy the patient is and whether the virus has progressed in the body:

This study reported that majority of study population has lower CD4 counts suggesting ongoing infection. This research works shows that regardless of the CD4 count the patient can get infected with HSV 1. The only difference is that those with CD4 above 350 would look and feel healthier than those below. All the patients in this study were placed on antiretroviral therapy to boost their CD4 counts. This study showed that regardless of antiretroviral drugs the study population were still tested infected with herpes. We hypothesized that the ART might not have a direct positive effect on HSV. None of the patients showed any clinical manifestation of AIDS, this is because all the patients complied with their antiretroviral drugs.

4.3. CONCLUSION

The results generated from this research has shown that most HIV infected persons are also infected with HSV-1, although HSV infections are often mild or asymptomatic.

4.2. RECOMMENDATION

The general public should be educated on HSV-1 and its mode of transmission, prevention, control and the management therapy. Early detection of HSV-1 IgG in HIV patients will encourage prompt treatment of its symptoms.

The implications for clinical practices are:

Doctors and other medical personnel should attend seminars and provide health talk to provide better knowledge of the coinfection.

Aseptic techniques should be used to collect samples from patient to prevent cross infection. Doctors should take prophylaxis anti HIV drugs in case of accident prick.

The implications for policy makers

Policy makers should provide fund for workshops for doctors and other health personnel. The government must have political will to attend to all health programs. The policy makers should ensure aseptic techniques are used and violators should be punished accordingly.

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