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Research Article

Sero-Prevalence of Treponema Pallidum Infection and Immunological (Il-6 and TNF-A) Responses in HIV Sero-Positive Patients Attending Federal Medical Centre Gusau, Zamfara State, Nigeria

Abdullahi Y. Alkali[1], Kasim Muhtari[2] , Nura I. Shehu[3] , Saratu Ibrahim[4] , Hajara Yusuf [5], Mahmud Jibril,[6]

[1, 3, 4,5,6,7] *College of Health Sciences and Technology, Tsafe,*
[2]*Yusuf Maitama Sule University, Kano.*
[1, 2,3,4,5, ,7]*Department of Medical Laboratory Sciences, CHST-Tsafe,*
[2]*Parulniversity, Vadodara, Gujarat, India*

Abstract: Human Immunodeficiency Virus (HIV) infection is a global public health concern due to associated morbidity and mortality as a result of immunosuppression. HIV is complex and remains the subject of ongoing research. HIV patients can lead to adverse outcomes and this can be averted if detected and treated. However data on this subject matter are uncommon in Gusau, Zamfara state, Nigeria. This study was designed to determine the Sero-prevalence and immunological responses among HIV attending Federal Medical Center, Gusau. A total of 301sample were collected for Serological investigations and sero-positive patients were enrolled for the study. The samples were tested using antibodies detection kit (Unigold). Flow cytometry was used to enumerate $CD4^+$ T-cells and ELISA was use for quantitative detection of cytokines production. Questionnaires were used to obtain information on subject bio-data. Data were analyzed using SPSS software (Version 26.0 IBM corp, USA) to determine the relationship between socio-demographic factors, $CD4^+$ T-cells and cytokines production. The overall Seropositive infection tested was 2.7 %(8/301) were. Sero-positive and 97.3 %(293/301) were Sero-negative. The highest Sero-prevalence of 2.8% (6/208) was recorded among female followed by 2.2% (5/221) and 2.2 % (5/218) males and married with those that are engage in sexual behavior and lack of education background respectively. majority (2.7%; 8/301) of those that are Seropositive with the $CD4^+$ T-cell count in male, >500 ART are males were statistical significant compare to those that are on non-ART with the total average of 326 Cells/mml3 and the female with 608 Cell/mml^3 on those that are on therapy while the average of Non ART in female is < 350.The cytokines responses (IL-6 and TNF-alpha) were measure by the Used of Quantitative ELISA. Syphilis and HIV co-infection were associated with increase in IL-6 (18.5 pg/ml) and TNF- α (20 pg/ml). IL-6 and TNF- α is correlated with low CD4+ T cell count and high plasma cytokines values, syphilis infection was associated with significant increase cytokine production and significant decrease in the CD4+ T cell count. The findings underscore the importance of preventing and promoting treating of HIV infected individuals and there immunological responses, the cytokines production in HIV individual's increases conferred to the HIV-infected and not significant in the HIV-uninfected group. Serological test leads to false positives result and its result should be treated with caution. Sero-positive result should be confirmed by the use of PCR, especially in patients with low CD4+ T cell count to reduce unnecessary administering drugs to individuals that does not require therapy.

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1.Introduction

The Human Immunodeficiency Virus (HIV) infection is complex and remains the subject of ongoing research worldwide (Peyriere et al., 2018). Epidemiologic studies demonstrate that sexually transmitted diseases (STDs) including syphilis, and particularly genital ulcers associated with primary syphilis, are associated with an increased risk of HIV acquisition (CDC.2018). The World Health Organization (WHO) estimated that the majority of 12 million annual new cases of HIV occur in South and Southeast Asia, Latin America and Africa including Nigeria [1, 2]. Worldwide, about 45.4 million people were infected with HIV as of 2015[1, 2]. In 2015, it caused about 107,000 deaths, down from 202,000 in 1990 [1, 2]. Phagocytes are a group of cells responsible for recognition, capturing of foreign invaders and destroyed them outside the body by the use of enzymatic reaction [1]. The phagocytes include the monocytes, macrophages, neutrophil granulocytes, or dendritic cells [1]. These cells express germ-line encoded pattern recognition receptors (PRR) that detect conserved microbial structures not being present in the host [6]. The immune response to Retrovirus involves humoral and cell-mediated immunity, and both of these are implicated in resistance to reinfection [2]. Tumor necrosis factor (TNF) is a cell signaling protein (cytokine) involved in systemic inflammation and is one of the cytokines that make up the acute phase reaction [3].

2. Methodology

Specimen Collection, Preparation and Storage**.**Five milliliters (5ml) venous blood of each of the study participant was collected into a Vacutainer with Advanced Semi-separator gel (SST II), (Belliver Industrial Estate, Plymouth, PL6 7BP, United Kingdom) using standard method [9]. A total of 301 HIVpatients attending STDs clinic at Federal Medical Center (FMC), Gusau-Zamfara State, located in Gusau local Government Area (LGA) of Zamfara State-Nigeria. The study population involved individual's attending sexually transmitted diseases (STDs) Clinic at Federal Medical Centre, Gusau-Zamfara State.

Ethical clearance.Was obtained from the Ethical Committee of Federal Medical Centre, Gusau- Zamfara State before the commencement of the study. The Sample size was determined using the formula for cross sectional studies which was proposed by Lwanga and Lemeshaw (1991), Grad and Araoye (2006). The sample size was determined based on the estimated syphilis prevalence of previous studies; the prevalence of HIV infection was 16.0 % [1, 7].

Venereal Disease Research Laboratory. Rapid test strip manufactured and described by ACON Laboratories, Inc. (USA) was used for the detection of T. palladium antibodies in serum respectively, with strict adherence to the manufacturer's instructional manual.

All patients attending STDs clinic and gave consent to participate were included in the study and those who did not give consent were excluded from the study. Written consent was obtained from the subjects prior to data and sample collection. The specimen was then dispensed in to well labeled container and was stored at -20^{o}C until use [8]. The collected samples were transferred from STDs Clinic to the Microbiology Laboratory Unit at FMC, Gusau where they were screened for Retrovirus using UNIGOLD kit.

Flow Cytometry.Flow cytometry is a procedure used for counting cells stained with an antibody conjugate with Chromogen. The flow Cytometry is designed to analyse and separate cells stained with fluorescent antibody. The flow cytometer uses a laser beam and light detector to count single intact cells in suspension. Flow cytometry was used for counting cells stained with an antibody conjugate with Chromogen.

ELISA. A solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (ELISA). A monoclonal antibody specific for TNF-α coated was used for determination of TNF-α content and IL-6. All the data generated were analyzed using SPSS software Version 26.0(IBM corp, USA) and levels of IL-6 and TNF-alpha response against T. pallidum infection were expressed in pg/ml. The sero-prevalence of T. pallidum infection in HIV patients was expressed in proportion and percentages.

Data Analysis.All the data generated were analyzed using SPSS software Version 26.0(IBM corp, USA) and levels of IL-6 and TNF-alpha response against T. pallidum infection were expressed in pg/ml. The sero-prevalence of T. pallidum infection in HIV patients was expressed in proportion and percentages. The Chi-square was used to determine the relationship between the T. pallidum and HIV co-infection and socio – demographic factors of the patients. A p-value of < 0.05 was considered as significant.

3. Results

A total of 301 HIVsero-positive patients consisting of 33 (11 %) ART-naïve and 268 (89 %) ART experienced patients attending STDs clinic at Federal Medical Center (FMC), Gusau-Zamfara State were screened for T. pallidum infection (syphilis). Of the 301 patients, 89 (29.5%) were males 212 (70.5 %) were females (Table 1). Out of the 89 males 2 (2.2%) had syphilis and 6 (2.8%) of the 212 females were infected with Treponema pallidum (P= 0.0018). Out of the 33 ART-naive patients tested, 6 (18.2%) were positive for syphilis while 2 or 0.8% (2 out of 268) of the ART-Experienced patients were infected. The Overall prevalence of T. pallidum infection was 2.7% (8 of 301) Out of this figure 2 %(6/301) were ART-naïve while 0.7% of (2/301) were ART-experienced patients (Table 3.1). Statistical analysis showed that there was significant association (P= 0.0018).Relationship between HIV infection and Socio-demographic factors of HIV Sero-positive Patient at Medical Center, Gusau.According to the age range, the distribution of HIVinfection showed highest prevalence among patients aged 11-20 (2, 10%), 21-30(4, 3.5%), and 31-40 (2, 1.6 %). However, No HIV infection was detected patients aged 0-10 and 41-70 years (P> 0.05). Based on the educational status of the patients, frequency of HIV was higher among those with primary educational level and least among those who had secondary and Islamic education (Table 2). Distribution of HIV Infections based on $CD4^+$ T-cell Counts. Six (6, 2%) of the HIV sero-positive patients (who were ART-naïve)had CD4+ cell counts of <200 cells/ul (mean $CD4^+$ cells count = 143 cells/ul). Two (2, 0.6%) of these patients had CD4+ cell counts between 200-350 cells/ul (mean $CD4^+$ cell counts=263 cells/ul), while the rest of those patients had $CD4^+$ cell counts of >350 cells/ul (mean CD4+ cell counts=356 cells/ul) (Table 3).Levels of Cytokines Production (IL-6) in Response to T. pallidum infection among HIV Sero-positive Patients.A total of 8 HIV sero-positive patients were analyzed for the levels of IL-6 production. Out of the 8 patients, 3(37.5%) (S=1, S=4 and S=8) had the highest levels of IL-6 production (Table 4.5) while 5(62.5%) (S=2, S=3, S=5, S=6 and S=8) had the lowest level of cytokines production among the patients. On cytokines production in HIV patients, there was a consistent decrease in IL-6 production among syphilis negative individuals (Table.4).

Cytokines Levels (TNF-α) in Response to T. pallidum infection in HIV Sero-positive Patients.A total of 8 HIV sero-positive patients were analyzed for the levels of TNF-α production. Out of the 8 patients, 3(37.5%) (S=1, S=4 and S=8) had the highest levels of production (Table 4.5) while 5(62.5%) (S=2, S=3, S=5, S=6 and S=8) had the lowest level of cytokines production in normal patients. On cytokines production in HIV-experienced patients, there was a consistent decrease in IL-6 production among those who were negative (Table 5).

Table 1 Gender of HIV Sero-positive patients at Federal Medical Center, Gusau

	T. pallidum Detection ;		P Value	
Parameters	No. (%) Screened	No. (%) positive	No. (%) Negative	
Gender				
Males	89 (29.6%)	2 (2.2%)	87 (97.8%)	
Females	212 (70.4%)	6 (0.5%)	206 (97.2%)	
Total	**301(100%)**	**8 (2.7%)**	**293 (97.3 %)**	P= 0.0018

ART-Antiretroviral therapy

Table 2 Relationship between HIVinfection and Socio-demographic factor of patients at Medical Center, Gusau.

	T. pallidum Detection ;			
Demographic	No (%).	No. (%)	No. (%)	s CharacteristicScreened
Positive	Negative			
Gender				
Males	89 (29.5%)	2 (2.2%)	87 (98.8%)	
Females	212 (70.5%)	6 (2.8%)	206 (97.2%)	
Total	**301(100%)**	**8 (2.7%)**	**293 (97.3 %)**	
Age range(years)				
0-10	13	0(0.0%).	13 (100%)	
11-20	20	2(10.0%)	18 (90%)	
21-30	113	4 (3.5%)	109(96.5%)	
31-40	121	2 (1.6%)	119 (98.4%)	
41-50	19	0 (0%)	19 (100)	
51-60	10	0(0%)	10 (100%)	
61-70	5	0 (0%)	5 (100%)	
Total	**301(100%)**	**8 (2.7%)**	**293 (97.3%)**	
Education status				
Islamic school	63	2(3.2%)	61 (20.3%)	
Primary	108	4(3.8%)	104 (34.5%)	
Secondary	83	2(2.4%)	81 (26.9%)	
Tertiary	55	0 (0%)	55 (18.9%)	
Total	**301 (100%)**	**8(2.7%)**	**293(97.3%)**	

Table 3 Distribution of T. pallidum Infection among ART- naïve HIV patients Based on $CD4^+$ T cells counts.

$CD4^+$ Counts (Cells/ul)	Mean $CD4^+$ Counts (Cells/ul)	No (%) of ART Naïve patients for T. pallidum	No (%) positive for T. pallidum	P-Value
<200	143	33(11.3%)	6(2%)	0.0410*
200-350	263	220 (75.0)	2(0.6%)	0.0205*
>350	356	40(13.6%)	0(0%)	0.009
Total	**524**	**293(97.4%)**	**8(2.6%)**	**0.0042**

CD= cluster of differentiation
ul= microliter

Table 4 Distribution of T. pallidum Infection among ART-Experienced HIV patients Based on CD4⁺ T cells counts.

CD4⁺ Counts (Cells/ul)	Mean CD4⁺ Counts (Cells/ul)	No (%) of ART Experienced for T. pallidum	No (%) of positive for T. pallidum	P-Value i
<450	329	288 (98.3%)	5(1.7%)	0.0013*
450-650	393	2 (0.7%))	2(0.7%)	0.0025*
>650	715	3 (1.0%)	1(0.3%)	0.0018*
Total	**479**	**293**	**8**	

CD= cluster of differentiation
ul= microliter

Table 5: Levels of Cytokines Production (IL-6 and TNF-alpha) in Response to T. pallidum Infection among HIV Sero-positive Patients.

No of HIV/syphilis Positive Sample Tested	IL-6 levels in ART-naïve patients (pg/ml) TestedTested	IL-6 levels in HIV positive ART-Experienced patients (pg/ml)	Control (N=8) Tested	p-value
S=1	20.7 (pg/ml)	11pg/ml	10.26pg/ml	0.023*
S=2	18.8 (pg/ml)	12.3 pg/ml	10.26pg/ml	0.011*
S=3	16.4 (pg/ml)	10 pg/ml	10.26pg/ml	0.002*
S=4	21.3 (pg/ml)	12 pg/ml	10.26pg/ml	0.031*
S=5	12.2(pg/ml)	11 pg/ml	10.26pg/ml	0.001*
S=6	16.5(pg/ml)	13 pg/ml	10.26pg/ml	0.002*
S=7	18.6(pg/ml)	11 pg/ml	10.26pg/ml	0.011*
S=8	22.1 (pg/ml)	10 pg/ml	10.26pg/ml	0.032*

Key
S= Sample
ART= antiretroviral therapy
IL= interleukins
Pg/ml=pictogram per mills
***Significant association**

Table 6Cytokines Levels (TNF-α) in Responses to T. pallidum Infection in HIV Sero-positive Patients.

No of HIV/syphilis Positive Sample Tested	TNF-α levels in ART-naïve patients (pg/ml) TestedTested	TNF-α levels in HIV positive ART-Experienced patients (pg/ml)	Control (N=8) Tested	P-value
S=1	24 (pg/ml)	10.2pg/ml	10.26pg/ml	0.013*
S=2	19 (pg/ml)	12.3 pg/ml	10.26pg/ml	0.021*
S=3	18 (pg/ml)	10 pg/ml	10.26pg/ml	0.012*
S=4	20 (pg/ml)	12 pg/ml	10.26pg/ml	0.001*
S=5	19(pg/ml)	11 pg/ml	10.26pg/ml	0.031*
S=6	20(pg/ml)	13 pg/ml	10.26pg/ml	0.012*
S=7	16(pg/ml)	11 pg/ml	10.26pg/ml	0.016*
S=8	24(pg/ml)	12 pg/ml	10.26pg/ml	0.023*

Key
S= Sample
ART= antiretroviral therapy
Pg/ml=pictogram per mills
TNF-α= tumor necrotic factor alpha
***Significant association**

4. Discussion

Human immunodeficiency Virus constitutes a significant public health problem and it is associated with an increased risk of acquiring opportunistic resulting in aggravated morbidity and mortality [1, 2, 3.]. The immunological response to syphilis in HIV infection can lead to a high production levels of the cytokines especially IL-6 and TNF-Alpha. This occurs as a result of improper response of CD4+ T-cells response to macrophages to eliminate the virus, contrasting to our result which indicates that the infection rate was lower among HIV sero-positive individuals attendees in FMC, Gusau (2.7%) and higher among sexually transmitted disease (STD) clinic attendees in Argentina (9.7%) [1, 2]. The overall sero-prevalence of 2.7% recorded in the study was higher than the prevalence of 0.9%-2.5% reported in Kenya [1, 3], and 1.8% in Uganda [1, 3], However, the present result was lower than the figures reported elsewhere including 4.8% in older men in Kenya [1,],; 11.7 % in Kigali [1, 3],. The lower seroprevalence of HIV patients observed in this study as compared to other countries in sub-Saharan Africa may be due to increased awareness campaign about the infection and control strategies being implemented in Nigeria. It is widely believed that the high prevalence of syphilis in HIV infected patients depends on pre-ART period of individual HIV patients [1, 3]. This is consistent with the observations made in this study and other studies elsewhere [3], which showed that highly active ART appears to reduce the incidence of syphilis in HIV infected patients [1, 6],. The study recorded a low prevalence rate of HIV/syphilis co-infection and it established that there was significant association ((P= 0.0018) between the prevalence of syphilis and ART status of the HIV sero-positive patients attending Federal Medical Centre (FMC), Gusau. Although the prevalence of syphilis infection appears to be low, but the detrimental health impact on HIV patients resulting in severe morbidity and mortality has been suggested to be significant if left untreated [1, 3, 5]. It is particularly significant among those with primary educational level and less among those with secondary and Islamic education when compared between educational statuses of the patients. However, the $CD4^+$ T-cell counts of ART-Naïve patients were lower when compared with the HIV sero-negative in this study. Thus, higher active ART has a significant role to play in reducing the incidence of. On the other hand, the IL-6 production was low among patients compared to seropositive patients. The TNF-α production levels was also low compared to the HIV-experienced patients in this study. it's recommended that the HIV sero-positive result should be confirmed by the use of PCR, especially in patients with low CD4+ T cell count to reduce unnecessary administering of drugs to negative individuals that do not require therapy and There is need to for awareness campaign among the populace on the impact of HIV to limit the spread of the disease. The importance of ART should be emphasized to all HIV positive. HIV patients with low CD4+ T-cells counts should be educated on the need to adhere to treatment and adequate management to boost their $CD4^+$ T-cells count so that the Efficacy of drugs should be monitored periodically to regulate the emergence and spread of the drug resistance particularly HIVwhich are sexually transmitted that leads to the decrease in $CD4^+$ T cells counts in HIV as well as increase in cytokines (IL-6 and TNF-a) production. Future researches are recommended, including, In vitro analysis such as identification of different antigens that share similar epitope with antigen that lead to the decrease in CD4+ T-cells and increase in cytokine (IL-6 and TNF-alpha) production. There is need for identification of different classe of antibody (IgM or IgG) against HIV infection in other to identify whether the infection is primary or secondary infection.[1, 2, 3]'

Disclosure

This article contain study with Human participant and is hospital base by the Author

Conflicts of interest

There are no conflicts of interest between the Authors.

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