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Evaluation of the immunological response to HIV-Nef antigen among HIV positive clients in Anambra state Nigeria: A pilot study

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Abstract

Human Immunodeficiency Virus (HIV) is a global public health challenge that has resulted in millions of deaths worldwide. Despite the introduction of the Highly Active Antiretroviral Therapy (HAART) regimen, which has significantly improved the life expectancy of HIV-positive individuals, there is still no cure for this disease. Therefore, alternative treatment approaches are urgently needed to address the unmet medical needs of HIV patients. Our pilot study aims to assess the formation of naturally occurring HIV-specific Nef antibodies among HIV-positive clients.

This study was carried out in two hospitals in Anambra State, accredited for the management of people living with HIV (PLWH). After the issuance of the ethical approval, a total of 16 HIV-positive clients were recruited, blood samples were withdrawn with consent and subsequently subjected to ELISA for the detection of HIV-specific Nef antibodies. R version 4.3.2 was utilized for the analysis.

The mean age of participants was 40 years with more female participants recruited for the study. HIV-specific Nef natural antibodies were detected in 87.5% of these participants. IgG and IgM were the most prevalent (31%) and IgG1 was the least prevalent (19%). Amongst the demographic and clinical information retrieved, none showed any association with HIV-positive clients who possessed these antibodies.

This pilot study provides preliminary evidence for the development of HIV accessory protein antibodies in HIV-positive clients in Anambra State, Nigeria.

Keywords: Vaccine development; Immune response; HIV; Natural occurring antibodies

1. Introduction

The current management approach to Human Immunodeficiency Virus/Acquired Immune Deficiency Syndrome (HIV/AIDS) infection offers a chronic and lifelong treatment option, often with associated long-term side effects such as liver complications, renal and metabolic disorders, and osteoporosis, among others (1). Although the current treatment regimen requires the use of a single pill in a bid to promote adherence, studies have shown that many patients routinely seek alternative forms of treatment which can often negatively affect their treatment outcomes (1,2). Additionally, epidemiological data published by the Joint United Nations Programme on HIV/AIDS (UNAIDS) has shown a reduction

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in the trend of the HIV incidence-to-prevalence rates, recording 11.2% in 2000, 6.6% in 2010 and 4.6% in 2018 (3). While this metric brings us closer to the global target of 3% and below to foster the reduction in the incidence rates, it is clear that focused interventions to protect seronegative individuals will play a major role in achieving this goal.

The development of an HIV vaccine has been a daunting task primarily due to the high genetic variability of HIV, across different countries, and different viral subtypes (1,4). As such, developing a universal vaccine candidate has been made with less success as evidenced but the vast majority of unsuccessful studies identified in the literature (1,2,5,6). Currently, the RV144 vaccine clinical trial represents the only recent clinical evidence to show vaccine-driven protection among study participants (7). This was placed at 60% after 12 months but reduced to 31% over 3.5 years. Incidentally, while the current focus and vaccine strategies had primarily been on neutralizing and broadly neutralizing antibodies, the RV144 study provided an immune protection correlate linked to a non-neutralizing IgG antibody. This antibody was shown to target the V1V2 portion of the HIV envelope, thus opening the road to investigation of non-neutralizing antibodies for HIV immune protection.

The HIV Nef is an essential accessory protein linked to viral immune escape due to its interference in the antigen presentation mediated by the Major Histocompatibility Complex-1 (MHC-1) on cells (8–10). However, while studies have identified the development of immune response in preliminary data, clinical trials incorporating its use are yet to identify a protective correlation between antibodies developed and protection (11). However, with the improvement in technology and precision in targeting viral epitopes in recent years, its potential remains primarily due to its role as an important mechanism by which the virus evades the immune response (6,12).

Our study therefore provides a pilot documented evidence for the development of naturally occurring Nef antibody response within HIV-positive persons in Nigeria.

2. Material and method

2.1. Ethical Consideration

Due approval was obtained from the Nnamdi Azikiwe University Teaching Hospital (NAUTH) ethics committee. The nature of the study was presented to the committee, including how the patient's consent would be obtained before being recruited into the study. The right of the patients to participate or withdraw from the study was also fully honoured without any adverse consequence to the patient during the execution of this study.

The approval number from NAUTH was NAUTH/C5/66/VOL.15/VER.3/109/2020/075.

2.2. Study Population

The participants consisted of HIV-I-positive persons, who were either single or married. These participants were primarily recruited from antiretroviral (ARV) clinics in our study sites. The inclusion criteria were both male and female individuals aged between 18 and 60 years who were confirmed HIV-positive clients. Additionally, an exclusion criterion was placed for Pregnancy (in women) and for falling outside the age bracket (18-60 years).

2.3. Sample Size and Sampling Approach

This study was designed as a pilot study to confirm the occurrence of naturally occurring HIV Nef antibodies. As such, a sample size of 16 participants was recruited using a convenient sampling approach. Demographic information of all recruited participants was collected and subsequently utilized for further downstream analysis.

2.4. Study Area

Between August 2022 and February 2023, a pilot study was conducted at two healthcare facilities in Anambra State - Nnamdi Azikiwe University Teaching Hospital (NAUTH) and Regina Caeli Hospital, Awka (RCH). These institutions are fully equipped to provide comprehensive care for both adults and children living with HIV/AIDS.

2.5. Sample Storage

A phlebotomist withdrew 10 ml of blood from the participants and then transported them to the laboratory in a vaccine bag within two hours of collection. The samples were centrifuged and stored at -20°C to ensure their viability over a long period. In certain cases, the samples were collected, centrifuged and frozen at the collection sites before transportation.

2.6. Enzyme-Linked Immunosorbent Assay (ELISA) Detection of Immune responses

This was done using an in-house optimized protocol which was adapted from literature (13). ELISA plates were coated with a 1:1000 dilution of HIV Nef antigen and then incubated overnight. The plates were subsequently washed with 0.05% Tween 20 and blocked with 2% bovine serum albumin (BSA). Participants' serum samples were diluted to 1:400 (1:800 for samples tested against IgG), added to their assigned wells and incubated for 2 hours at +37°C. After washing, HRP-conjugated secondary antibodies (IgG (1:1,024,000), IgG1 (1:512,000), IgG2 (1:32,000), IgG3 (1:16,000), IgG4 (1:512,000), and IgM (1:256,000)) were added and incubated. The plates were washed again and 50 ul of TMB substrate was added. The end product was blue which turned yellow with the addition of 50 ul of 1M sulphuric acid. The optical density (OD) was measured at 450 nm with the AMR-100 ELISA plate reader.

2.7. Statistical Analysis

R version 4.3.2 was used for statistical analysis (14). The *changepoint.np* package was used to determine the breakpoints of the ELISA assays (15). The ELISA readings were sorted in ascending order with the readings from the blank wells included and passed through the nonparametric changepoint package (16). Finally, variables such as age, time of diagnosis, and time of initiation of therapy were correlated with the inferred HIV Nef status of the participants.

3. Result

A total of 16 participants were recruited for this pilot study with a mean age of 40 years. More female participants were recruited with most of the participants having their highest education at the secondary level. Table 1 summarizes the demographic information of all recruited participants.

Furthermore, this study observed a mean value of 5.8 years as the mean number of years post-confirmatory test for participants, with a maximum of 16 years and a minimum of 6 months. Additionally, the participants' viral load levels ranged from undetectable to 237 copies/ml while $CD4^+$ levels ranged from 144 cells/mm^3 to 712 cells/mm^3 with a mean value of 438 cells/mm^3. Finally, all participants were on the same treatment regimen comprised of Tenofovir, Lamivudine and Dolutegravir.

Table 1 Demographic data distribution among study participants

Demographic	Description	Summary
Age	Mean age	40 years
Health Institution	Public	10 participants
	Private	6 participants
Gender	Male	7 participants
	Female	9 participants
Education	Secondary	6 participants
	Tertiary	2 participants
	Postgraduate	1 participant

Figure 1 visualizes the range of Optical Density (OD) values for each of the HIV Nef-specific antibodies assessed using a boxplot. The data indicated that all OD values were within the range of 0.1 and 1, which is the recommended OD range for maximum reliability of results. Furthermore, all OD values showed similar interquartile range values but considering the fact the ELISA protocol was not repeated, it might be difficult to tell to what extent the OD values obtained in this experiment can be reproduced.

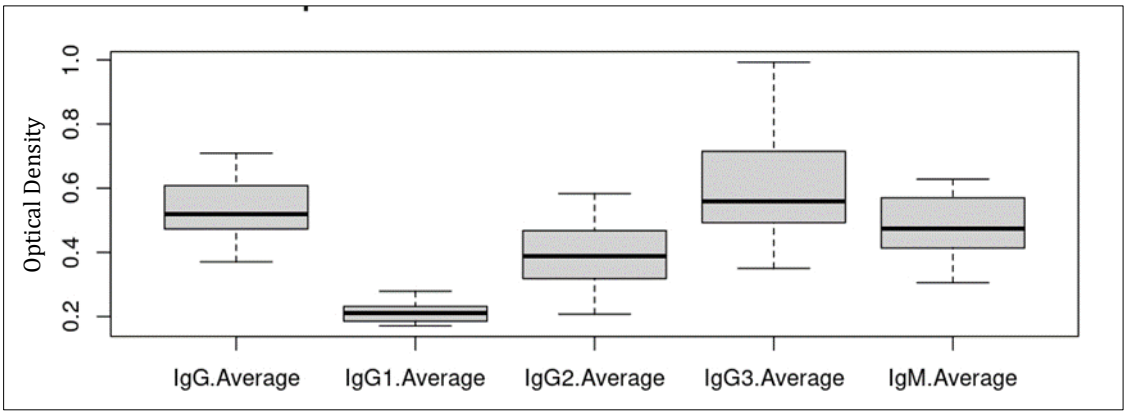

Figure 1 ELISA Optical Density values for different HIV-specific Nef antibodies

Using the breakpoints determined by the *changepoint*.np package, the OD values were qualitatively inferred for the presence of specific antibody response. Figure 2 indicates that HIV-specific Nef IgG and IgM had the highest (31%) immune response while IgG1 had the least (19%) immune response. Also, our data showed that 87.5% (n = 14) of participants displayed at least one immune response against the HIV Nef antigen tested with Figure 3 indicating that HIV-specific Nef immune response was predominantly higher in these HIV seropositive participants. Furthermore, no participant simultaneously tested positive for all antibody subtypes assessed in our study.

The chi-squared test performed additionally indicated that there was no signification relationship/dependence between the presence of at least one type of HIV Nef-specific antibody and the demographic data, viral load and $CD4^+$ cells.

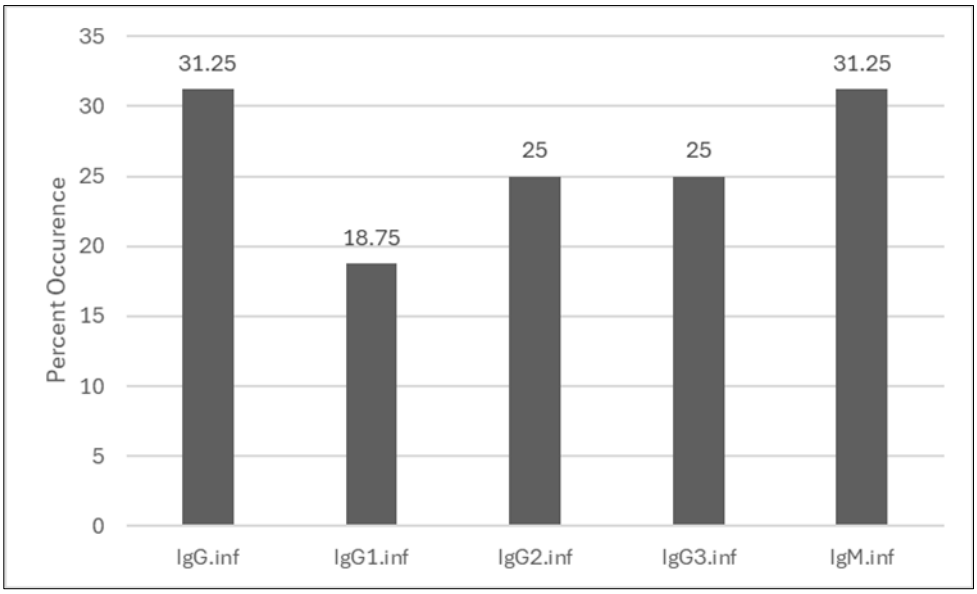

Figure 2 Occurrence of different HIV-specific Nef antibody subtypes

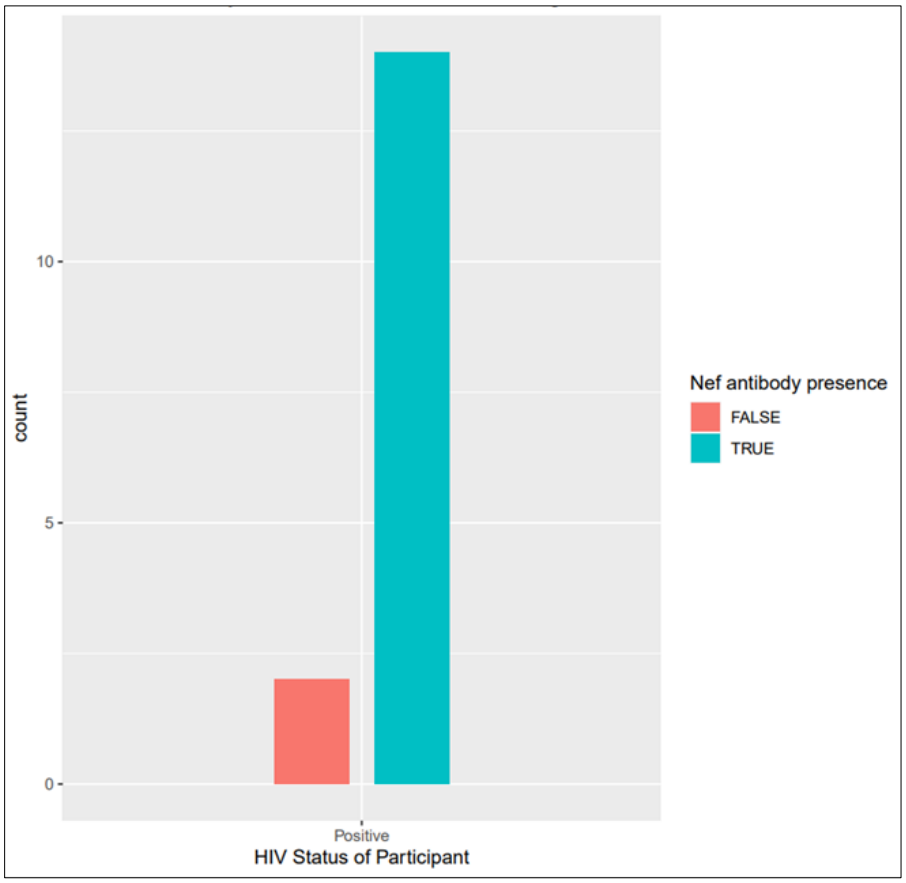

Figure 3 Distribution of the presence of at least one subtype of HIV-specific antibody subtype in HIV-positive clients

4. Discussion

Our study provides evidence for the development of a humoral immune response to HIV Nef antigen within HIV seropositive participants in Nigeria. More important is the fact that the different antibody subtypes observed in our study had different levels of occurrence among participants. This is similar to what can be found in the RV144 clinical trial where the presence of IgG and IgG3 were correlated with the protective efficacy of the vaccine candidate via antibody-dependent cell cytotoxicity (ADCC) and phagocytosis (ADCP) (2,7). Other literature has also supported the roles of these antibody subtypes in humoral responses via these same mechanisms.

However, the presence of the antibodies does not automatically translate into protection as shown by the STEP study which utilized Env, Gag, Pol and Nef immunogens (11). The study recorded frequent and consistent CD8+ cellular responses but ultimately did not translate into a protective effect on participants. More recent evidence has suggested that this outcome was associated with HIV susceptible CD4+ cells targeting the adenoviral vector used in the study(2). This underscores the need for more reliable vaccine-targeting strategies to bypass the viral immune escape mechanisms. A possible approach would use intracellular targeting antibodies such as cell-penetrating peptides as reported in a previous study(4). Additional use of nanotechnological approaches such as nanoparticles could further improve vaccine stability and targeting while minimizing vector-specific immune response(10). While HIV Nef is not currently the main target of HIV vaccine research, it represents an important aspect of the virus's mechanism for evading the immune system and could be a valuable target in the broader context of HIV treatment and cure strategies.

5. Conclusion

Our study presents preliminary evidence for the occurrence of HIV-specific Nef antibodies within our participants. Considering that current trends in vaccine strategy and design revolve around the development of immunogens drawn from the viral envelope, our preliminary results provide evidence for more focus on other HIV-specific immunogens beyond the viral envelope.

Compliance with ethical standards

Acknowledgements

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Disclosure of conflict of interest

The authors declare no conflict of interest.

Statement of ethical approval

This study involved human participants. The Nnamdi Azikiwe University Teaching Hospital Ethics Committee provided the ethical approval for this study (approval number: NAUTH/C5/66/VOL.15/VER.3/109/2020/075).

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

Authors contribution

UCO, GOC, GN and COE conceptualized and designed this study; UCO, IMA and ECO performed the clinical and laboratory aspects of the work; UCO, CKE and OAE developed the manuscript and GOC, GMN and IMA reviewed the manuscript.

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Data availability

The primary data for this work is not available due to the need to protect the identity of participants. However, aggregated data with no client-identifying information can be made available for research purposes on reasonable request from the corresponding author.

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