Assessment of HIV p24 antibody response in HIV discordant couples in Anambra state, Nigeria.

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Submitted: 23rd April, 2024; Accepted: 25th April, 2024; Published online: 30th April, 2024
DOI: https://doi.org/10.54117/jcbr.v4i2.4
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Abstract

The current management approach of HIV/AIDS is not associated with curative potential via the HAART regimen, thus necessitating alternative treatment approaches. Current vaccine target options have limited protective efficacy and thus create the need for the identification of better and more reactive immunogenic HIV epitopes. Our study therefore aims to assess the formation of naturally occurring HIV-specific p24 antibodies among HIV heterosexual serodiscordant couples.

This study was carried out in two hospitals, all of which can manage HIV-positive clients and also offer counselling and case management services. After the issuance of the ethical approval, a total of 64 participants were selected for the ELISA to detect HIV-specific antibodies for p24 antibodies. R version 4.3.2 was utilized for the analysis.

The mean age of participants recruited stood at 38 years with an equal number of males and females, since all participants were HIV-serodiscordant heterosexual couples. Thus, the antigen-antibody ELISA results indicated that IgM and IgG3 predominantly reacted with p24 (29.7% and 25% of samples testing positive). Further binomial regression analysis identified no predictors for the formation of HIV-specific p24 antibody immune response. However, it was established that the development of this antibody in HIV-seronegative partners was not dependent on their partners, suggesting the potential benefit of incorporating HIV p24 antigenic epitopes in vaccine development.

This study therefore provides substantive evidence for the natural development of antibodies to other proteins of the HIV molecule beyond the HIV Envelope region that has been consistently targeted in previous studies. Thus, future studies need to explore the protective efficacy of these antibodies.

Introduction

The global estimates of HIV infections currently stand at 39 million with evidence suggesting that more women currently live with HIV compared to men (World Health Organization, 2022) . In Nigeria, recent estimates have placed HIV infection rates at between 1.4% and 2.1% with more than 2 million persons currently living with HIV

(Onovo et al., 2023; UNAIDS, 2017). Current management of HIV via HAART has achieved significant milestones within the country. However, several challenges persist such as issues with adherence, and complications, such as lipid abnormalities and lactic acidosis, which arise from the use of these drugs (Oturu et al., 2024; Zachová et al., 2024).

From a contextual perspective, qualitative studies have linked poor perception and willingness to access HIV testing and management to factors such as poor formal education, poor household wealth quintile, younger age and religious beliefs which lead to stigma and shaming of people living with HIV (PLWH) (Dahlui et al., 2015; Odimegwu et al., 2017) . The authors' data confirm a reduction in the rate of avoidance of PLWH in recent years, with more recent estimates placing the percentage who avoid PLWH at about 50% when compared with initial estimates of 85% (Odimegwu et al., 2013, 2017) . It is clear that while government policies and interventions are partly responsible, issues relating to the context-specific implementation of such policies may hinder their optimum impact. Additional reliance on alternative treatment options such as traditional medicine also has an impact on HIV management within the country, with a study showing that while 77% of 640 recruited participants had sort alternative (herbal and spiritual) solutions to HIV management, 73% of participants indicated that they had denied this fact when asked by their clinician (Onifade et al., 2013).

Due to these challenges, recent strides in HIV research are focused on curative treatment approaches which target reservoir sites while promoting optimum immune response. Thus, the development of vaccines, vaccine targets and strategies is paramount, particularly due to evidence which has shown the propensity for the development of broadly neutralizing antibodies (BNabs) against HIV, particularly in serodiscordant couples (Cheng, 2014; Liu et al., 2018; Ruiz et al., 2017). BNabs such as 10E8 and 2F5 have been identified to target the envelope region of the virus and have been credited with conferring protection in seronegative HIV partners (Cheng, 2014; Lorenzi et al., 2021) . However, a major challenge in broadly neutralizing antibody research includes the inability to stimulate the production of BNabs artificially. Prospective research trends seeking to identify more immune-stimulatory sites of the virus, as potential targets for vaccine development are currently ongoing.

The p24 capsid protein is a relatively conserved region of the virus but has been cited to be poorly immunogenic by several studies. However, a study has optimized the delivery of this p24 antigen directly to antigen-presenting cells in lymph nodes using nanostructured lipid carriers, resulting in significant T-cell and antibody proliferation (Bayon et al., 2018) . While the protective potential of this approach was not explicitly measured, this study demonstrated that further exploration of p24 for vaccine development shows promise. An earlier study further explored the potential for the use of a particle-based p24-based vaccine, administered subcutaneously (Liard et al., 2011) . The results identified that Subcutaneous (SC) immunization induced HIV-1 p24 specific IgG without CD8 T cells, intradermal (ID) immunization induced both cellular and humoral responses, and transcutaneous (TC) immunization targeted epidermal Langerhans Cells (LCs) inducing CD8 effector cells and mucosal IgA in the vagina (Liard et al., 2011). The study suggests that different skin layers can be targeted to direct specific immune responses, which could be significant for developing innovative vaccination strategies against HIV1

However, considering the high rate of genetic variability in HIV strains, it is imperative to assess the potential for LMICs, including Nigeria to benefit from these biotechnological advances. Considering the paucity of data on the identification of p24 antibodies in serodiscordant couples within Nigeria, our study aims to fill this gap while also seeking to explore potential factors which may optimize the development of these p24-specific HIV antibodies.

Methods

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.

Study Population

The participants consisted of HIV-I heterosexual serodiscordant couples, recruited using a convenient sampling technique. The inclusion criteria were both male and female individuals aged between 18 and 60 years who have a partner who is a confirmed HIV-positive patient. Additionally, an exclusion criterion was placed for Pregnancy (in women), for falling outside the age bracket (18-60 years) or not having a partner who is a confirmed HIV patient.

Sample Size

The sample size for this study was calculated using Cochran's formula using the following expression (Nanjundeswaraswamy *et al.*, 2021):

$n = (Z^2\ pq)/e^2$

Where: e= desired level of precision = 5%

P = estimated proportion of the population that has the attribute (prevalence rate) = 1.4% (UNAIDS, 2017)

q = 1-p

Z = confidence level = 95% = 1.96

$n = 21.21 \approx 21$ HIV-positive participants

Therefore, since our work dealt with HIV-serodiscordant couples, this sample size translated to imply that a minimum of 42 serodiscordant couple participants would be included.

Study Area

The study was carried out at Nnamdi Azikiwe University Teaching Hospital (NAUTH), Nnewi, and Regina Caeli Hospital, Awka (RCH), both located in Anambra State from August 2022 to February 2023. These institutions are well-equipped for the management of adults and children living with HIV/AIDS.

Proforma Validation

This was designed to serve as a supporting document, and as such, only a construct validity was performed. This was done by presenting the proforma draft to two persons, both of whom are well-experienced in conducting clinical research (Strauss *et al.*, 2009). These persons provided constructive feedback, chief of which was the need to include the viral load and CD4 count data as part of the data which would be collected for the patient folder before sample collection, in a bid to save cost. Additionally, they also advised on the need to obtain information on when the spouse got married as this would

serve as the beginning of HIV exposure for the seronegative partner.

Transport and Storage of Blood Samples

Ten millilitres of blood was withdrawn from participants by a Phlebotomist. The samples were conveyed to the laboratory in a vaccine bag (cold-chain transport) within 2 hours of collection. The samples were centrifuged and stored at -20ºC to ensure that samples would be viable over a long period. In certain cases, the samples were collected, centrifuged and frozen at the collection sites before being transported.

Indirect ELISA Assessment of Samples

An in-house ELISA protocol was obtained and optimized in line with the steps described in the literature (Alandijany et al., 2020).

A multi-channel pipette was used to transfer 100 µl of 1:2000 dilution of p24 antigen, utilized for coating the ELISA plates. The microtiter plates were incubated at +4°C overnight. The next day, the plates were washed four times using the washing buffer (0.05% tween 20), followed by blocking the ELISA plates using 200 µl of 2% bovine serum albumin (BSA) as the blocking buffer for 1 hour at 37°C. Thereafter, the plates were washed once more with a washing buffer.

The primary antibody for this study was the participants' samples. The serum samples were diluted with 1X PBS (phosphate-buffered saline) to 1/400 (1/800 for microtiter plates testing for HIV-specific IgG antibodies. Afterwards, 100 µl of these dilutions were added to their assigned wells and the setup was incubated for 2 hours at +37°C. After this incubation, the plates were washed six times with a washing buffer.

The Horseradish peroxidase-conjugated secondary antibodies were then diluted (IgG (1:256,000), IgG1 (1:64,000), IgG2 (1:32,000), IgG3 (1:16,000), IgG4 (1:8,000), and IgM (1:128,000) using 1X PBS, after which 100 µl of each dilution was added to wells of their respective ELISA plates. The plates were covered once more and incubated for another 1 hour at +37°C. After this time, the plates were washed six times with a washing buffer.

The substrate for HRP used for this study was the BioFx TMB One component substrate. Using a micropipette, 50 µl of the substrate solution was placed per well. After 15 minutes, 50 µl of stop solution (sulphuric acid) was added to the wells. The end product was blue which turned yellow with the addition of 1M sulphuric acid (H_2SO_4). The optical density was measured at 450 nm with the AMR-100 ELISA plate reader.

Statistical Analysis

Statistical analysis was conducted using R version 4.3.2 (R Core Team, 2022) . The *changepoint. np* package was used to statistically determine the breakpoints of the ELISA assays (Killick *et al.*, 2014). To do this, each batch of ELISA readings was sorted in ascending order with the readings from the blank wells included and passed through the nonparametric changepoint package (Lardeux et al., 2016) . This was followed by a McNemar's test to test for the independence of paired HIV-specific antibody results. The *glm* function and the *blorr* package in R were then subsequently utilised to perform a logistic regression to identify the potential of other data collected in predicting the development of HIV-specific p24 antibodies (Hebbali, 2020; Marschner *et al.*, 2022).

Results

A total of 64 participants who satisfied the inclusion criteria were recruited into the study. Since our participants were serodiscordant heterosexual couples, Table 1 below shows an equal number of male-to-female participants; however, we can observe that there was a slightly higher population of HIV seropositive

participants among the male population. Additionally, a higher proportion of recruited participants had secondary education as their highest form of formal training.

Table 1: Demographic characteristics of recruited participants

Demographic	Item	Serological status (n = 64 participants)		
		Seronegative	Seropositive	N
Age	Mean Age	38.6	38.2	
Serological status		50%	50%	32
Health Institution	Public	50%	50%	32
	Private	50%	50%	32
Gender	Male	46.9%	53.1%	32
	Female	53.1%	46.9%	32
Highest Education	Primary	33.3%	66.7%	3
	Secondary	47.4%	52.6%	19
	Tertiary	42.9%	57.1%	7
	Postgraduate	50%	50%	2
Ethnicity	Igbo	45.2%	54.8%	42
	Urhobo	100%	--	1
	Calabar	--	100%	2

The boxplot represented in Figure 1 below shows the relative variation in OD?? values obtained. The IgG OD values had the highest interquartile range of 0.25 while IgG2 had the smallest interquartile range with a value of 0.05. Additionally, all antibody responses measured except for IgG had a minimum OD value of less than 0.1 but a maximum OD of less than 1.

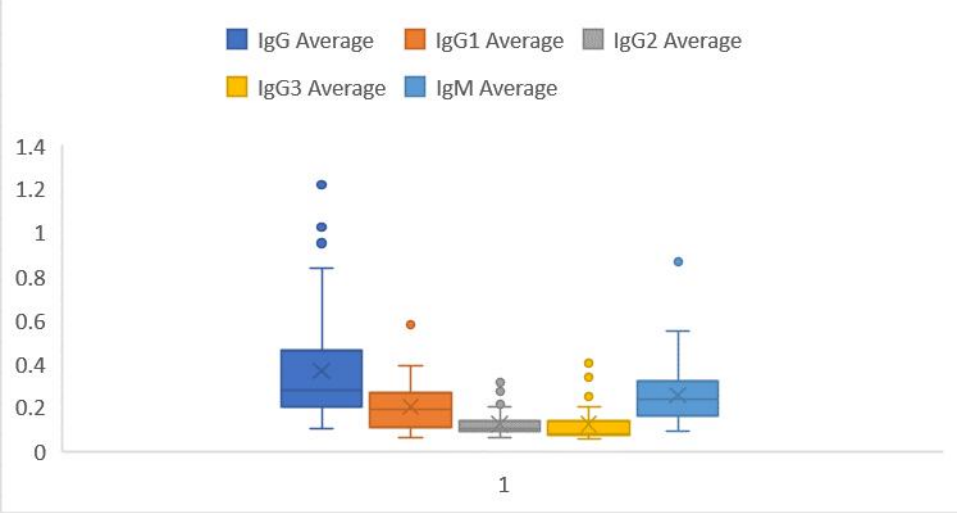

Figure 1: Boxplot of Optical Density values obtained for HIV-specific p24 antibody response assessment via ELISA

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 p24 IgM had the highest immune response while IgG1 had the least immune response. Also, our data showed that 62.5% (n = 40) of participants displayed at least one immune response against the HIV p24 antigen tested with Figure 3 indicating that HIV-specific p24 immune response was preferentially more among HIV seropositive participants. Furthermore, only one (1.6%) HIV seropositive participant tested positive for all antibody subtypes assessed in our study.

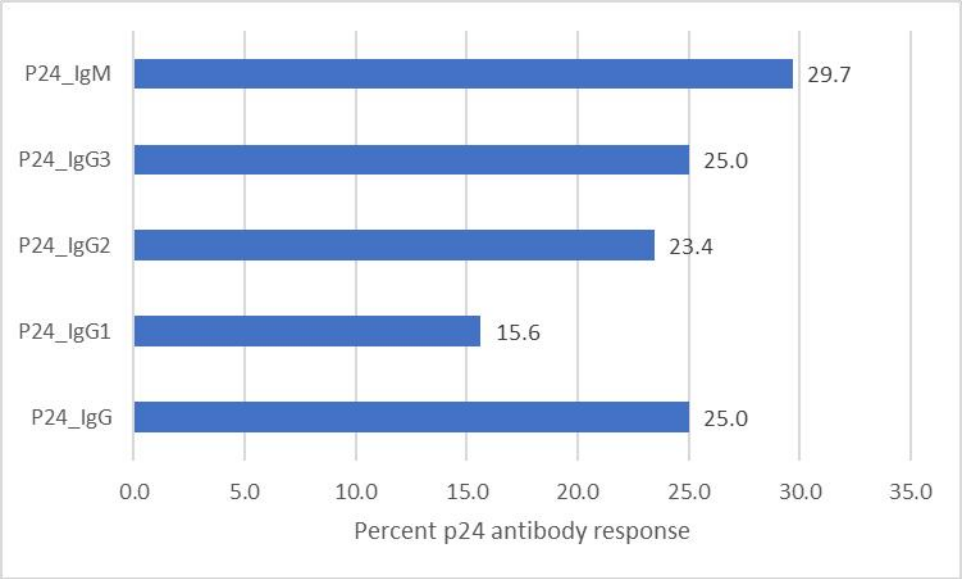

Figure 2: Immune response pattern of HIV-specific antibody subtypes.

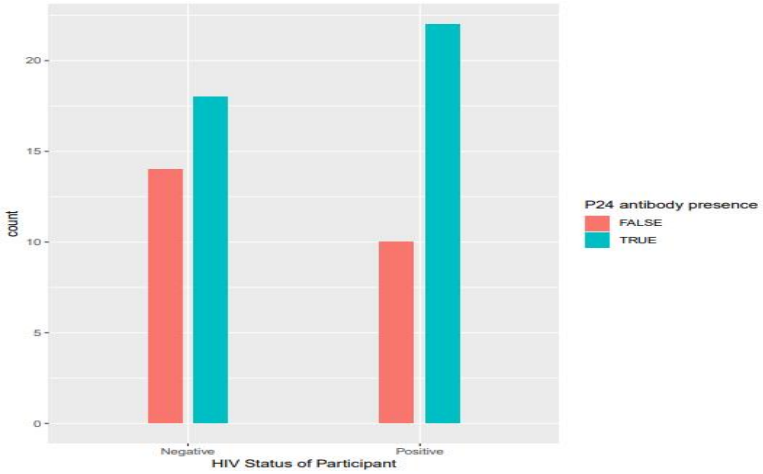

Figure 3: HIV-specific P24 antibody response across the HIV serological status of participants

Having identified HIV-specific p24 antigen immune response in seronegative partners, a McNemar's test was conducted which gave a non-significant (Table 3) result indicating that the presence of these antibodies in seronegative participants was not dependent on its presence in their seropositive partners.

Finally, a binomial logistic regression was fit for the immune response binary outcome variable using potentially predictive information obtained via the Proforma, including the participants' viral load, $CD4^+$ count, number of years of exposure and year of diagnosis. However, there was no significant predictor for the formation of HIV-specific p24 antibodies based on all predictive data collected thus far in this study.

Table 2: McNemar's test for HIV p24 antibody responsible between heterosexual partners

Antigen	McNemar's X^2	p-value
p24	0.75	0.39

Discussion

The results indicated that serodiscordant participants within Nigeria were able to generate HIV-specific antibodies. However, our current study does not provide sufficient evidence to support that these antibodies observed translate to protective efficacy for the participants involved. This was supported by a different study which tested the MRKAd5 HIV-1 Gag/Pol/NEF vaccine (McElrath et al., 2008) . The study utilized 3000 seronegative participants and confirmed the production of interferon-gamma-HIV specific T-cells targeting the HIV antigens utilized in the vaccine. However, it noted that despite being immunogenic, this T-cell production did not translate to protective efficacy. Recent approaches to vaccine development have been able to sufficiently improve the immunogenicity of HIV p24, although more research into protective efficacy remains as a gap (Bayon et al., 2018; Liard et al., 2011).

From our study, we can speculate on the possible activity of the observed antibodies based on literature evidence. For example, IgG3 is predominantly known to possess invitro neutralization activity while IgG1 have been associated with antibody-dependent cellular cytotoxicity (ADCC) of HIV-1-infected cells, both of which confer protection (Neidich *et al*., 2019; Tomaras *et al.*, 2009). The neutralizing activity of IgG3 may account for its ready deployment in the acute phase of HIV infection with its downregulation over time (Yates et al., 2011) . Thus, it is possible that HIV p24 antigenic epitopes targeted to stimulate IgG3 production would improve the protective efficacy of vulnerable populations. Our study also indicates a higher proportion of IgG3 antibody immune response, thus, implying a possible protective impact on a subset of our participants.

Additionally, the McNemar test indicated that the development of HIV-specific p24 antibody immune response in HIV-seronegative partners was not dependent on its presence in their partner. This supports the potential for the stimulation of HIV p24 antibodies as potentially beneficial vaccine candidates. However, considering that none of the typical predictive variables had any influence on its development, more research in determining the correlation of protection and stimulation of antibody-protective response is crucial.

We also observed from the boxplot visualization in Figure 1 that IgG antibody subtype had the highest interquartile range. Considering that these samples were evaluated in batches, the relatively wide range

of OD values seen may indicate a lack of precision in this optimized indirect ELISA. As such, several studies have suggested the use of batch-specific OD breakpoints; an approach which we applied to our analysis. Finally, considering the relatively smaller sample size used in our study, it is possible that predictors were not sufficiently powered to significantly influence the study data.

Conclusion

Our study provides evidence to support the hypothesis on the development of a natural immune response against HIV p24 within heterosexual HIV-serodiscordant couples. The immune response pattern identified IgM and IgG3 as the predominant antibody subtypes. However, no predictive variables assessed displayed any potential to predict the formation of these antibodies. This might suggest that a complex interaction is responsible for the development of these antibodies.

Acknowledgement

We wish to acknowledge the Chantal Biya International Reference Centre (CIRCB), Yaounde Cameroun for providing the HIV p24 antigen for this study at no additional cost.

Conflict of interest

The authors declare no conflict of interest.

Funding source

This study was funded partly by the American Society for Tropical Medicine and Hygiene (ASTMH) ACGH student support.

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 either the first or corresponding authors.

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