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Assessment of the Patterns of Immunoglobulin G and M Levels in HIV Serodiscordant Couples

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ABSTRACT

This longitudinal study assessed the patterns of immunoglobulin G and M levels in HIV serodiscordant couples. A total of 20 serodiscordant HIV couples (40 patients) and 20 controls (40 HIV seronegative couples) were randomly recruited for the study. Five milliliters of venous blood samples were collected from each of the participants at baseline, after 6 months, and after 12 months follow up period respectively for the determination of Immunoglobulin G (IgG) and Immunoglobulin M (IgM) levels using immunoturbidimetric method. Results showed thatthere was a statistically significant decrease in the mean serum level of IgG in the HIV discordant test group at baseline and 6 months than in the control group (p=0.000) but remained similar after 12 months follow up (p=0.081). The mean serum IgM level observed in the HIV discordant test group did not differ significantly (p>0.05) compared to the control group at baseline, after 6 months, and 12 months follow up respectively. The mean serum levels of IgG was significantly decreased in the male test group compared to male controls at baseline and 6 months but were similar at 12 months whereas there were no significant differences observed in serum levels of IgG in the female test groups at baseline, 6 months and 12 months follow up respectively. Furthermore, the mean serum IgM levels in both the male and female test groups did not differ significantly at baseline and after 6 months follow up compared with the male and female control group respectively. However, the mean serum IgM levels in the male test group was significantly decreased after 12 months follow up compared with the male control group. Therefore, HIV serodiscordance status may influence the serum IgG and IgM levels in affected individuals. Further studies are necessary to unravel the mechanism behind these findings.

Keywords: HIV, AIDS, Serodiscordance, Immunoglobulin G, Immunoglobulin M, Couple.

1. INTRODUCTION

The genus Lentivirus, subfamily Orthoretrovirinae of the Retroviridae family, is where the human immunodeficiency virus (HIV) is classified.[1,2] Types 1 and 2 of HIV are distinguished from one other by genetic traits and variations in the viral antigens (HIV-1, HIV-2). It is the causative agent of acquired immunodeficiency syndrome (AIDS) and it is transmitted through infected body fluids such as blood, semen, and vaginal fluids as well as breast milk and during intravenous drug use by sharing unsterilized needles. However, it is vital to emphasize that people with HIV who are taking antiretroviral therapy (ART) and have their viral load suppressed do not transfer the virus to their sexual partners.[3] HIV is characterized by immunosuppression, which renders the affected individuals susceptible to myriads of pathogenic organisms that can cause numerous infections.[4-6] With 40.1 million [33.6-48.6 million] deaths caused by HIV to date, it is still a significant global public health concern. In 2021, 1.5 million [1.1-2.0 million] people acquired HIV, and 650 000 [510 000-860 000] people died from HIV-related causes. At the end of 2021, there were an estimated 38.4 million [33.9-43.8 million] HIV-positive individuals worldwide, with 25.6 million of them living in the WHO African Region.[3]

The worldwide HIV response has come under growing danger over the past two and a half years

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because of the AIDS and COVID-19 pandemics, as well as the economic and humanitarian crises. Millions of pupils have missed school due to COVID-19 and other instability, which has interrupted health services around the world and made them more susceptible to HIV.[7] 60% of the world's poorest nations are in debt distress or are at great risk of entering it, and an estimated 75 to 95 million people have been pushed into poverty, an increase without precedent.[8,9] As a result, communities that were previously more vulnerable to HIV have come under intense pressure to improve their response to AIDS. The newest figures gathered by UNAIDS show that while new HIV infections decreased globally last year, the drop was only 3.6% compared to 2020 the smallest yearly reduction since 2016.[10] This suggests that rather than accelerating, global progress against HIV is slowing. As a result, many areas, nations, and communities are left to deal with other current problems as well as rising HIV infections.[10]

Discordance is an important aspect of HIV infection and is becoming a key focus area in HIV prevention research.[11-13] When one partner in a romantic or sexual relationship has HIV while the other does not, the partnership is said to be serodiscordant.[14] About 50% of people living with HIV in high-incidence regions like Sub-Saharan Africa are serodiscordant partners.[15,16]

In Sub-Saharan Africa, transmission within serodiscordant married or cohabiting couples accounts for a sizable part of new infections, and transmission within this crucial demographic is a major and preventable driver of the HIV epidemic.[17,18] Varied prevalence has been documented across Nigeria ranging from 16.3 – 60.7%.[19-22] Sero-discordant relationships are hence a focus of HIV prevention and care initiatives. Significant research has shown that people living with HIV (PLWH) can effectively prevent HIV transmission to their sexual partners by using suppressive ART.[23,24]

Immunoglobulins are synthesized by B lymphocytes (B cells) and are both synthesized and secreted by plasma cells. An immunoglobulin molecule that is specific for an antigen's epitope is referred to as an "antibody." Structurally, immunoglobulin is a "Y"-shaped molecule that consists of four polypeptide chains; two identical heavy chains, and two identical light chains connected by disulfide bonds.[25] Each chain is composed of structural domains called immunoglobulin domains. These domains contain about 70-110 amino acids and are classified into different categories according to their size and function.[26] The mammalian immunoglobulin heavy chain has five sub-types which are denoted by the Greek letters: α, δ, ε, γ, and μ.[27] The type of heavy chain present defines the class of antibody. IgA, IgD, IgE, IgG, and IgM are the five major types of antibodies that are produced by B cells. Each main type of antibody recognizes and neutralizes a particular pathogen and has biological properties that are very distinct from one another. During the acute stage of infection, antibodies are crucial in controlling viral proliferation. Out of the five existing isotypes of antibodies, those of HIV-1 specific antibodies are mostly IgG and to a lesser extent IgM and IgA.[28]

Studies have shown elevated levels of IgG among HIV-positive persons.[29-31] Also, some authors have documented elevated levels of IgM in HIV seropositive individuals, although some other studies found no significant differences in mean serum IgM levels in HIV-positive persons compared to controls.[29-32] It is important to assess these immunoglobulin levels, particularly concerning determining their levels in HIV serodiscordant couples, given the documented variations in the levels of these immunoglobulins in the sera of HIV-positive individuals and the crucial roles they play in the ongoing monitoring and management of HIV infection and its progression.

Furthermore, despite the fact that serodiscordant couples are at an elevated risk of HIV transmission and are neglected or under-evaluated in clinical settings, particularly in Nigeria, the levels of these immunoglobulins have not yet been thoroughly assessed in this population.

2. MATERIALS AND METHODS

2.1 Study Area and Location

The study area for this work was Jos North Local Government Area of Plateau State and the location includes the APIN (Aids Preventive Initiative of Nigeria) section of Our Lady of Apostles (OLA) Hospital, Faith Alive Foundation Hospital, and Plateau State Specialist Hospital where HIV screenings are carried out.

2.2 Study Design and Subject Selection

The present study adopted a longitudinal study design. The participants were partners who were known to be HIV positive and exposed to seronegative's and were between the ages of 18 and 49 years. In addition, controls consisting of HIV-negative couples in the aforementioned age group who appeared to be in healthy condition were used. The HIV-positive individuals were already taking antiretroviral drugs, but the negative individuals in the discordant relationship were not. Baseline samples were collected from the participants and subsequently, the participants were followed up for 12 months. Triplicate sample collection was carried out on each of the participants at baseline, 6 months, and 12 months following the study.

2.3 Study Population

The study population included male and female subjects in discordance relationships between the age of 18 to 49

years attending the APIN section of Our Lady of Apostles Hospital, Faith Alive Foundation, and Plateau State Specialist Hospital. A total of 20 discordant HIV couples (40 patients) and 20 controls (40 non-HIV couples) were included in the study.

2.4 Sample Collection

Five milliliters of venous blood samples were collected from each participant at baseline, 6 months, and after 12 months follow-up period respectively into plain containers and allowed to clot, retracted, and serum was recovered after centrifugation at 3000 rpm for 10 minutes. Serum samples were kept frozen at -20°C until analyzed.

2.5 Inclusion Criteria

HIV-negative individuals who had been in a committed, discordant relationship for at least three months were eligible to participate. Participants in the study were between the ages of 18 and 49, registered patients at the hospitals where the study was carried out and have the required status documentation. A control group of participants in the same age range who seemed healthy was also included in the study (HIV seronegative subjects).

2.6 Exclusion Criteria

Participants who were already bedridden due to AIDS, diabetics, contraceptive users, those who were not registered patients or had improper documentation with the institution where the study was carried out, and those who refused to give informed consent were all excluded from the study.

2.7 Ethical Approval

Ethical approval was obtained from the Ethics Committees of the hospitals: Plateau State Specialist Hospital (PSSH/ADM/ETH.CO/2019/005); Faith Alive Foundation Hospital (FAFEC/08/34/25) and Our Lady of Apostles Hospital (dated 13th June 2018) where the study was carried out.

2.8 Laboratory methods

IgG and IgM levels were assayed by using the immunoturbidimetric method.

2.9 Statistical Analysis

The data obtained were analyzed using an independent t-test and one-way analysis of variance (ANOVA) with the aid of SPSS statistics tool version 23.0 software. A significant level was assumed at $p < 0.05$.

3. RESULTS

The mean age of the HIV discordant test group was significantly higher (p=0.001) compared to the control group. There was a significant decrease in the mean serum level of Immunoglobulin G in the HIV discordant test group than in the control group at baseline (1294.83±259.73 vs 1504.47±234.60; p=0.000), and after 6 months (1363.74±189.79 vs 1566.45±190.91; p= 0.000). However, there was no statistically significant difference (p=0.081) observed in the mean serum Immunoglobulin G level in the HIV discordant test group when compared to the value obtained in the control group after 12 months.

On the other hand, the mean serum IgM level observed in the HIV discordant test group did not differ significantly when compared to the control group at baseline, after 6 months, and 12 months (p=0.081, 0.123, and 0.214) respectively, Table 1 and Figure 1.

The mean age of the male control group was significantly higher than in the female control group (p=0.000). Also, the mean age of the female test group was significantly higher than in the female control group (p=0.000). Additionally, the mean age of the male test group was

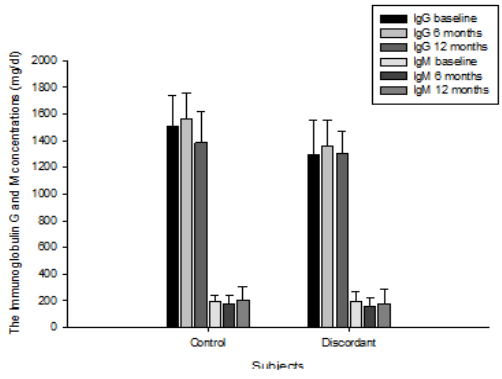

Figure 1: The immunoglobulin G and M concentrations in normal and discordant couples.

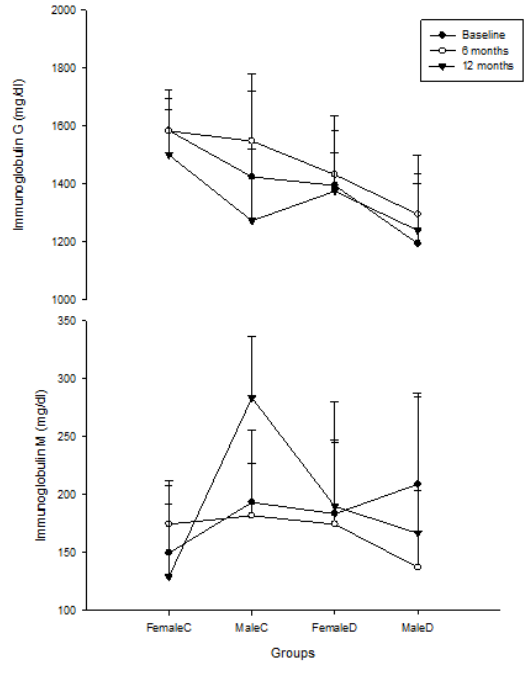

Figure 2: The IgG and M concentrations in female and male controls and discordant couples.

Table 1: Mean ± SD of IgG and M levels in the HIV discordant group studied at baseline, 6 months, and 12 months follow up.

Group	*Age (years)*	*Ig^G_0 (mg/dl)*	*Ig^G_1 (mg/dl)*	*Ig^G_2 (mg/dl)*	*Ig^M_0 (mg/dl)*	*Ig^M_1 (mg/dl)*	*Ig^M_2 (mg/dl)*
1: Control (n=40)	35.6 ± 6.9	1504.47 ± 234.60	1566.45 ± 190.91	1386.98 ± 234.11	191.50 ± 51.65	178.18 ± 57.64	206.33 ± 96.89
2: Discordant (n=40)	40.5 ± 6.1	1294.83 ± 259.73	1363.74 ± 189.79	1307.60 ± 160.79	196.23 ± 71.81	155.84 ± 69.76	178.18 ± 104.05
t-value	3.350	3.788	4.739	1.768	1.768	1.561	1.252
p-value	0.001*	0.000*	0.000*	0.081	0.081	0.123	0.214

*p-value is statistically significant at p<0.05; KEY: IgG0= Immunoglobulin G value at baseline, IgG1= Immunoglobulin G value at 6 months, IgG1= Immunoglobulin G value at 12 months; IgM0= Immunoglobulin M value at baseline, IgM1= Immunoglobulin M value at 6 months, IgM2= Immunoglobulin M value at 12 months.

Table 2: Mean ± SD of Immunoglobulin G and M levels in the male and female participants studied at baseline, 6 months, and 12 months follow up.

Group	Age (years)	Ig^G_0 (mg/dl)	Ig^G_1 (mg/dl)	Ig^G_2 (mg/dl)	Ig^M_0 (mg/dl)	Ig^M_1 (mg/dl)	Ig^M_2 (mg/dl)
Female Control (1)	30.5 ± 4.5	1584.12 ± 110.55	1582.71 ± 142.63	1500.61 ± 155.76	149.66 ± 58.03	174.55 ± 36.97	129.06 ± 62.83
Male control (2)	40.7 ± 4.6[a]	1424.81 ± 295.62	1548.19 ± 232.03	1273.35 ± 247.09[a]	193.34 ± 33.22	181.80 ± 73.65	283.60 ± 52.45[a]
Female test (3)	37.5 ± 5.6[a]	1395.09 ± 242.02	1432.25 ± 149.89	1375.33 ± 132.19	183.45 ± 63.62	174.47 ± 70.05	189.72 ± 90.24[b]
Male test (4)	43.5 ± 5.2[a,c]	1194.57 ± 242.36[a,b,c]	1295.22 ± 203.92[a,b]	1239.88 ± 161.06[a]	209.00 ± 78.70[a]	137.22 ± 65.97	166.65 ±1 17.46[b]
f-value	25.13	9.434	9.775	8.555	3.429	2.025	12.104
p-value	0.000*	0.000*	0.000*	0.000*	0.021*	0.117	0.000*

*p-value is statistically significant at p<0.05. KEY: a: compared with (1), b: compared with (2), and c: compared with (3).

significantly higher compared to the values observed in the female control group and female test group (p=0.000) respectively. There was a significant decrease in the mean serum level of IgG in the male test group when compared to the values observed in the male control group as well as in the female test and female control group respectively at baseline (p=0.000). However, there was no significant difference in the mean serum level of IgG in the female test group when compared to the values observed in the female control group at baseline as well as after 6 months and 12 months (p>0.05) respectively. Nevertheless, the mean serum IgG level was significantly decreased in the male test group compared to the observed values in both the male control group and female control group (p=0.000) respectively after six (6) months follow up. On the other hand, the mean serum IgG levels in both the male and female test groups did not differ significantly after 12 months of follow-up compared to the observed values in both the male control group and female control group (p>0.05) respectively, although the values obtained in the male test and control groups was significantly decreased compared to the female control group (p=0.000) after 12 months.

Furthermore, the IgM levels in both the male and female test groups did not differ significantly at baseline and after 6 months when compared with the male and female control group (p>0.05) respectively. However, the IgM levels in the male test group were significantly increased at baseline when compared with the value observed in the female control group (p=0.021).

Observably, the IgM levels in the female test group did not differ significantly after 12 months when compared with the female control group (p > 0.05). On the other hand, the IgM levels in the male test group significantly decreased after 12 months when compared with the male control group (p=0.000). More so, the IgM levels in the male control group significantly increased after 12 months when compared with the values obtained in the female control group (p=0.000), whereas the IgM levels in the female test group significantly decreased after 12 months when compared with the values obtained in the male control group (p=0.000). See Table 2 and Figure 2.

4. DISCUSSION

In this investigation, the mean serum immunoglobulin G level in the HIV serodiscordant group was significantly lower than the levels seen in the control participants at baseline and after the six-month follow-up period.

This suggests that, although the exact mechanism is unknown, immunoglobulin G levels may be influenced by discordance status. A decrease in polyclonal B-cell activation, which is often shown to be enhanced in HIV infection as the disease progresses, may be implied by the decline in IgG levels in the HIV serodiscordant

group. It may suffice to say that the current result could also point to an improvement in the humoral immune response to control the underlying infection. Thus, it could be that HIV infection presentsa different pattern of antibody response depending on the stage of the disease. It is important to keep in mind that the study's HIV-positive partners received antiretroviral medication (ART) throughout the investigation. This study is notable since it is the first of its kind to assess the immunoglobulin G and immunoglobulin M levels in HIV serodiscordant couples in Nigeria. In the past, several comparable studies have been conducted in HIV seropositive individuals in this regard and diverse findings have been published; most of these data point to hypergammaglobulinemia, which is contrary to the current findings. In the study titled "Levels of Immunoglobulin Classes Are Not Associated with Severity of HIV Infection in Nigerian Patients," Akinpelu *et al*. found that HIV-positive participants had significantly higher mean serum IgG levels than healthy control persons.[33] Following their study on "Effect of HIV Infection on some haematological parameters and immunoglobulin levels in HIV patients in Benin city, Southern Nigeria," Ifeanyichukwu *et al*. also observed higher levels of immunoglobulin G in HIV-positive subjects on ART and those not on ART compared to control subjects, noting that the rise in IgG concentration in HIV infected participants may suggest evidence of increased opportunistic infection requiring IgG response.[30] Higher IgG levels have been observed in HIV-positive individuals in similar studies.[29, 31]

However, the present investigation found no statistically significant difference in the mean serum IgG level between the HIV serodiscordant group and the control participants when comparing the baseline value and post-12-month follow-up period. This may suggest that 12 months of continuous antiretroviral therapy may be capable of normalizing immunoglobulin levels and thus improving humoral immune response in HIV-discordant couples. Thus immunoglobulin G estimation may serve as an important marker in the monitoring of HIV progression and response to treatment. In line with this study, Onifade and colleagues in their study on "The role of IgG, IgA and IgM as immunological markers of HIV/AIDS progression" recorded no significant difference in the mean serum IgG levels in HIV subjects compared before and after 3 months treatment follow up period.[34]

Interestingly, the mean serum IgM levels in the HIV serodiscordant group did not significantly differ from those seen in the control group at baseline, 6 months, and 12 months follow-up periods, respectively. this agrees with the reports of some other similar previous studies which found no significant differences in mean serum IgM levels in HIV positive persons compared to controls, but is not in keeping with the findings of other authors who documented elevated levels of IgM in HIV seropositive individuals.[29-32,34]

However, when compared to the male control group, the male test group had significantly lower mean serum IgG levels at baseline and 6 months, but these differences were not significant at 12 months follow-up. Additionally, at baseline, 6 months, and 12 months after the test, there were no significant variations in the IgG values between the female test group and the female control group. Furthermore, the mean serum IgM levels in the male HIV discordant group remained similar at baseline and after 6 months follow up but lower after 12 months follow up compared to the male control group, while they remained similar at baseline after 6 months and 12 months follow up in the female HIV serodiscordant group and the female control group, respectively. This suggests that the length of ART may affect the IgG and IgM levels in the HIV-serodiscordant male group but not in the HIV-discordant female group.This is partially in line with the study of Ezeugwunne *et al*., which showed that after six months of treatment; mean serum IgM levels in both male and female HIV-positive subjects were not significantly different.[32] Also, this study recorded significantly lower levels of serum IgG and IgM in the male test group than in the female control group which may be due to gender disparity.

5. CONCLUSION

There was a significant decrease in the mean serum level of Immunoglobulin G in the HIV discordant test group at baseline and 6 months than in the control group but remained similar at 12 months follow up.The mean serum Immunoglobulin M level observed in the HIV discordant test group did not differ significantly when compared to the control group at baseline, and after 6 months and 12 months follow up respectively. The mean serum levels of Immunoglobulin G were significantly decreased in the male test group compared to male controls at baseline and 6 months but were similar at 12 months whereas there were no significant differences observed in serum levels of IgG in the female test groups at baseline, 6 months and 12 months follow up respectively.Furthermore, the mean serum IgM levels in both the male and female test groups did not differ significantly at baseline and after 6 months of follow-up compared with the male and female control groups respectively. However, the mean serum IgM levels in the male test group significantly decreased after 12 months of follow-up compared with the male control group. Therefore, HIV serodiscordance status may influence the serum IgG and M levels in affected individuals. Further studies are necessary to unravel the mechanism behind this influence.

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