

Interferon alpha (IFN- α) among Human Immunodeficiency Virus (HIV) patients exposed to *Plasmodium falciparum* infection in selected Hospitals in Niger State, Nigeria

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Abstract

Both Human Immunodeficiency Virus (HIV) and *Plasmodium falciparum* (*P. falciparum*) infection are associated with production of several cytokines such as type I Interferons which may be beneficial to the host during acute infection. The plasma level of type I interferons could vary among HIV patients co-infected with *P. falciparum*. This study therefore, aims to quantify the level of IFN- α , a type 1 interferon, among HIV patients exposed to *P. falciparum* infection in selected Hospitals in Niger State, Nigeria. This was a cross-sectional study. Blood samples were collected from 300 HIV patients for blood film microscopy using Giemsa staining technique and CD₄ count using flow cytometer. Of these, 65 were selected for IFN- α analysis (22 controls and 43 HIV/*P. falciparum* patients) determined using enzyme-linked immunosorbent assay (ELISA). (Chi-square test, $P < 0.05$). Higher levels of IFN- α were observed in patients with mild *P. falciparum* infection and in those with low CD₄ below 200 cells/ μ L. Conclusively, mild *P. falciparum* infection in HIV patients is characterized by up-regulated IFN- α activity.

Keywords: *Plasmodium falciparum*; IFN- α ; HIV; Malaria; CD₄

1. Introduction

Plasmodium falciparum and Human Immunodeficiency Virus (HIV) infections continues to be a public health concerns in the tropical and sub-tropical regions of the world, accounting for massive morbidity and mortality in sub-Saharan Africa (Alaofin *et al.*, 2019). It is estimated that about 241 million people suffers from malaria worldwide and 37.7 million were infected with HIV in 2020 (WHO, 2021; UNAIDS, 2021). Due to overlap in the geographical distribution of these infections in the sub-Saharan Africa, HIV infection is very common in most areas where *P. falciparum* infection is endemic, and these infections may induce interferonemia (Cai *et al.*, 2017). Also the host immune defenses of HIV individuals which is being weakened by HIV infection may promotes occurrences of co-infection especially with malaria in regions where it is endemic (HIV/gov. 2019; Roberds *et al.*, 2020).

Innate immunity is the first line of host defense against an invading pathogen. Interferons (IFNs) are a multigene family of inducible cytokines produced by the host immune system which are among the key molecules in the early defense against infections and are in many cases the first line of resistance to many viral and protozoan infections (McNab *et al.*, 2015; Silva-Barrios and Stager, 2017). IFN-Is, particularly IFN- α , appear to be protective in human infections of *P. falciparum* (Subramaniam *et al.*, 2015) and HIV infections (Nuvor *et al.*, 2016). Studies have also shown associations between high levels of IFN- α produced early during an infection with protection (Subramaniam *et al.*, 2015). On the other hand, excess production of IFN- α have been shown to cause immunopathology leading to immunosuppression (Davidson *et al.*, 2014; Lukhele *et al.*, 2019).

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Many regulatory mechanisms of IFN- α production in *Plasmodium* and virus infections are similar because the IFN- α responses during both infections shared the same pathways. However, *Plasmodium* species are more complex organisms, and as a result, IFN- α responses and regulation during *Plasmodium* infections are likely more sophisticated (He *et al.*, 2020). Furthermore, in what way do co-infection with HIV influences IFN- α responses during *P. falciparum* infection is still only partly known and none to our knowledge has been investigated in the study area. Thus, the aim of this study was to quantify the levels of IFN- α among HIV patients exposed to *P. falciparum* infection attending selected hospitals in Niger State, Nigeria.

2. Material and methods

2.1. Study Area

The study was conducted in three major hospitals in the three senatorial zones of Niger State, Nigeria. These are General Hospital Bida in Niger East (Zone A), General Hospital Minna in Niger South (Zone B) and General Hospital New Bussa in Niger North (Zone C) respectively. Niger state is located in the North-Central part of the country and lies between the latitude of 8 - 11.3⁰N and longitude 3.30 - 7.40⁰E covering a total area of 76,363 sqkm². The climate of the area is tropical with an average annual rainfall of 1090 mm³ and temperature ranging 27.38 – 36.9⁰C as reported by Niger State Ministry of Land and Housing, (2020). Majority of the population are farmers.

2.2. Study Population

A hospital-based cross-sectional study was carried out among HIV patients attending these facilities from a period of February to June, 2021. Participants were recruited irrespective of their age, sex and with or without malaria-related signs and symptoms. Only those who agreed to participate in the study were enrolled, those that did not consent and non-HIV patients were excluded. Three hundred (300) HIV patients were selected out of which 22 that do not have malaria after microscopy were randomly selected as control group.

2.3. Sample Size Determination

A prevalence of 24.8% for *P. falciparum* by Daniel *et al.* (2016) was used to determine the sample size using the equation of Vinodh, (2018).

$$N = Z_{(1-\alpha/2)} P(1-p) / d^2$$

Where N = sample size, $Z_{(1-\alpha/2)}$ = standard normal variate (at 5% type 1 error = 1.96), P = prevalence in study area and d = precision (5%).

Therefore, $n = 1.96^2 \times 0.248(0.744) / (0.05)^2 = 286.577 \approx 300$.

2.4. Ethical Approval

Ethical approval was obtained from the Ethical committee of Niger State Hospital Management Board/General Hospital, Minna, Niger State (HMB/GHM/136/VOL.III/590). Consent of participants or guardians of the children was also obtained prior to collection of samples.

2.5. Blood Sample Collection

Using sterile vacutainer needle/holder, 4 mL of blood samples were drawn aseptically by venipuncture from the patients and transferred into a sterile Ethylene Diamine Tetra Acetate (EDTA) anticoagulant bottle and mixed immediately (Yohanna *et al.*, 2019).

2.6. Detection of Parasitaemia

Thick and thin blood films were made on glass microscope slides. Thin films were fixed using absolute methanol and both films were stained with 3% of Giemsa stain. The stained blood films were examined microscopically under x 100 objective lens for the detection and identification of malaria parasites (WHO, 2015). Malaria parasites were counted in all the positive thick blood films with an aid of a differential counter, the number of parasites seen (asexual stages) and the number of white blood cells (WBCs) were counted separately but simultaneously field by field. These was done till 200 WBCs were counted in all fields. The parasitaemia value was calculated and expressed as parasites per microliter of blood (WHO, 2015). The level of parasitaemia was graded as low (<1000 parasites/ μ L), moderate (1000–9999

parasites/ μL) and high/severe ($\geq 10,000$ parasites/ μL) (Atroosh *et al.*, 2015). The parasite density is calculated mathematically as:

Malaria parasites/ μL of blood = No. of observed parasites counted x estimated WBC (8000) / No. of WBC counted (200) ... (1)

2.7. Determination of CD₄ Count

The CD₄ count was determined using a two-color single platform flow cytometer (Partec Cyflow) within 6 hours of sample collection. Twenty microliter (20 μL) CD₄ phytoerythrin (PE) antibody was dispensed into labeled Partec (Rohren) tubes and 20 μL of well mixed whole blood was added, mixed with a vortex mixer and incubated in the dark for 15 minutes at room temperature. The mixture was mixed at 5 minutes' interval during incubation. After incubation, 800 μL of CD₄ diluting buffer was added to the mixture of antibody and samples mixed gently before analyzing. CD₄ count was categorized according to WHO, 2018 as low or advanced stage (< 200 cells/ μL), moderate or chronic stage (200 – 499 cells/ μL) and high or asymptomatic stage (≥ 500 cells/ μL).

2.8. Determination of Interferon alpha using Enzyme Linked Immunosorbent Assay

Interferon alpha was measured in plasma samples from 43 participants co-infected with *P. falciparum* (detected microscopically) and 22 participants as controls using ELISA (ABclonal Biotechnology Co., Ltd, USA). Plasma samples were diluted at 1 in 10 in sample diluents and used with their respective serially diluted standards on monoclonal antibody-coated 96-well plates. The plates were incubated at 25°C for 2 hours with shaking and then washed five times to remove the unbound antigens from the coating antibodies. This was followed by addition of Working Biotin-Conjugate Antibody to bind to the antigen- antibody complex. Plates were washed following 1 hour incubation at 25°C. After 5 washes, Working Streptavidin horse-radish peroxidase (HRP) was added, incubated for 1 hour, and then washed again four times before the substrate, tetramethyl-benzidine (TMB) was added. Development of a blue colour occurred within 15 minutes, the intensity of which depended on the extent of the antigen-antibody complex formed. The reaction was stopped by addition of an acid and the absorbance was read with the Microtitre plate Reader at 450 nm within 15 minutes.

2.9. Data analysis

All the data collected were entered into Microsoft Excel files and analyzed using STATA/MP 12.0 (StataCorp, College Station, TX) and Prism 5.0 (Graph pad Inc., San Diego, CA). Univariate analysis which includes descriptive statistics such as frequencies, percentages and exploration of the distribution of all variables were performed. Groups were compared with non-parametric Wilcoxon-matched paired and Kruskal-Wallis tests. Categorical variables were compared with Pearson's correlation co-efficient (Chi-square) and Odds ratio. Multiple logistic regression was performed to assess whether cytokine (IFN) levels were independently associated with the likelihood of *P. falciparum* infection. All tests were performed at 95% confidence interval ($p \leq 0.05$).

3. Results

3.1. Profile of Co-infected participants and Controls

Table 1 shows the profile of the forty-three (43) study participants co-infected with *P. falciparum* and twenty-two (22) randomly selected study participants that acted as control. Out of the 43 co-infected participants, 15 (34.9%) of them had CD₄ count ≥ 500 cells/ μL , a mean age of 38.8 years with a standard error of ± 10.71 , a mean CD₄ Count of 1581 ± 430.56 cell/ μL and a mean IFN- α of 44.4 ± 18.31 pg/mL while the control group had a mean age of 31.36 years with a standard error of ± 14.11 , a mean CD₄ Count of 1253 ± 682.32 cell/ μL and a mean IFN- α of 39.03 ± 20.04 pg/mL. There is no significance difference in IFN- α levels between the two groups ($p > 0.05$).

3.2. Multiple logistic regression analysis with dependent variables of Malaria Parasitaemia

The logistic regression analysis shows a statistically significant relationship (p -value = 0.02) between the presence of malaria parasitemia (MP) and the independent variables of age, sex, CD₄ count and IFN- α levels. The model explains 13.9935% of the deviance in MP, and the adjusted percentage is 1.97448%.

After adjustment for cofactors, the results of the likelihood ratio tests indicate that CD₄ count (p -value = 0.0012) have a significant effect on the presence of malaria parasitemia. The p -value for age ($p = 0.1694$), sex ($p = 0.9816$) and IFN- α levels ($p = 0.4065$) are not statistically significant (Table 2).

Table 1: Profile of Co-infected participants and Controls

Groups	CD ₄ Count/ μ L	No	Male	Female	Age Mean + SE	CD ₄ Count Mean + SE	IFN- α Mean + SE
Co-infected participants	>500	15	3	12	38.8+10.71	1581+430.56	44.4+18.31
	200 – 499	7	4	3	32.86+19.28	275.29 + 60.75	44.58+29.84
Control	<200	21	8	13	29.24+12.6	135.86+47.25	44.5+33.154
	-	22	7	15	31.36+14.11	1253.23+682.32	39.03+20.04

P < 0.0012 Key: No = Number of Participants, SE = Standard error

Table 2: Multiple logistic regression analysis with dependent variables of Malaria Parasitaemia

Variables	Odd ratio	95% Confidence Interval	P-value
Age	1.03092	0.985383 – 1.07856	0.1694
Sex	1.01455	0.289592 – 3.55432	0.9816
CD ₄ count	0.99875	0.997932 – 0.999583	0.0012
IFN- α	1.01052	0.984743 – 1.03698	0.4065

Key: IFN- α = Interferon alpha.

3.3. Relationship between parasitaemia and Interferon alpha

Logistic regression correlation coefficient values and p values are shown in Fig. 2. Analysis for possible correlational relationship was done. There is a statistically moderate negative correlation between IFN- α and parasitaemia.

3.4. Relationship between parasitaemia and CD₄ count

To determine whether cytokine levels in our study were associated with CD₄ counts that are a standard marker of progression of HIV infection, we measured the IFN- α levels in the patients. IFN- α plasma concentrations showed a trend toward higher levels in patients with lower CD₄ counts compared with those with higher CD₄ counts (Kruskal-Wallis test; P = 0.06 for both comparisons) (Figure 2). When considering *P. falciparum* infection as a confounder, the trend toward a negative association between IFN- α levels and CD₄ counts was confirmed only in patients who were parasitemic (not indicated here).

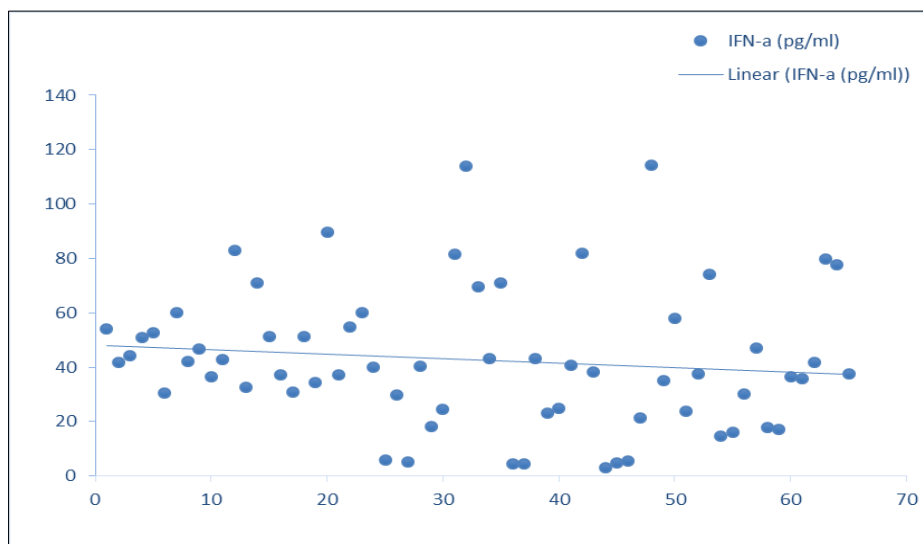


Figure 1 IFN- α (pg/ml) vs MP

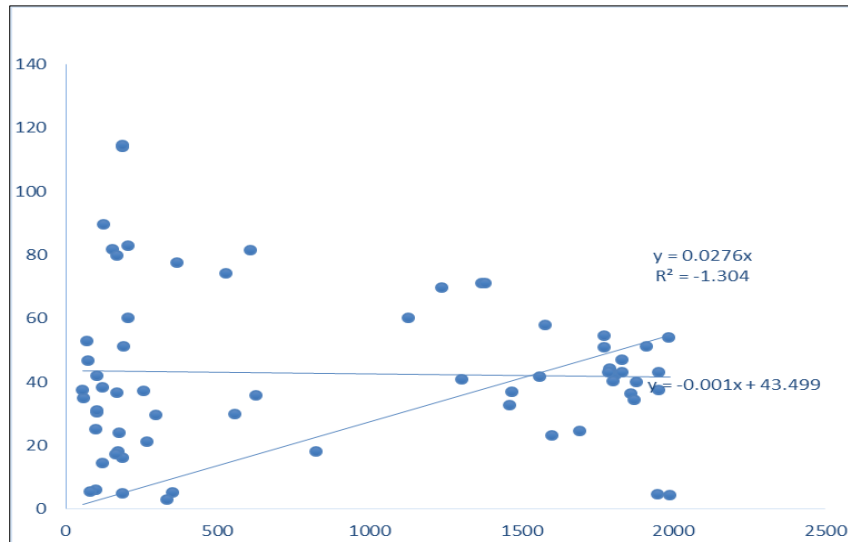


Figure 2 IFN- α (pg/ml) vs CD $_4$ (cells/ μ l)

4. Discussion

4.1. Cytokine profile (IFN- α) of the study participants with *falciparum* malaria

Type I interferons have been shown to play important roles against malaria and viral infection (Silva-Barrios and Stager, 2017). IFN-I, especially IFN- α response has been reported to be protective leading to suppression of parasitemia and better survival during malaria parasite infections. These protective effects are mediated through adequate levels of IFN- γ , proper activation of immune cells, and production of antibodies (He *et al.*, 2020). The principal findings of this study concern IFN- α activity against *P. falciparum* infection, which is characterized by the higher levels in plasma production capacity of this cytokine in patients with mild compared to severe malaria. In addition, IFN- α showed inverse correlations with parasitemia. This may be due to fact that high secretions of IFN- α produced early in infection leads to clearance of parasites and/or improved host survival rate and limit the progression from uncomplicated malaria to severe and life-threatening complications. This is in line with reports by Hardy *et al.* (2013) and Silva-Barrios and Stäger (2017). In another study conducted on Higher Type 1 Interferon Levels in Plasma of Asymptomatic HIV-2 than in HIV-1 Individuals, it was reported that Type 1 interferon produced in the early immune response to infection acts as an effective regulator of adaptive immunity as well as inhibiting viral replication. Their results suggest that the levels of IFN- α are crucial for control of HIV infection (Nuvor *et al.*, 2016),

The HIV infection has been shown to impair proinflammatory cytokine production (Roberds *et al.*, 2021; Klatt, 2022) which could be considered as being beneficial for the persistence of malaria parasites. CD $_4$ cells have been reported to play a critical role in HIV/malaria immune control. Earlier studies have shown that stimulation of an early IFN- α response influence the direction of host immune responses such as CD $_4^+$ T cell activation (He *et al.*, 2020). This study shows that the presence of infection with *P. falciparum* is associated with low CD $_4$ counts, themselves primarily related to the concurrent HIV infection, which lead to a stronger likelihood that these participants have plasmodial infections. Though, it should be stressed in this context that our sample size is small, and that some of our data only revealed trends that show borderline significance: multivariate analyses adjusted for multiple confounders however confirmed most of the findings obtained in univariate analyses.

Another reason for increased levels of IFN- α could be low CD $_4$ count which may be due to the fact that infection of HIV as well as *P. falciparum* has been known to induce depletion of CD $_4$ T-cells and to reduce CD $_8$ T-cells causing down-modulation, reduction in T-cell sub-population and defective cell mediated immunity against any microbial infection (Kirinyet, 2019). This study however, contradicts the findings of Nuvor *et al.* (2016) which suggests that high levels of IFN- α may be supporting the persistently high CD $_4^+$ T cell numbers in HIV infection from an early stage of infection. Whether and how IFN- α regulate host immune mechanisms, including T cell activation and antibody production require additional investigation.

5. Conclusion

High plasma levels of IFN- α were associated with mild *falciparum* malaria and low CD₄ counts. IFN- α levels were also found to be correlated with *falciparum* malaria infection.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declare no conflict of interests regarding the study and findings of this research work.

Statement of ethical approval

Ethical approval was obtained from the Ethical committee of Niger State Hospital Management Board/General Hospital, Minna, Niger State (HMB/GHM/136/VOL.III/590).

Statement of informed consent

Consent of participants or guardians of the children was also obtained prior to collection of samples.

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