DETERMINATION OF INTERFERON GAMMA ASSOCIATED WITH MALARIA PARASITAEMIA AMONG PATIENTS ATTENDING SELECTED HOSPITALS IN ZARIA, KADUNA STATE, NIGERIA

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Abstract

Background

Malaria has been recognized as a severe and life- threatening illness for thousands of years. It is still one of the most common diseases affecting humans worldwide. Results gathered in primates challenged by *Plasmodium falciparum* and, to some extent, in humans, point to interferon gamma as a possible immune mediator or at least a surrogate marker significantly associated with protection against *Plasmodium falciparum* and actually, the only surrogate available to-date.

Aim

This study was aimed at determining malaria parasitaemia and interferon gamma concentration among patients attending selected hospitals in Zaria, Kaduna State, Nigeria.

Methods

Four hundred blood samples were collected from four hospitals in Zaria, Kaduna State.The samples were microscopically screened for malaria parasites. The concentrations of interferon gamma were determined using ELISA, and the results obtained were analysed using Chi square.

Results

Only the ring trophozoites of *Plasmodium falciparum* were observed in the infected samples. In the whole study population, males had a higher parasitaemia than females.The individuals with *Plasmodium falciparum* infection had the highest mean concentrations of interferon gamma with 121.32pg/ml than those in the negative control group, that is, those without the infection (75.69pg/ml). There was no statistically significant difference (p=0.079).

Conclusion

This study shows a higher *Plasmodium falciparum*parasitaemia in males than females, with a relatively higher concentration of interferon gamma in the group with parasitamia than the control group.

Key words: Parasitaemia, interferon gamma, *Plasmodium falciparum*, ELISA

INTRODUCTION

Malaria has been recognized as a severe and life- threatening illness for thousands of years. It is still one of the most common diseases affecting humans worldwide. The major impact of the disease is almost entirely on the developing countries, with the heaviest burden in Africa. Almost half of the world's population is exposed to the risk of contracting malaria (Ananya, 2015). Malaria is caused by protozoan parasites of the genus *Plasmodium*, belonging to the phylum Apicomplexa.The *Plasmodium* species which are known to cause human malaria include;*P. falciparum, P.vivax,P. ovale*, *P. malaria*, and *P. knowlesi*,which has been recently documented to cause human infections in many countries of Southeast Asia (Daneshvar *et al*., 2009;WHO, 2016).

About 3.4 billion people (half of the world's population) are at risk of malaria (WHO, 2014).Six countries; Nigeria, the Democratic Republic of Congo, Burkina Faso, Mozambique, Code d'Ivoire and Mali account for 60% or 390,000 of malaria deaths (WHO, 2011).

Results obtained from studies on primates challenged by *Plasmodium falciparum* and to some extent, in humans, point to interferon gamma (IFNɣ) as a possible immune mediator or at least a surrogate marker significantly associated with protection against *Plasmodium falciparum* and actually, the only surrogate available to-date. In vitro studies with *P. falciparum* in primary cultures of human hepatocytes revealed a very potent effect of IFNɣ. Indeed, very low concentrations of IFNɣ were efficient against *P. falciparum* liver forms and moderate concentrations were able to fully block the liver schizogonic development (Perlaza *et al*., 2011).

Plasmodium falciparum, the causative agent of the lethal form of malaria, elicits a complex immune response. The parasite exhibits sophisticated mechanisms of immune-evasion and antigenic variation, and these may be the reasons why, even after a hundred years of research on malaria, we do not have an effective malaria vaccine. However, immunity to malaria does exist. It develops gradually, after many attacks and over many years, in adults living in highly endemic areas (Mannan *et al*., 2003).

In human malaria, the naturally-acquired immune response can result in either the elimination of the infectious agent or a persistent response mediated by cytokines that leads to immunopathology, with activated T. cells and macrophages, although the mechanisms are not well understood. High levels of pro-inflammatory cytokines, such as tumor necrosis factor (TNF), interferon-gamma and interleukin-6 have been associated with severe pathologies (Day *et al*., 1999), whereas low levels of regulatory cytokines, such as TGF-b and IL-10, have been associated with acute malaria (Peyron *et al*., 1994). Many aspects of this process remain to be understood, including the effects of cytokines on the control of the immuneresponse and the differences between the protective and pathological modulatory effects.In *P. falciparum* malaria, several studies have described an association between severe infections and enhanced pro-inflammatory cytokine response, including TNF, IL-1b,IL-6,andIFN-g (Prakash *et al*., 2006). These cytokines are responsible for all the symptoms, pathological alterations and the outcome of the infection depends on the reciprocal regulation of the pro and anti-inflammatory cytokines (Medina *et al*., 2011).

METHODOLOGY

Study area

The study was carried out in four selected hospitals in Zaria Nigeria; Major Ibrahim B. Abdullahi Memorial Hospital Zaria (former Limi hospital), Hajiya Gambo Sawaba Hospital Kofan Gaya Zaria, Salama Hospital and St.Luke's Hospital Wusasa Zaria. Zaria is a city found in Kaduna state, Nigeria. It is located at 11.11 latitude and 7.72 longitude and it is situated at elevation 644 meters above sea level. Zaria has a population of 975,153 making it the second largest city in Kaduna (World Atlas, 2015).

Sample size

The sample size was determined using a prevalence of 23.45% (Bechemagbor, 2010) and the following formula as described by Naing *et al.,* 2006*:*

$$n = \frac{z^2 p(1-p)}{d^2}$$

n= number of samples

p=prevalence rate of previous study = 23.45% = 0.2345 (Bechemagbor, 2010)

z=standard normal distribution at 95% confidence limit = 1.96

d=absolute desired precision of 5% = 0.05

z=1.96

$$n=\frac{1.96^2 * 0.2345(1-0.2345)}{0.05^2}$$

n=275 samples

Four (400) blood samples were however collected for this study.

Administration of structured questionnaire

The use of a well-structured questionnaire was employed for the collection of patient's data. Only consenting individuals were enrolled.

Sample collection

A total of 400 blood samples were collected from Major Ibrahim B. Abdullahi Memorial Hospital Zaria (former Limi Hospital), Hajiya Gambo Sawaba Hospital Kofan Gaya Zaria, Salama Hospital and St.Luke's Hospital Wusasa Zaria (100 samples from each hospital). Venipuncture technique was used for blood sample collection. A soft tubing tourniquet was fastened to the upper arm of the patients to enable the index finger to feel a suitable vein. The puncture site was then cleansed with Methylated spirit (methanol) and venipuncture was made with the aid of a 21 G needle attached to a 5 ml syringe. When sufficient blood (3ml) was collected, the tourniquet was then released and the needle removed immediately while the blood was transferred into an EDTA bottle (Epidi *et al*., 2008).

Determination of malaria parasitaemia

Thick and thin blood films were prepared from the blood samples collected as outlined by Cheesebrough (2004). The presence of ring forms ortrophozoites of *Plasmodium* indicated positive results. A blood smear was considered negative if no parasite was seen after 10 min of search or examination under 100 high power fields of microscope.

The malaria parasitaemia was determined using a grading scheme of + =1-10 parasites, ++ = 11-20 parasites, +++ = more than 20 parasites per microscopic field was used to establish the levels of parasitaemia (Bechemagbor, 2010).

Determination of interferon gamma (IFNγ) concentrations

The concentrations of interferon gamma were determined in eighty eight (88) serum samples, using Boster's interferon gamma ELISA kit purchased from Boster Biological Technology Co. Ltd. Fremont, CA in the United States of America. The samples were divided into two groups; the malaria positive group (59) and the control group (29).The interferon gamma concentrations were determined according to the instructions contained in the ELISA kit manual provided by Boster Biological Technology Co. Ltd.

Statistical analysis

The data was analysed using chi square.

RESULTS

Table1 Distribution of *P.falciparum* malaria in relation to hospitals

Hospital	No. examined	No. positive (%)
HGSH	100	38(38.0)
MIBA	100	15(15.0)
SH	100	29(29.0)
SL	100	31(31.0)
Total	400	113(28.3)

χ^2=13.752, p=0.03, df=3Key:HGSGH=Hajiya Gambo Sawaba Hospital, MIBA=Major Ibrahim B. Abdullahi Memorial Hospital, SH=Salama Hospital, SL=St. Luke's Hospital

Table 2 The intensity of *P.falciparum* parasitaemia in males and females based on number of parasites count per microscopic field.

			Parasitaemia		
Gender	No. examined	Nil	+ (%)	++ (%)	+++ (%)
Male	113	71(62.8)	30(26.5)	8(7.1)	4(3.5)
Female	287	216(75.3)	53(18.5)	15(5.2)	3(1.0)
Total	400	287(71.8)	83(20.8)	23(5.8)	7(1.8)

χ^2=7.665, p=0.05,Key No.=number,+=1-10,++=11-20, +++=above 20 parasites per microscopic field

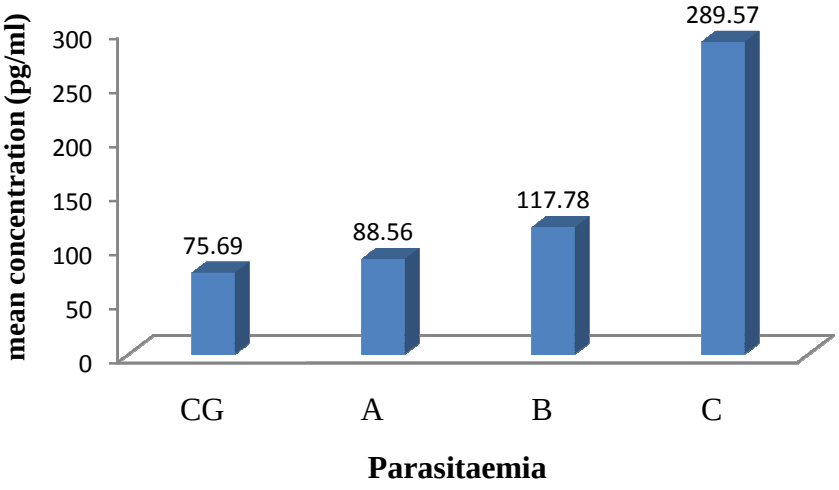

Figure 1 Mean concentrations of IFN gamma in relation to malaria parasitaemia
P=0.000,Key: CG= control group, A=+ (1-10 parasites per microscopic field), B=++ (11-20parasites per microscopic field), C=+++ (above 20 parasites per microscopic field)

Table 3 Mean concentrations of IFN gamma among malaria positive individuals and control group

Malaria status	No. examined	Mean(pg/ml)	SE	P value
Positive	59	121.32	16.869	0.079
CG	29	75.69	12.580	
Total	88	106.28	12.217	

Key: SE=standard error, No. =Number

Discussion

Gambo Sawaba General Hospital and St. Luke's Hospital which are both in Zaria local government area, had higher prevalence (38.0% and 31.0% respectively) than MIBA Hospital and Salama Hospital (15.0% and 29% prevalence respectively) which are in Sabon-gari local government area.

Only *Plasmodium falciparum* was encountered during this study. Males had + (26.5%), ++ (7.1%) and +++ (3.5%) parasitaemias which were progressively higher than females with + (18.5%), ++ (5.2%) and +++ (1.0%) parasitaemias respectively. This agrees with the findings of Reza and Taghi (2011). The low rate of infection among females suggests that females were less exposed to mosquitoes that transmit malaria parasite. The high prevalence of malaria in males suggests that males may be more prone to malaria than females (Umaru and Uyaiabasi, 2015). This may also be justified by more exposures to mosquito bites in males due to their outdoor activities.According to the World Health Organization report, men are less likely to sleep under the insecticide treated nets than females (WHO, 2010).

Severe *P. falciparum* malaria is characterized by marked changes in cytokine production resulting from the immuneresponse to the infection. Interferon gamma is the most widely studied interferon in malaria infection since it is primarily involved in host defence against intracellular pathogens. In this study, the mean levels of interferon gamma were seen to increase with increase in parasitaemia.

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This suggests that, more interferon gamma cytokines were released as the *Plasmodium falciparum* parasite thrived in infected individuals. This is in consonance with the report of Moore *at al*., (1999).Blood stage *Plasmodium* parasite infection generates robust innate immune responses that have been extensively studied and predominantly mediated by interferon gamma, tumour necrosis factor α and IL-12 (Riley and Stewart, 2013). In this study, the mean level of interferon gamma was also higher in individuals with *Plasmodium falciparum* infection than in those without (control group). This study also shows that interferon gamma concentration is significantly (p=0.000) associated with the level of *Plasmodium falciparum* parasitaemia. Previous study had shown the protective effect of IFN-γ responses to *P. falciparum* malaria in children living in malaria endemic regions (Nnaemeka *et al.*,2009). In a similar study by Tiago *et al.*(2011), it was reported that the levels of a pro- (IFN-gamma) and an anti-inflammatory cytokine (IL-10) were significantly higher in *P. vivax* infected individuals as compared to healthy controls.

Conclusion

In conclusion, the study reveals a higher malaria parasitaemia in males than females. The concentration of interferon gamma in the group with malaria parasitaemia was higher than the concentration of interferon gamma in the control group. Also, there was a progressive increase in the concentrations of interferon gamma as the malaria parasitaemia increased.

Recommendation

Having seen part of the role interferon gamma plays in malaria infection, it is recommended that further studies should be carried out to elucidate the actual role of interferon gamma in malaria pathology.

Authors' contributions

All authors contributed significantly to the writing of the manuscript. Allauthors read and approved the final manuscript.

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