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RESEARCH ARTICLE

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Haematological Profiles of Plasmodium-Induced Changes in Serum Amyloid-A levels among the Internally Displaced Persons in Maiduguri

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ABSTRACT

The menace of malaria has constituted a health challenge in Nigeria. Internally Displaced Persons' Camps are available temporary accommodation provided for victims of war in war torn zones around the world. A study was undertaken to investigate relationship between Serum Amyloid A (SAA), haematological profiles and malaria infection among internally displaced person in three (3) different camps (GGC: Government Girls' College Camp, GSS: Girls' Secondary School Camp, CAN-: Christian Association of Nigeria) within Maiduguri, Borno state capital between March and September, 2017. A total of 400 sample subjects were recruited for the study, malaria parasite test was done using the Giemsa thick and thin peripheral blood film, SAA was analysed using the Enzyme linked immune-sobent assay (ELISA) kit while Haematological parameters were analyse using the automatic full blood count analyser. The result of the study shows a positive correlation between malaria infection and these parameters. A mean SAA of 32.76mg/l, PCV of 28.2%, haemoglobin of 8.88g/dl, platelets of $116.54\times10^3/\mu l$, leucocyte of $6.02\times10^3/mm^3$ and neutrophils of $3.34\times10^3/mm^3$ was recorded in the positive subjects compared to the 14.11mg/l, 41%, 14.81g/dl, $261.46\times10^{3/}\mu l$, $8.23\times10^3/mm^3$, $4.11\times10^3/mm^3$ reported in the negative patients group, this results shows that there are abnormal haematological alterations in malaria subjects compared to the negative control group, hence SAA and haematological parameters can be used as markers of plasmodium infection.

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Introduction

Malaria has become a major public health concern for the past years, with more prevalence in developing countries especially in Africa. There are five (5) species of *Plasmodia* that causes malaria, *namely; P. falciparum, P. vivax. P. ovale, P. knowlesi, P. malariae*. Of the 5 human malaria infectious species, *P. falciparum* and *P. vivax* are responsible for over 90% of the malaria cases hence are regarded as the major parasites species [1]. Majority of the malaria mortality worldwide had been attributed to *P. falciparum* [2], which are considered as a lethal species responsible for about 247million cases and approximately 1million death each year especially in the sub-Saharan Africa [3]. Liver and renal dysfunction, severe anaemia, acute respiratory distress syndrome (ARDS), hypoglycaemia, cerebral manifestation and multiple organ involvement are associated symptoms due to *P. falciparum* infection [3,4]. The innate immune system responds to inflammatory stimuli of infection, trauma and neoplasia. This inflammatory respond is called Acute Phase response (APP) [5,6]. APP defined as a complex systemic response is characterized by elevation and depression of blood level proteins such as C-Reactive Protein (CRP) and Serum Amyloid-A (SAA), tissue injury and trauma [7]. Concentration of APP had been used for diagnostic and prognostic analysis of several diseases like cancer, sepsi and cardiovascular disease [8,9]. Haematological changes and alteration due to infection involves cell types like RBCs, leucocytes, thrombocytes. These plays major roles in the clinical signs and pathogenicity of the disease [10-12]. Previous studies had shown the variation in the different haematological parameters significantly lowered platelets, WBCs, lymphocytes, Haemoglobin (Hb), Eusinophils and RBCs, but proportionately elevated neutrophils and monocytes [10,11].

Microscopy is very helpful and important diagnostic method of *Plasmodium* infection. However, this technique is very laborious and requires the expertise of an experienced technicians and scientist [13,14]. There is therefore the need to look out for some alternative and easy- to- interpret parameters, which would be use as an indicator of the haematological changes and alteration with malaria necessitated infection. The study is therefore aimed at assessing plasmodium induced changes in serum amyloid A and haematological profiles of Internally Displaced persons in Maiduguri.

Materials and Method

Study Area

Maiduguri, (110 50' 42" N and 13^0 09' 36" E) is the capital city of Borno State, North-Eastern Nigeria, with a population of

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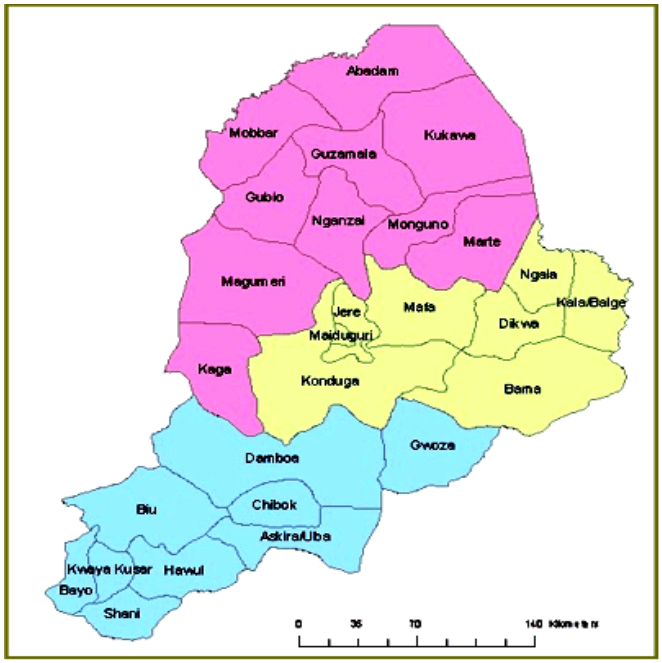
543,016 according to 2006 census. It lies in the hot semi-arid, Temperatures are high with annual rainfall just a little above 500mm. Mean annual temperatures are as high as 27.4^{0}C [15]. The study locations are: Government Girls' College Camp (GGC) (N11.8329 and E013.14091), Girls' Secondary School Camp (GSS) N11.83225 and E013.14010 and Christian Association of Nigeria (CAN) (N11.84394 and E013.13693) respectively [16].

Plate 1: Map of Borno State.
Source; www.thenigeriangazette.com



Research Design

The study adopted a descriptive cross-sectional study to assess the *Plasmodium* - induced changes in serum amyloid A and haematological profiles of Internally Displaced Persons in Maiduguri.

Ethical Consideration

Permission for this study was obtained from the Research Ethics Committee, University of Port Harcourt. Authorities of the three Camps were informed and a written approval for the study was obtained. Consent of each subject was also obtained before assessment.

Study Population

The population of the study comprised the secondary schools located at the Internally Displaced Person's Camp in Maiduguri the capital city in Borno State. Over 2,114,000 Internally Displaced Persons had been identified [17].

The Exclusion Criteria

i. Patients not residing in any of the camps.
ii. Patients with recent chemotherapeutic record of malaria.
iii. Patients with recent prophylactic treatment of malaria.
iv. Patients with recent blood transfusion record.

Inclusion list include

i. Persons who consent and presents themselves for the research work within the camps.

Sampling Techniques and Microscopic Analysis

The samples of the study comprise of all students from the selected three school at the Internally Displaced Person's Camp in Maiduguri. Two millilitres (2ml) of Peripheral venous blood samples were collected from 400 students; (93 from GGC, 245 from GSS and 62 from CAN) into an EDTA bottles duly labelled. The procedures of Rapid Diagnostic Test (RDT), GIEMSA staining and microscopic screening for *Plasmodium* parasites followed standard techniques as described by Cheesbrough [18]. The RBCs, WBCs, Haemoglobin, Packed cell Volume, Platelets counts, neutrophils and lymphocyte counts were analysed. Quantitative analysis of Serum Amyloid-A was done with the Abcam's Serum Amyloid-A Human following standard ELISA procedures [18]. Personal data collection of the consented subjected were also collected using self-structured questionnaire that contains demographic information of respondents and other items that illicit the response of the subjects.

Analysis of Serum Amyloid-A

Serum Amyloid-A (SAA) analysis was done using Abcam's Serum Amyloid A Human ELISA kit. It is an in-vitro enzyme-linked immunosorbent assay for the numerical measurement of Human Serum Amyloid A in plasma, Serum, and Cell culture supernatants. This Assay uses an antibody specific for Human Serum Amyloid A coated on a 96-well plate. Blood samples were pipetted into the wells. Serum Amyloid-A present bind to the wells by the immobilized antibody. The wells were washed and biotinylated anti-Human Serum Amyloid A antibody was added. After washing away, the unbound biotinylated antibody HRP-conjugated streptavidin was pipetted to the wells. The wells were washed again, a TMB substrate solution were added to the wells and colour developed relative to the amount of Serum Amyloid A present. The stop solution changed the colour from blue to yellow, and the intensity of the colour will be measured at 450nm as per Abcam, and Priyamvada, et al., 2014.

Data Analysis

Statistical analysis was done using SPSS version 19. Data obtained were presented as prevalence (%) and intensities. Chi-squared tests were used to determine significant association between malaria parasitaemia and age, sex and camps at p= 0.05.

Results and Discussion

Overall Prevalence of Malaria among the Internally Displaced Persons in Maiduguri

The overall prevalence of malaria in the study locations was 90.2%. Females accounted for a prevalence of 72.3% while males accounted for the prevalence of 27.7% respectively. The differences of sex related prevalence were significant p<0.05). The infection was highest (45.4%) among age bracket 11-20 years, followed by age bracket 0-10 years (24.4%). The least (2.2%) infection was among age bracket 61-70yrs. The

Table 1: Pooled prevalence of malaria among displaced persons in the three camps.

Variables	No. Examined (%)	No. Infected (%)	No. non-infected (%)
Sex			
Male	114 (28.5)	100 (27.7)	14 (12.3)
Female	286 (71.5)	261 (72.3)	25 (8.7)
Age			
0-10 yrs	97 (24.3)	88 (24.4)	9 (9.3)
11-20 yrs	182 (45.5)	164 (45.4)	18 (9.9)
21-30 yrs	49 (12.3)	45 (12.5)	4 (8.2)
31-40 yrs	35 (8.89)	32 (8.9)	3 (8.6)
41-50 yrs	19 (4.8)	16 (4.4)	3 (15.8)
51-60 yrs	10 (2.5)	8 (2.2)	2 (20)
61-70 yrs	8 (2.0)	8 (2.2)	0 (0)
Camps			
GGC	93 (23.3)	81 (87.1)	12 (12.9)
GSS	245 (61.3)	223 (91.0)	22 (9.0)
CAN	62 (15.5)	57 (91.9)	5 (8.1)

Table 2: Overall comparison of Serum Amyloid A, Packed Cell Volume, Haemoglobin, Platelet, Leucocyte and Neutrophils SAA-Serum Amyloid A, PCV-Packed Cell Volume, HGB: Haemoglobin, PLT: Platelet, LEU: Leucocyte, NEUT: Neutrophil.

Para-meters	Mean SAA (Mg/L)		Mean PCV ± SD (%)		Mean HGB \ SD (g/dl)		Mean PLT ± *SD* (10^3/µL)		Mean LEU ± *SD* (10^3/mm^3)		Mean NEUT ± *SD* (10^3/mm^3)	
	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative
0-10 yrs	32.81 ± 3.95	13.90 ± 1.34	28.24 ± 2.53	40.99 ± 1.48	8.82 ± 0.39	15.12 ± 0.14	116.39 ± 7.53	282.56 ± 6.77	6.51 ± 0.25	8.28 ± 0.29	3.34 ± 0.24	3.78 ± 0.65
11-20 yrs	32.38 ± 3.33	13.39 ± 1.12	28.15 ± 2.53	41.69 ± 2.18	8.92 ± 0.41	14.77 ± 1.40	116.81 ± 7.08	256.78 ± 58.43	6.54 ± 0.26	8.27 ± 0.29	3.32 ± 0.21	4.21 ± 0.50
21-30 yrs	33.20 ± 3.07	13.75 ± 2.09	28.5 ± 2.50	40.65 ± 1.39	8.93 ± 0.42	15.18 ± 0.31	115.20 ± 7.12	280.50 ± 4.43	6.48 ± 0.27	8.43 ± 0.18	3.33 ± 0.21	4.07 ± 0.58
31-40 yrs	33.46 ± 3.07	12.57 ± 0.64	28.1 ± 2.26	40.57 ± 1.37	8.82 ± 0.40	15.07 ± 0.15	117.47 ± 6.41	282 ± 8	6.54 ± 0.25	8.23 ± 0.15	3.39 ± 0.21	4.13 ± 0.54
41-50 yrs	33.28 ± 3.18	20.57 ± 10.51	27.6 ± 2.46	37.10 ± 8.26	8.83 ± 0.34	13.10 ± 3.47	115 ± 9.11	169.67 ± 96.42	6.54 ± 0.27	7.70 ± 0.87	3.33 ± 0.13	4.12 ± 0.89
51-60 yrs	33.90 ± 4.17	14.85 ± 2.05	28.9 ± 2.62	41.80 ± 0.42	9.0 ± 0.39	15.25 ± 0.07	121.25 ± 5.57	277.50 ± 4.95	6.51 ± 0.29	8.05 ± 0.21	3.38 ± 0.24	4.63 ± 0.12
61-70 yrs	32.63 ± 2.98	-	29.7 ± 3.01	-	8.75 ± 0.49	-	115.0 ± 6.35	-	6.36 ± 0.24	-	3.40 ± 0.27	-
Total	**32.76 ± 3.44**	**14.11 ± 3.32**	**28.2 ± 2.51**	**40.99 ± 2.81**	**8.88 ± 0.41**	**14.81 ± 1.34**	**116.54 ± 7.21**	**261.46 ± 53.83**	**6.52 ± 0.26**	**8.23 ± 0.36**	**3.34 ± 0.21**	**4.11 ± 0.57**

difference in the malaria prevalence among age bracket was significant ($p<0.05$). The prevalence of malaria in GGC, GSS and CAN were 81 (87.1), 223 (91.0) and 57 (91.9) respectively.

Overall Analysis of Serum Amyloid A (SAA) and Haematological Parameters

PCV was significantly as low as 28.2% in the positive patients compared to 41% recorded in the positive subjects, it ranges between 27.6% to 29.6% in the positive subjects compared to a range of 37.10% - 41.30% recorded in the negative non-infected patients (Table 2). The mean haemoglobin of 8.88g/dl was recorded in the positive subjects relative to the 14.81g/dl recorded in the negative subjects. The mean haemoglobin in the positive subject ranges from 8.82g/dl recorded in the negative 9.0g/dl as the positive subjects while the range in the negative subject was between 13.10g/dl to 15.25g/dl (Table 2). Mean platelet in the positive subject was significantly as low as 116.54 ×10^3/µl compared to the high 261.46×10^3/µl recorded in the negative study subjects, the mean platelet value in the positive subject ranges from 115.0 to 121.25×10^3/µl compared to the high mean range of 169.67 – 282.86×10^3/µl in the positive group (Table 2). A low mean leucocyte of 6.02×10^3/mm^3 was recorded in the positive study cases with a range of 6.36 – 6.54×10^3/mm^3 compared to 8.23×10^3/mm^3 recorded in the negative subjects with a mean range of 7.70 – 8.1 ×10^3/mm^3 (Table 2). Mean neutrophils in the positive subjects, was as low 3.34×10^3/mm^3 compared to 4.11×10^3/mm^3 in the negative subjects, while the mean range of 3.32 – 3.4×10^3/mm^3 and 3.78 – 4.63×10^3/mm^3 were recorded in the positive and negative subjects respectively (Table 2).

Discussion

The high malaria prevalence observed in this study among (90.2%) is alarming. The lifestyle and socio-economic well-being, lack of access to basic amenities in conjunction with the poor housing patterns of these displaced person, which may have made the displaced person vulnerable to mosquito bites. The high prevalence of 90.2% observed in this present study was many fold higher than 17% recorded by Charchuk, et al., [19] among internally displaced children in the Democratic Republic of Congo, 49% reported by Henry, et al., [20] among pregnant women in internally displaced persons camp in Uganda, 78.5% recorded by Eze et al., [21] among Fulani pastoralist, 54.5% by Adefioye et al., [22] in Oshogbo, South-West, Nigeria. The observed higher prevalence could be due to the fact that the females experience the immune-suppression during pregnancy to accommodate the foetus, this immunological process lowers their immunity and pre-disposes them to several infection including malaria. This observation is consistent with earlier report [23]. The age bracket, 11-20years old had higher prevalence. This is not surprising as this age group is the most active residents of the camps, as they are always seen

loitering around the camps even till late in the night, which make them vulnerable to mosquito bite and hence malaria. A high mean Serum Amyloid A level of 32.7mg/l observed in the malaria positive patients was abnormally higher than the malaria negative patients. This is an indication that SAA can be a good biomarker for malaria parasites. The result of this work agrees with the findings of Kassa et al., [24,25] who recorded a higher SAA of 55.9mg/l in malaria patients compared to the non-malaria patient in his research work. The PCV of 28.2% recorded in malaria positive patients was lower than a 41.0% in the non-malaria subjects, high light the fact that a malaria infected person always develops a reduced PCV. This observation is in conformity with the report of Imoru, et al., [26] who recorded a very low PCV of 29.0% in non- malaria children compared to 32.3% in the control group. Several haematological parameters have been used to assess malaria parasitaemia in malaria endemic area [27-30]. Heamoglobin had shown a positive correlation with malaria infection [12]. He observes that that the higher the malaria parasitaemia, the more reduced the haemoglobin count. In this present study, the mean haemoglobin reduced as much as (8.88g/dl) in the malaria positive subject in relative to 14.81g/dl in the negative subjects. The haemoglobin level is far below the threshold of 10.9g/dl documented as the normal level. This observation agrees with the findings of Zeeba, et al., [31] who recorded a mean haemoglobin of 10.0g/dl in positive malaria patients. This abnormal reduction in the haemoglobin is due to the fact that haemoglobin is the main target of malaria parasite hence continually destroying it during infection. The low Platelet recorded in malaria patients (116.54×10^3/µl) compared to 261.46×10^3/µl in the non-malaria patients high light a drastic reduction in platelet value in malaria related infections [12,23]. The reduction could be caused by a general slowdown in erythropoiesis due to the plasmodium infection on one hand and peripheral destruction and excessive removal of the platelets by spleen in response to malaria infection on the other hand.

A mean leucocyte count in malaria positive patients (6.52×10^3/mm^3) was higher than non-malaria patients ($8.23\times10^3/mm^3$). This contradicts the work of Zeeba, et al., [31] who recorded a higher leucocyte count in the malaria positive patients compared to the non-malaria patients. This could be due to the suppressed peripheral removal of WBC by the immune system in response for malaria infection as also documented by Zeeba, et al., [31]. A mean low neutrophil in malaria positive patients than malaria negative patients in this present study agrees with the report of Senthilkumaar, and Sarojini [32]. The observe report may be attributed to the abnormal destruction of the WBCs by the *Plasmodium* during malaria infection.

Conclusion

It is evident from the study that SAA is a good biomarker of malaria diagnosis. The haematological parameters associated with malaria infections were packed cell volume, haemoglobin count, platelets, leucocyte count and neutrophil. From this study, all these haematological parameters decrease with increase in malaria parasitaemia. However, SAA level increases with increase in malaria parasitaemia. Human Serum Amyloid A in plasma, Serum, and Cell culture can therefore be used as a foundational diagnosis for both asymptomatic and symptomatic malaria patients.

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