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Pathophysiology of Eosinophilia in Malarial Infection in Patients Attending Usmanu Danfodiyo University Teaching Hospital (Uduth) Sokoto, Nigeria.

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Abstract: *To determine the pathologic relevance of eosinophilia in malaria infection.A total of two hundred and fifty (250) patient attending UsmanuDanfodiyo University Teaching Hospital Sokoto were used in this study. Comprising of 200 malaria positive patient and 50 apparently healthy individual as test sample and control sample respectively. Thin and thick blood film were made from each client and stained with leishman and giemsa stain for the microscopic determination of differential leucocyte count and malaria parasite density respectively.The pathologic relevance of eosinophilia in malaria infection and the effect of malaria infection on increase in eosinophils count were determined using SPSS computer software version 18.0. In all out of 91(36.4%) patient with mild infection (+), 68(27.2%) patient have mild eosinophilia (5-8), and 23(9.2%) have moderate eosinophilia (5-8) and out of 57(22.8%) patient with moderate infection (++), 12(4.8%) patient have mild eosinophilia (5-8), 39(15.6%) patient have moderate eosinophilia (9-12), and 6(2.4%) patient have severe eosinophilia (13-16), and then out of 52(20.8%) patient with severe infection (+++), only 1(0.4%) patient have mild eosinophilia(5-8),12(4.8%) patient have moderate eosinophilia (9-12), and the majority of the population with severe infection (+++), 39(15.6%) patient have severe eosinophilia (13-16).Out of the 50(20.0%) client that were used as control, 25(10%) client have normal eosinophil count(1-4), 24(9.6%) client have mild eosinophilia(5-8), and only 1(0.4%) client have moderate eosinophilia(9-12).It was found that there was decrease in the incidence of eosinophilia with increased incidence of malaria infection and it was also found that other infection can cause eosinophilia other than malaria infection as observed in the control population.*

I. Introduction

Malaria infection is caused by invasion of red blood cells with protozoan parasites of the genus Plasmodium. The female anopheles mosquito is the carrier of the parasite and transmits it to man by next blood meal. Malaria is widespread in tropical and subtropical regions because of the significant amounts of rainfall and consistent high temperatures and high humidity, along with stagnant waters which provide mosquitoes the environment needed for continuous breeding *(3).*

The bite of mosquito that carries the plasmodium leads to the transmission of the parasite in red blood cells, causing symptoms that typically include fever and headache, in severe cases progressing to coma and death. The four Plasmodium species that infect humans are P. falciparum, P. vivax P. ovale and P. malariae. Occasional infections with monkey malaria parasite, such as P. knowlesi, also occur *(4)*

II. Eosinophilia

Eosinophilia is a condition in which the eosinophil count in the peripheral blood exceeds 0.45×10^9/L (450/µl). Eosinophils usually account for less than 7% of the circulating leukocytes. A marked increase in non-blood tissue eosinophil count noticed upon histopathologic examination is diagnostic for tissue eosinophilia*(12).*

Eosinophils are bone marrow-derived leukocytes whose development and terminal differentiation are under the control of several cytokines (IL-3, GM-CSF and IL-5), with IL-5 being the cytokine that is primarily responsible for eosinophilopoiesis. Eosinophils and neutrophils share a common morphology but the eosinophils are a little larger than the neutrophils and measure 12-17µm in diameter. They usually have two nuclear lobes and the cytoplasm has distinctive spherical orange granules. The underlying cytoplasm which is usually

obscured by granules is pale pink. Eosinophils are predominantly tissue dwelling cells and express a specific chemoattractant receptor and respond to a specific chemokine, eotaxin. They are moderately effective as a phagocyte for bacteria, yeast and protozoa but less effective than neutrophils *(12).* The major function of eosinophil as a cytotoxic cell is against parasitic infections. Eosinophils can kill a wide variety of parasitic organism especially in their larval stages, by depositing cationic proteins on the surface of the parasite *(10).*

Conventionally eosinophils have been considered as an end-stage cells involved in host protection against parasites. Numerous lines of evidence however have now changed this perspective by showing that eosinophils are pleiotropic multifunctional leukocytes involved in initiation and propagation of diverse inflammatory responses, as well as modulators of innate and adaptive immunity. The circulating life span of eosinophil is 6-12 hrs before it migrates to tissue but unlike the neutrophils it can recirculate and have a much longer life *(12).* The normal eosinophil count is up to 600/cmm,When the levels go beyond the normal the condition is called as eosinophilia. There is considerable diurnal variation in the eosinophil count which may be as much as 100% *(Rothenberg, 5,9).*

Eosinophils may be produced as a result of the T- helper-2 (Th-2) immune response *(6,2)* and apparently by other immune pathways as well *(1).* The balance between Th-1 and Th-2 mediated immune responses is of central importance for the body's response to parasitic infections. Based on data from rodent models and some human studies, a Th-1 immune pathway appears to predominate in the acute phase of malaria infection, while a Th-2 type immune response is characteristically observed in chronic malaria or the recovery phase*(7,8,11).*

III. Materials And Methods.

Study Area

The selected area for this study or research work was UsmanuDanfodiyo University Teaching Hospital Sokoto, sokotostate,Nigeria. UsmanuDanfodio University Teaching Hospital (UDUTH) Sokoto is a tertiary health institution, located in north western Nigeria.

Study Population

The population of subjects used in this study were 250 individual irrespective of age, gender, and medical condition. Comprising of 200 test sample from malarial infected patients, and 50 apparently healthy individuals as the control.

Study Design

This is a cross-sectional comparative study.

Sample Size

The sample size (N) used in this study were 200 test sample from malarial infected patients, and 50 apparently healthy individuals as the control.

Ethical Approval

The ethical clearance for the study was obtained from the ethical committee of the UsmauDanfodiyo University (UDUTH) Sokoto. Before collecting blood sample, subjects consent was obtained after explanation about the study.

IV. Sampling Techniques

Sample Collection

A volume of 2.5ml of venous blood sample obtain into tubes containing the anticoagulant potassium ethylenediamine-tetra acetic acid (EDTA) for making a thick blood film for malaria parasite identification and thin blood film for differential leucocytes count.

Test Sample

The test samples were obtained from 200 malaria infected patient that were malaria positive, irrespective of age, gender and medical condition attending UsmanuDanfodiyo University Teaching Hospital Sokoto.

Control Sample

The control samples were obtained from 50 apparently healthy individuals that are malaria negative attending UsmanuDanfodiyo University Teaching Hospital Sokoto.

Laboratory Analysis

Sample Processing

All sample collected were processed and maintained under aseptic condition and stored under correct temperature if not used immediately. The sample collected in a plane tube was processed immediately for the study while those collected in anticoagulant bottles were kept under a correct temperature before use.

Peripheral Thick Blood Film, Making And Staining Procedure:

1. A drop of whole blood was placed at the centre of a clean grease free glass slide.
2. Using a smooth edge slide spreader the drop of blood was spread uniformly to cover an area of 15×15mm.
3. The film was allowed to air dry by waving the slide back and front.
4. When completely dried, the film was fixed in absolude methanol for 2 minute.
5. It was allowed to air dry and ready for staining.

Giemsa Stainig Method

1. A prepared thick blood film was covered with giemsa stain and allowed to act for 30 minute.
2. The stain was washed with tap water and it was allowed to air dry.
3. The prepared stained thick blood film was examined microscopically using oil immersion objective (×100)
4. The result obtained were recorded.

Peripheral Thin Blood Film, Making And Staining Procedure:

1. A drop of whole blood was placed on the end of a clean grease free glass slide.
2. Using a clean smooth edge spreader (cover slip), the spreader was drawn back to touch a drop of blood so that it extends along the edge of the spreader and moved gently and smoothly.
3. The film was allowed to air dry by waving the slide back and front.
4. When completely dried, the film was fixed in absolute methanol for 2 minute.
5. It was allowed to air dry and ready for staining.

Leishman Staining Method

1. A prepared thin blood film was covered with leishman stain and allowed to act for 2 minutes.
2. The stain on the film was diluted with twice the volume of stain pH 6.8 buffered water and allowed to act for 8 minutes.
3. The stain was washed with tap water and it was allowed to air dry.
4. The prepared stained thin blood film was examined microscopically using oil immersion objective(×100).
5. The result obtained were recorded.

Differential Leucocyte Count Method

1. A drop of immersion oil was placed on the third of the stained dried blood film and covered with a cover slip.
2. The film was examined microscopically and focus using ×10 objective with the condenser iris closed sufficiently to see the cell clearly.
3. The part of the film where the cells are just beginning to overlap are focused and the ×40 objective was brought into place and iris diaphragm opened.
4. Systematically the blood film was examined and the white blood cells seen in each field are counted preferably using an automatic differential cell counter.
5. When a total of 100 cells have been counted. The number of each cell type is expressed as the percentage of that cell type present in the blood film of that individual. It also represents the number of each cell type present in the system of that individual.

V. Malaria Parasite Detection Method

1. A drop of immersion oil was placed around the edge of the stained dried thick blood film.

2. The slide was then mounted unto the stage of microscope and first examined using 40x objectives. The objective was then changed to 100x.

3. The malaria parasites were examined and the approximate numbers of parasites were recorded. The following plus signs were used to report parasite numbers:

1. 1 – 10 per 100 high power fields …… +
2. 11 – 100 per 100 high power fields …… ++

3. 1 – 10 in every high power field …… +++
4. More than 10 in every high power field …… ++++

VI. Result Analysis

Two hundred and fifty (250) subjects were used in this research work irrespective of age, gender and medical condition, which comprises of 200 malaria positive patient and 50 apparently healthy individual that are malaria negative which were used as test sample and control sample respectively. Out of 200 malaria positive patient in table 4.1 91(36.4%) of patient fall under mild infection (+), 57 (22.8%) fall under moderate infection (++) and 52 (20.8%) fall under severe infection (+++) while the control consist of 50 apparently healthy individual that comprises of 20% of the total population. Out of the 250 total population size in table 4.2 25(10%) of the population falls within the range of eosinophil count 1-4(normal), 105(42%) falls within the range 5-8(mild), 75(30%) falls within the range of 9-12(moderate), and 45(18%) falls within the range of 13-16(severe).In table 4.3 the population of subject that falls under mild, moderate and severe infections in table 4.1 were correlated with the ranges of eosinophil count of table 4.2.The 91(36%) of the total population in table 4.1that falls under mild infection were distributed within the range of eosinophil count in which 5-8 consist of 68(27.2%) and 9-12 consist of 23(9.2%) number of subject respectively, 57(22.8%) that falls under moderate infection were distributed within the range of 5-8 which consist of 12(4.8%) , 9-12 consist of 39(15.6%) and 13-16 consist of 6(2.4%) respectively, 52(20.8%) that falls under severe infection were distributed within the range of 5-8 which consist of 1(0.4%), 9-12 consist of 12(4.8%), and 13-16 consist of 39(15.6%) respectively, and the control was also distributed within the range of 1-4 which consist of 25(10%), 5-8 consist of 24(9.6%) and 9-12 consist of 1(0.4%).

Table: 1 shows the percentage of cases according to malaria parasite density (+, ++, +++) and Control.

Malaria parasite density	number of cases	% of cases
+	91	36.4
++	57	22.8
+++	52	20.8
Control	50	20.0
Total	250	100

Key:
+ = Mild infection
++ = Moderate infection
+++ = Severe infection

Table: 2 shows the percentage of cases according to category of eosinophilic count.

Eosinophil count	number of cases	% of cases
1-4	25	10.0
5-8	105	42.0
9-13	75	30.0
13-16	45	18.0
Total	250	100.0

Key:
1-4 = Normal
5-8 = Mild eosinophilia
9-12 = Moderate eosinophilia
13-16 = Severe eosinophilia

Table: 3. Shows the correlation between malaria parasite density and ranges of eosinophilic count.

Malaria parasite density	Eosinophil count				Total
	1-4	5-8	9-12	13-16	
+	0	68	23	0	91
++	0	12	39	6	57
+++	0	1	12	39	52
Control	25	24	1	0	50
Total	25	105	75	45	250

Key:
+ = Mild infection
++ = Moderate infection
+++ = Severe infection

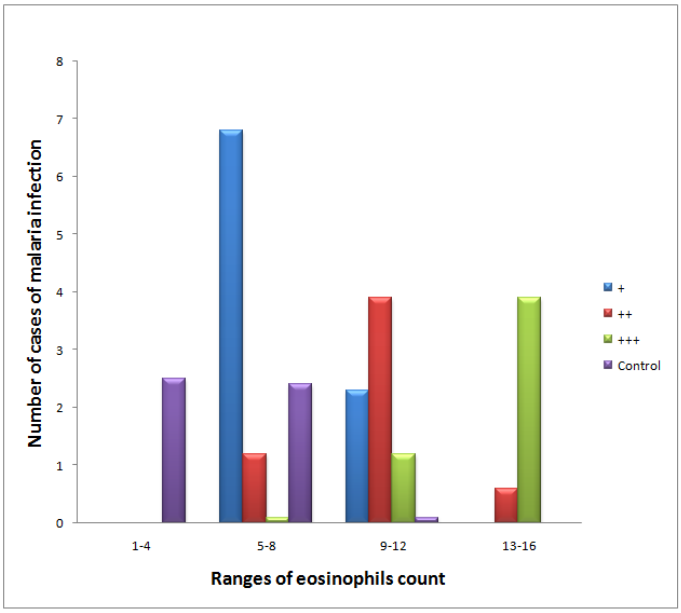

Fig:1. Bar chart showing correlation between malaria parasite density and ranges of eosinophil count.
Key:
On the vertical axis
1 unit = 10 unit.

VII. Discussion

In this study 200 malaria positive patient attending UsmanuDafodio University Teaching Hospital Sokoto, Sokoto State along with 50 control obtained from apparently healthy individual were analysed for malaria parasite density and eosinophil count from which percentage of cases according to malaria parasite density(+, ++, +++) and percentage of cases according to ranges of eosinophil count(1-4, 5-8, 9-12, and 13-16) (5) were obtained, which confirm that there is higher incidence of cases with regard to + which consist of the total number of 91(36.4%) subjects out of 200 client, followed by ++ with the total number of 57(22.8%) and then lastly +++ with the total number of 52(20.8%). these confirm that there is decrease in incidence with increase in malaria parasite density which is in consistence with the results of (1). The control which consist of 50 apparently healthy individual remain undistributed due to the absence of malaria parasite within their system. And also incidence with regard to eosinophil count follows the pattern of a normal distribution curve in which the range of eosinophil count 1-4 consist of the total number of 25(10%) subject out of 250 client, followed by 5-8 with the total number of 105(42%), and then 9-12 with the total number of 75(30%)and then lastly 13-16 consist of the total number of 45(18%).The correlation between malaria parasite density and the ranges of eosinophil count in table 3 shows that 91(36.4%) of patient with mild infection were distributed between the range of 5-8 consisting of 68(27.2%) patient and 9-12 consist of 23(9.2%) patient respectively. This confirmed that there is the production of eosinophil during the early phase of infection in which 68(27.2%) patient have mild response while 23(9.2%) of patient have moderate response. The difference of eosinophil ranges in patient with mild infection may be due to either difference in the time of exposure or may be due to the difference in which an individual immune system response to an infection. 57(22.4%)of patient with moderate infection were found to be distributed within the range of 5-8 which consist of12(4.8%) patient,9-12 consist of39 patient and 13-16 consist of 6 patients. This indicate that majority of caseswithin moderate infection fall within the range of 9-12, this confirmed that as the level of parasiteamia increases also individual immune responsealso increases to show the pathological relevance of eosinophil to malaria infection. Also 47 patientwith severe infection fall within the range of 5-6 consisting of 1 patient, 9-12 consist of 12 patients and 13-16 also consist of 36 patients. The case is similar with severe infection in which the majority of cases fall within the range of 13-16 which consist of the total number of 36 patient. This confirmed that increases in parasiteamia is accompanied by increase in eosinophil count, this shows that there is a high degree of relationship between malaria infection and eosinophil count. The control which consist of 50(20%) of subjects were distributed between the range of 1-4

which consist of 25 subjects, 5-8 consist of 24 subjects and 9-12 also consist of 1 subject. These shows that only people without parasiteamia fall within the range of 1-4 and the ranges 5-8 and 13-16 consist of 24and 1 subject respectively confirmed that there are other underline factors that can cause increase in eosinophil count other than malaria infection.

VIII. Conclusion

In conclusion, this study has shown that, there was pathologic relevance of eosinophilia in malaria infection among malaria patient attended UsmanuDanfodioUnversity Teaching Hospital Sokoto. which was determined based on increase in eosinophil count at different level ofparasiteamia as shown in Fig 1, and this increase in eosinophil count in relation to the level of parasiteamia depend upon duration of exposure to the parasite or an individual difference in the strength of the immune system to mount an effective immune response against the parasite. And there are other underline factors that can induced the production of eosinophil other than malaria infection. It was also observed during the study that most of the malaria patient have an increase in neutrophil count and few have decrease in neutrophil count while others were within the normal range.

IX. Recommendations

1. Differential leucocyte count should be done to any malaria infected patient to determine the level of eosinophil in the peripheral blood circulation.
2. Sign and symptoms of eosinophilia should be included in the clinical diagnosis of malaria patient.
3. Immediate treatment is required for any eosinophilic patient with malaria to avoid further damage due to infiltration of eosinophil in the tissue.
4. Treatment of eosinophilia should start from treating the underline factor that causes the eosinophilia.
5. Prescription of drugs that can cause extensive eosinophilia should be avoided
6.Prescription of drug that can induced the development of eosinophilia should be combined with other drugs that can suppressed the production of eosinophil by pluripotent stem cell.

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