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Research Article

Evaluation of Haemolysin Activity in Malaria Patients in Owerri Metropolis

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Abstract

The aim of the study was to determine the prevalence of haemolysin activity in malaria patient. This prospective study was carried out in Owerri Metropolis from July 2012 to September 2012. Fifty voluntary groups confirmed malaria patients (23 males and 27 females with mean age 22.46 ± 4.53) were screened for alpha (Anti A) and beta (Anti B) haemolysin. The overall prevalence of haemolysin activity in malaria patient show 12% prevalence in 50 malaria patients screened with the mean ± standard deviation (0.32 ± 0.0721) considered significant for packed call volume PCV ($P < 0.05$) and mean ± standard deviation value of l ib estimation (10:67 V 2.4295) ($P < 0.05$) when compared with the 88% non-haemolysin malaria patient of mean l - standard deviation of PCV (0.42 ± 0.0463) and Hb estimation 13.82 ± 1.519 respectively. The mean ± standard deviation level (in weeks) between haemolysin activity in malaria patient were significant ($P < -0.005$) (3.33 ± 1.033) when compared to the negative result of haemolysin (1.5 ± 0.590) thus prevalence of haemolysin activities in malaria patient would likely be due to prolonged untreated malaria.

Keywords: *Haemolysin; IgM; IgG; Malaria Patient*

Introduction

Haemolysin is a type of antibody that has the ability of combining with its specific antigen on the red blood cells and together with complement causing destruction of the cells [1]. Haemolysin can be either IgM or IgG immunoglobulin. Hence its characteristics depend on the immunoglobulin class of the haemolysin antibody. It can be divided into alpha, beta and alpha plus beta haemolysin types - alpha haemolysin may be detected in ABO blood group B and O individuals while beta haemolysin may be present in group A and O persons, immune haemolysin production may occur following ABO heterospecific pregnancy, incompatible blood transfusion or immunization with A or B substances [2].

Alpha and beta haemolysin occur more in blood group O donors than in other blood group donors types put together. Ritter., *et al.* [3] stated that prolonged haemolysin may accompany infection with *Plasmodium falciparum* having observed prolonged haemolysin in 4 of 10 patients with extended type of haemolytic malaria after glycolytic enzyme triosephosphate isomerase were detected in these patients sera [3]. Clinical recovery and a decrease in haemolysin coincided with a fall in these autoantibodies. *In vitro*, affinity purified autoantibodies isolated from the sera directed against triosephosphate isomerase induced lysis of erythrocytes and activation of complement as shown by the $^{cr}51$ release assay. It was assumed that autoantibodies against triosephosphate isomerase to the development of prolonged haemolysin and anaemia in *Plasmodium falciparum* malaria [3].

Malaria is the most common infectious cause of haemolytic anemia worldwide [4]. Haemolysis in malaria results directly from erythrocytic infestation by *Plasmodium* organism. Infested erythrocytes are selectively removed from the circulation by the spleen, with some red cells re-entering circulation after splenic pitting of parasites. The severity of the haemolytic process is related to the degree of parasitemia

and may be increased by hypersplenism. *Plasmodium vivax and P. ovale* only invade reticulocytes while *P. malariae* only invades mature erythrocytes, *plasmodium falciparum* invades erythrocytes of all ages and therefore leads to a higher degree of parasitism with *plasmodium falciparum* infection, intravascular haemolysis may be severe and associated with haemoglobinuria (black water fever), as well as cerebral erythrostasis due to decreased deformability of infested erythrocytes.

Materials and Methods

Study area

The study took place at Imo State University Medical Laboratory in Owerri Metropolis.

Study design

A cross sectional study on 50 subjects was conducted, comprising of 23 males and 27 females and there were between the age of 16 and above they were non-smokers, non-drinker and were not pregnant or lactating. They were not on any medication that will affect the parameters concentration of the patients.

Blood collection

About 5 ml of venous blood samples were collected from each patient and into EDTA bottle for the preparation of blood for thick film preparation for malaria parasite, PCV, haemoglobin and cell grouping. Then the serum from the clotted blood was used for serum grouping.

Methodology

ABO cell and serum grouping (tube technique)

Procedure

About 0.5 ml of EDTA blood (or red cells from a donor blood sample) was added to about 5 ml physiological saline. It was centrifuged at about 1000g for 2 - 3 minutes. The supernatant fluid was discarded, resuspended the sediment red cells in a further 5 - 7 ml saline and centrifuged. The supernatant fluid was discarded. A 3 - 5% red cell suspension was made by mixing 1 drop of red cell suspension by mixing 1 drop of sedimented cells in 20 - 25 drops of saline. Five small tubes are taken and labeled them 1 to 5. The following were pipette into each tube:

- Tube 1: 1 volume anti A serum, 1 volume 3 - 5% patient's red cells
- Tube 2: 1 volume anti-B serum, 1 volume 3 - 5% patient's red cells
- Tube 3: 1 volume patient's serum, 1 volume 3 - 5% A cells
- Tube 4: 1 volume patient's serum, 1 volume 3 - 5% B cells
- Tube 5: Volume patient's serum, (auto-control) 1 volume patient's 3 - 5% red cells.

The contents of the tube are mixed by gently tapping base of each tube with the finger. The tubes are left at room temperature for 5 minutes. It was centrifuged a lowest setting for 1 minute or at 500 - 100g 10 - 15 seconds. The tubes in the rack are replaced in the same position as before centrifuging. The results are read by tapping gently the base of each tube looking for agglutination or haemolysis. The

results are recorded.

Haemoglobin estimation (Cyanmethemoglobin method)

Procedure

About 0.02 ml of blood was added to 5 ml of Drabkins solution in a test tube (1:250 dilutions). It was mixed well and allowed to stand for 10 minutes. It was read calorimetrically at 540 nanometers (green filter) with Drabkins solution as blank:

Calculation: $\frac{\text{OD of test}}{\text{OD of standard}} \times \text{concentration of STD}$

PCV (Packed cell volume)

Capillary tube method

Procedure

About three quarter of capillary blood was filled in an heparinized capillary tube. The unfilled end of the capillary was sealed using a sealant. The filled capillary tube was carefully located in one of the number slots the microhematocrit rotor with the sealed end against the rim gasket (to avoid breakage). It was then centrifuge for 5 minute (rpm 12000 - 15000) and was read with microhaematocrit reader.

Method of identification of malaria parasite in thick film using geimsa staining method

Procedure

A drop of patience blood was drop at the center of a clean grease free slide. The blood was spread with the edge of another slide. The film was stained with 1 in 10 dilution of geimsa stain. It was allowed to dry in a dust free corner. The stain was washed off with distilled water and was air dried. It was then viewed under microscope using oil immersion (x 100 objective).

Result: Malaria parasite if present will be seen as ring form of early trophozoites gametocytes or as schizonts.

Statistical analysis

Values obtain were expressed as mean ± SD the statistical significant was evaluated by the z- score and simple percentage and values with $p < 0.05$ were considered significant.

Result

Table 1 show the mean haematological values or pcv and Hb estimate of malaria patients with Haemolysin (Test group) and malaria patients without Haemolysin activity (control group). Statistical analysis showed that there is significant difference ($p < 0.05$) between P.C.V of the Haemolysin serum grouping of malaria patients (0.32 ± 0.0729) and non-haemolysin serum grouping of malaria patients (0.42 ± 0.0463) and Hb estimate of haemolysin of malaria patients (10.67 ± 2.4295) and non-haemolysin malaria patients (13.82 ± 1.519) respectively.

Parameter	Haemolysin Malaria patients N = 6	Non-haemolysin Malaria patients Control (n = 44)	P values
PCV (l/l)	0.32 ± 0.0729	0.42 ± 0.0463	P < 0.05
HB (g/dl)	10.67 ± 2.4295	13.82 ± 1.519	P < 0.05

Table 1: *Comparison of mean haematological value of haemolysin activity in malaria patients and non-haemolysin patients. All values recorded as mean standard deviation ( ). P values < 0.05 are considered significant.*

In table 2, out of 50 malaria patients screened for haemolysin activity person with a percentage distribution of 12% were positive while 44% malaria Patients with percentage distribution of 88% were proved negative for haemolysin activity.

Parameter	Total no of sample (N = 50)	Percentage of total (%)
Haemolysin activity in malaria patients	6	12%
Non haemolysin activity in malaria patients	44	88%
Total	50	

Table 2: *Show the percentage distribution of Haemolysin activity in malaria patients and non-haemolysin malaria patients.*

In table 3 the mean level of Duration (in weeks) **(**176 0.89 (s) between haemolysin activity in malaria patients and non-haemolysin malaria individual (control) the statically analysis shows significant ($p < 0.050$) with the mean values of haemolysin patients (3.33 ± 1.033) and mean value of non-haemolysin malaria patients (1.5 ± 0.590).

Parameter	Haemolysin malaria patients (n = 6)	Non-haemolysin malaria patients (44)	P-value
Duration in malaria patient (weeks)	1.33 1.033	1.50.590	P < 0.05)

Table 3: *Show mean serum grouping between the duration in malaria patients amongst haemolysin and non-haemolysin individual.*

All value recorded as mean standard deviation (X ± s) →Duration (weeks).

Discussion

This study has confirmed the frequency of haemolysin in malaria patients. The prevalence observed in this study is higher than those reported by Wellek., *et al* [5]. The higher prevalence rate observed in this study could be due to the, duration of malaria patients. There was no statistically significant difference between male and female donors in the frequency of haemolysin. This is in conformity with the work reported by other work of Klein and Anstee [6]. Taking a visual titre in the serum grouping, adopted for this study show 12% prevalence in 50 malaria patients screened different from those reportedly free from Haemolysin activities. This prevalence of Haemolysin activity in malaria patient would be due to prolonged untreated malaria.

Conclusion

This study has shown that although the prevalence of haemolysin is prominent in prolonged malaria patients, the level of parasite infection is insignificant. Therefore, despite the level of illiteracy and the constant drug abuse, there is need to properly screen for malaria parasite before and after treatment.

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