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# Comparative study of the extent of haemolysis sickle cell patients in crisis and *Plasmodium falciparum* infected patients

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ABSTRACT: Serum lactate dehydrogenase (LDH), Potassium ($K^+$), Iron (Fe) and Packed Cell Volume (PCV) were studied in 22 patients with sickle cell disease in crisis, 30 patients with *Plasmodium falciparum* infection (malaria) and 50 apparently healthy subjects, as control. The serum levels of LDH, $K^+$ and fe in sickle cell patients and *Plasmodium falciparum* infected patients was significantly higher ($P<0.05$) than control subjects, while this parameters were significantly higher in sickle cell patients than *Plasmodium falciparum* infected patients ($P<0.05$). However, the mean value of Packed Cell Volume was significantly lower in sickle cell patients and malaria patients compared to that of control subject, and significantly lower in sickle cell patients compared to malaria patients.

These results show active haemolysis in sickle cell patients in crisis and patients infected with *P. falciparum*. The level of haemolysis was however more in sickle cell patients in crisis than that of patients infected with *Plasmodium falciparum*. While both patients require interventions that arrest clinical implication of haemolysis, these interventions are more necessitated in sickle cell patients in crisis.

Key words: LDH, Fe, $K^+$, PCV – Haemolysis, Sicklers, *P. falciparum* infection.

## Introduction

The sickle cell diseases are caused by a genetic mutation in the haemoglobin molecule resulting in the decreased ability to transport oxygen with subsequent clinical ramification secondary to the sickling of the red cell (1). Sickle cell anaemia is a commonly fatal hereditary haemolytic disease, which is characterized by acute episodes of exacerbation of the disease often referred to as crisis (2, 3 and 4). Sickle cell disease is the most common inherited haemoglobinopathy described.

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There is a higher concentration in black Africa primarily in the sub-sahara region (2,5,6). However, migration of people from these regions to other parts of the global spread this disease (2). Complications of sickle cell disease are due to chronic haemolysis of fragile red cells of 30 – 60 days life span or secondary to vascular occlusion by sickle red cells with subsequent tissue infarction (6,7,8).

Malaria, on the other hand, is a disease caused by sporozoa of genus *Plasmodium* transmitted by species of female anopheles mosquito (9,10). *Plasmodium falciparum* and *Plasmodium vivax* are the most common and most important plasmodium infections characterized by the destruction of red blood cell (9,10,11).

*Plasmodium falciparum* causative organisms of falciparum, malignant tertian or subtertian malaria invade all ages (old and young) of erythrocytes indiscriminately so that very high infections rates may occur and anaemia is most pronounced in falciparum infections with extensive and rapid destruction of red blood cell (9,11). Haemolysis in malaria infection may be due to antibody – antigen plus complement reaction and excessive destruction of erythrocytes by the invaded parasite. Haemolysis may also arise from autoimmune response or opsonization of infected erythrocytes in *Plasmodium falciparum* infection (9).

The pathophysiology of both sickle cell disease and malaria infection involves the destruction of red blood cells leading to loss of some intracellular substance into the extracellular fluid compartment affecting the levels of some biochemical parameters in the blood hence there elevated values in the plasma or serum.

In the present study, an attempt was made to investigate the extent of the relative influx of some biochemical parameters which may be use as a good index of extent of haemolysis, in the plasma of patients infected with *P. falciparum*; sickle cell disease in crisis and control subjects. These parameters include LDH, Fe and$K^+$. The packed cell volume, which is a good index of the level of red blood cell destruction or anaemia was also measured and correlated with the biochemical parameters. The degree of haemolysis and anaemia in sickle cell and *Plasmodium falciparum* infected patients were also compared.

## Materials and Methods

*Study Population*

A total of sixty patients were recruited into the study from the medical out-patients department of the Baptist Medical centre, Saki, Oyo State, Nigeria, after their consent had been sought. Thirty of which are diagnosed to have sickle cell disease in crisis aged between 15 and 51 years, 16 of which are male, and 15 females. Also thirty diagnosed to have malaria with *P. falciparum* infectation, aged between 14 – 71 years. 13 of which are males and 17 females. Fifty subjects of similar age and sex range (13 – 70 years, 25 males and 25 females) who are non-sickle cell patient (i.e. HbA) and who had no *plasmodium falciparum* infestation served as controls.

*Blood Sampling*

Six mls of venous blood was taken from each patient from the anterior cubital vein after the skin had been cleaned with methylated spirit. Blood specimens were divided into two sets of bottles for haemotological and biochemical analysis.

(1) Samples in $Na^+$EDTA bottles were used to determine packed cell volume, haemoglobin electrophoresis and thick film for malaria parasite smear.

(2) Samples in non-coagulant bottles were used for estimation of serum LDH, $Fe^+$ and $K^+$. The blood samples in the plain bottles were centrifuged at 1,000 rpm for 10 mins. The sera were separated and analysed.

Serum lactate dehydrogenase activity was determined by measurement of NADPH consumption (12). Serum iron concentration was determined by the method of Giovaniello *et al* (7). Serum potassium was estimated by colorimetric method of Terr *et al*., (13)). The blood packed cell volume was determined by

the capillary method (11). While the haemoglobin electrophoresis was done by cellulose acetate method (11). Thick film, prepared from EDTA blood samples were stained with Giemsa stain and examined microscopically to screen the subjects for malaria parasite (10).

*Statistical Analysis*

SPSS statistical software was used in all the statistical analyses (14). The results were expressed as Mean ± SD. The significant difference between study groups and within group were detected by analysis of variance (ANOVA), while significant difference in mean value of test and control was detected using paired sample student "t" test.

Table 1: Comparison of investigated parameters in the three groups of subjects.

| **Parameters** | **HbSS Patient** | ***P. falciparum*** | **Control** | **t** | **P-value** |
|---|---|---|---|---|---|
| PCV | 12.8±0.8 | 24.9±2.0 | 40.9±1.1 | $A_1 = 17.9$ | $P < 0.001$ |
| | | | | $A_2 = 7.9$ | $P < 0.001$ |
| | | | | $A_3 = 5.7$ | $P < 0.001$ |
| Fe (µg/dl) | 221.5±4.2 | 194.0±11.24 | 101±6.4 | $A_1 = 16.9$ | $P < 0.001$ |
| | | | | $A_2 = 6.9$ | $P < 0.001$ |
| | | | | $A_3 = 2.3$ | $P < 0.001$ |
| K (mmol/L) | 6.8±0.7 | 5.5±0.7 | 4.4±0.52 | $A_1 = 12.3$ | $P < 0.001$ |
| | | | | $A_2 = 7.3$ | $P < 0.001$ |
| | | | | $A_3 = 7.2$ | $P < 0.001$ |
| LDH (U/L) | 526.5±16.7 | 386.2±26.6 | 184.2±11.9 | $A_1 = 13.7$ | $P < 0.001$ |
| | | | | $A_2 = 7.3$ | $P < 0.001$ |
| | | | | $A_3 = 4.5$ | $P < 0.001$ |

$A_1$ = HbSS vs Control
$A_2$ = Malaria vs Control
$A_3$ = HbSS vs Malaria

ANOVA

$F = P < 0.001$.

## Discussion

Anaemia has been associated with sickle cell disease and malaria (3,4). This is evidence by significant low level of PCV in sickle cell and malaria patient investigated in this study compared to control subjects. The observed significant increase in serum K, Fe and LDH levels in test subject compared with control can be attributed to massive loss of this biochemical substances from intracellular compartment to extracellular compartment, possibly as a result of destruction of erythrocytes. The PCV shows significant inverse correlation with the biochemical parameters investigated ($P < 0.01$) in all study groups indicating that the lower the PCV value, the higher the biochemical values. These evidences shows that these biochemical parameters are good index of haemolysis and also that anaemia in sickle cell patients in crisis and

*Plasmodium falciparum* infected patients is due to excessive haemolysis (haemolytic anaemia). The later is in consonance with previous studies (3, 4, 5, 8).

Table 2: Pearson's correlation of biochemical parameters in each group of subjects investigated.

| | **PCV** | **Fe** | **K** | **LDH** |
|---|---|---|---|---|
| **Control** | | | | |
| PCV | 1.000 | -0.628* | -0.661 | -0.654* |
| Fe | -0.628* | 1.000 | +0.899* | +0.977* |
| K | -0.661* | +0.899* | 1.000 | +0.943* |
| LDH | -0.954* | +0.977* | +0.943* | +1.000 |
| **SS** | | | | |
| PCV | 1.000 | -0.887* | -0.933* | -0.941 |
| Fe | -0.889* | 1.000 | +0.828* | +0.821* |
| K | -0.933* | +0.828* | 1.000 | +0.900* |
| LDH | -0.941* | +0.821* | +0.900* | 1.000 |
| **PF** | | | | |
| PCV | 1.000 | -0.918* | -0.910* | -0.961* |
| Fe | -0.918* | 1.000 | +0.869* | +0.887* |
| K | +0.910* | +0.869* | -1.000 | +0.861* |
| LDH | +0.961* | +0.887* | +0.861* | 1.000 |

*Correlation is significant at the 0.01 level (2-tailed).

The relative significant increase in the K, Fe and LDH values of HbSS patients compared with malaria patient is a possible indication of higher degree of haemolysis in sickle cell compared with malaria patient. This is probably due to fragile red cell of life span 30 – 60 days found in HbSS patients (9,11), which subjected the cells to haemolysis. In malaria on the other hand, only the infected cells were destroyed (12). The same explanation holds for significant low PCV values in HbSS patients compared with malaria patients.

In conclusion, there is an evidence that sickle cell disease and malaria of *P. falciparum* are characterized by the destruction of erythrocytes resulting into massive loss of the red cell content into the extracellular compartment, leading to raised in serum levels of biochemical substances and reduced PCV. These biochemical substances could therefore be a good maker of haemolysis and degree of haemolysis in these patients. The degree of haemolysis in HbSS patients is significantly higher than that of malaria patients. However, both conditions might require blood replacement therapy or other interventions that may avert clinical implications of anaemia resulted from this diseases especially in severe cases.

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