The Reliability of Some Biochemical and Haematological Parameters in Predicting Uncomplicated Malaria Parasite Infection among Children in Anambra State, Nigeria

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Authors’ contributions

This work was carried out in collaboration among all authors. Authors OOP, EJE and OFC designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors OBU and ICA managed the analyses of the study. Authors CUU, AGU, UNP and AMI managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Malaria remains endemic in Sub-Saharan Africa. Haematological and biochemical changes that occur have been suggested as potential predictors of malaria. This study was aimed at evaluating the diagnostic relevance of some haematological and biochemical parameters in predicting malaria in children in Anambra State, Nigeria. A cross-sectional study involving 248 symptomatic and

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1. INTRODUCTION

Malaria has emerged as one of the top 10 killer diseases around the globe. It is the major cause of mortality in various tropical and subtropical regions. Malaria is a life-threatening disease caused by parasites that are transmitted to humans through the bites of infected female *Anopheles* mosquitoes [1]. Five species of the genus *Plasmodium* cause the disease (*P. falciparum*, *P. vivax*, *P. malariae*, *P. ovale* and *P. knowlesi*). *P. falciparum* is the most dangerous parasite that causes malaria and death especially in children less than five years. Between 2000 and 2013, an expansion of interventions helped to reduce malaria mortality rates worldwide by 47% and by 54% in the WHO African Region, where most malaria deaths occur [2]. Substantial reductions occurred as a result of a major scale-up of vector control interventions, diagnostic testing and treatment with artemisinin-based combination therapies (ACTs). An estimated 228 million cases of malaria occurred worldwide in 2018 compared with 251 million cases in 2010 and 231 million cases in 2017. The WHO African Region still bears the largest burden of malaria morbidity, with 213 million cases (93%) in 2018, followed by the WHO South-East Asia Region [3]. Also, in WHO African Region, case incidence levels declined from 294 in 2010 to 229 in 2018, representing a 22% reduction in incidence, although the rate of change also appeared to slow from 2014. According to WHO [4] malaria report, it is a major public health problem in Nigeria where it accounts for more cases and deaths than any other country in the world. Malaria is transmitted all over Nigeria and is a risk for 76% of Nigeria’s population. The remaining 24% of the population live in malaria low transmission areas [5].

It is suggested that haematological and biological changes are the most common complications in childhood malaria infection and may play a major role in fatal complications. These changes include anaemia, cytoadherence of infected red cells, leukocytic changes followed by the induction of cytokines, thrombocytopenia and coagulopathy, particularly disseminated intravascular coagulation [6, 7]. Okeke et al. [8] reported that symptomatic and asymptomatic malaria in children in Anambra State, Nigeria was associated with anaemia and haemoglobinuria. Others reported that haematological and hepatic dysfunction could be an indicator of malaria in endemic regions [9]. This study therefore, was aimed at evaluating the diagnostic relevance of some haematological and biochemical parameters in predicting malaria infection in children in Anambra State, Nigeria.

2. MATERIALS AND METHODS

2.1 Study Area

This study was carried out in Anambra State. Anambra is a State in South-eastern Nigeria made up of twenty-one Local Government Areas with a population of about 4 million comprising of 2,117,984 males and 2,059,844 females (population estimation of 2006) [10]. It is located between Latitude 5° 32’ and 6° 45’ N and Longitude 6° 43’ and 7° 22’E respectively. The study was conducted in thirteen communities and eleven secondary health care outlets purposively selected from thirteen local government areas in Anambra State. These communities have an average of 1 or 2 General
or Comprehensive Health Centres located in them.

2.2 Study Population and Sample

The study population include all the children between the ages of 0-14.9 years in Anambra State, Nigeria. Eighty-two children aged 0 – 14.9 years from the sampled households in the communities and 166 of their counterparts that attended the outpatient clinics of the selected General Hospitals in Anambra State were recruited into the study.

2.3 Sampling Technique

Stratified random sampling was used. All hospitalized or non-hospitalized patients (children 0-14.9 years) with acute febrile illness were randomly sampled two times in a month from April 2012 to March 2013. Fifty homes were randomly sampled by balloting in each of the 13 communities for 12 months.

2.4 Collection of Blood Sample

1 mL. venous blood was obtained from the children in the communities and hospitals after cleaning the site with spirit and put in ethylenediamine tetra-acetic disodium acid (EDTA) vacutainers to avoid clotting and ensure preservation of the samples. Their names, age and sex were recorded against the samples. Blood was sampled from malaria symptomatic and non-symptomatic children aged 0 – 14.9 years.

2.5 Preparation of Thick Films Blood Smears for Microscopy

Thick film blood smears were prepared from the blood samples according to WHO [11]. Large drop of blood samples was deposited at one end of the slide and were spread out evenly with the corner of another slide to a diameter of about 20mm. They were put in distilled water for 10 minutes for dehaemoglobinisation, dried in a flat position to ensure even distribution of blood and stained with Giemsa`s stain for 20 minutes. The stain was washed out with buffered water of pH 7.20 and stood upright to dry in the air, and viewed under x 100 objective (oil immersion) lens. The thick smears were used to confirm the presence or absence of malaria parasite. The asexual forms of the parasite were counted against 200 leucocytes. All children with the asymptomatic or symptomatic *Plasmodium* parasites during household survey were referred to the General hospital or nearest referral Health Centre.

2.6 Biochemical Analysis of Blood Sample

Four ml blood samples were obtained from the sampled children from the different communities and hospitals and put in a cleaned dried test tube. The blood samples were centrifuged for 45 minutes and serum separated into plain clean glass tube for biochemical tests. Parameters determined were: Sodium ion (Na⁺), Bilirubin, Alkaline Phosphatase (ALP), Serum Glutamate Oxaloacetate Transaminase (SGOT), Serum Glutamate Pyruvate Transaminase (SGPT) and Total iron concentration in blood samples of malaria parasitaemic children and controls. They were evaluated using the method of Sood [12].

2.7 Analysis

The SPSS for Windows statistical software version 20.0 was used for data analysis. The presence or absence of *Plasmodium* infection was determined. Data for the different haematological and biochemical parameters were expressed as mean (±SD). Haematological and biochemical parameters of infected and uninfected children from the communities and hospitals were compared using the Student’s t-test.

3. RESULTS

Table 1 summarize the serum levels of some biochemical and haematological indices of malaria-infected and uninfected children in Anambra State, Nigeria. In the community survey (household), infected children had higher mean value of SGOT, SGPT, total protein, bilirubin, total iron and PCV but the differences were not significant (p>0.05). Also, the serum level of all the parameters (with the exception of total iron (ug/dL) and total protein (gm/dL) in the infected children from hospital survey were more elevated compared to the uninfected but the differences were not equally significant (p >0.05).

Table 2 summarize the comparison of the biochemical and haematological indices, and the parasite density of infected and uninfected children from communities and hospitals in Anambra State, Nigeria. All the haematological indices except WBC of hospital and community infection did not differ significantly (P>0.05), but there were significant differences in their Na⁺,
total iron, SGPT and SGOT. To determine the diagnostic value of the different haematological and biochemical parameters, haematological and biochemical parameters for the malaria parasitaemia group were then compared with those of the non-parasitaemia group using the Student’s t-test as shown in Table 3. Ironically, sodium ion, bilirubin and the liver enzymes (ALP and SGOT) of the uninfected children from communities and hospitals also differed significantly (p < 0.05). Also, the PCV, Hb and WBC of the controls in community and hospital managed malaria infection showed no significant difference (p> 0.05). The parasite density of both infected and uninfected children in the hospitals and communities were significantly different (p <0.05).

Table 1. Biochemical and haematological indices of malaria infected and uninfected children from communities and hospitals in Anambra State, Nigeria

| Variables                  | Infected children | Uninfected children | Standard range | P – value |
|----------------------------|-------------------|---------------------|----------------|-----------|
|                            | (n = 38)          | (n =44)             |                |           |
|                            | **Communities**   |                     |                |           |
| AIP (iu/L)                 | 53.03 ± 12.55     | 55.18 ± 12.31       | 20 – 140 iu/L | 0.41ns    |
| SGOT (iu/L)                | 6.67 ± 6.81       | 6.28 ± 5.95         | 8 - 40 µL     | 0.84ns    |
| SGPT (iu/L)                | 6.21 ± 9.38       | 5.09 ± 2.31         | 5 - 35 µL     | 0.45ns    |
| Na⁺ (mmol/L)               | 153.80 ± 52.12    | 145.41 ± 25.60      | 133-155 mmol/L| 0.35ns    |
| Total protein (g/dL)       | 6.57 ± 1.16       | 6.77 ± 0.12         | 6.0 – 8.0 g/dL| 0.33ns    |
| Bilirubin (mg/dL)          | 0.51 ± 0.25       | 0.45 ± 0.16         | 0.02 – 10 mg/dL| 0.16ns    |
| Total iron (µg/dL)         | 389.63 ± 484.09   | 292.4 ± 70.79       | 6 – 150 µg/dL | 0.19ns    |
| PCV (%)                    | 30.26 ± 4.54      | 30.14 ± 5.00        | 25%           | 0.91ns    |
| Hb (gm/dL)                 | 10.21 ± 1.60      | 10.33 ± 1.56        | 10 – 13 gm/Dl | 0.72ns    |
| WBC (mcl)                  | 5131.58 ±1129.00  | 4551.14 ±1005.65    | 4,500 – 10,000mcl| 0.21ns    |
|                            | **Hospitals**     |                     |                |           |
|                            | (n=157)           |                     |                |           |
| AIP (iu/L)                 | 475.23 ± 5110.77  | 75.78 ± 39.29       | 20 – 140 iu/l | 0.815ns   |
| SGOT (iu/L)                | 29.35 ± 28.27     | 23.44 ± 20.0        | 8 - 40 µL     | 0.538ns   |
| SGPT (iu/L)                | 18.79 ± 46.52     | 17.93 ± 17.54       | 5 - 35 µL     | 0.966ns   |
| Na⁺ (mmol/L)               | 249.33 ± 83.50    | 222.56 ± 90.34      | 133 – 155 mmol/L| 0.353ns   |
| Total protein (g/dL)       | 8.99 ± 10.99      | 12.70 ± 17.49       | 6.0 – 8.0 g/dL| 0.334ns   |
| Bilirubin (mg/dL)          | 1.65 ± 10.16      | 1.14 ± 0.74         | 0.02 – 10 mg/dL| 0.882ns   |
| Total iron (µg/dL)         | 221.56 ± 148.04   | 248.76 ± 150.15     | 6 – 150 µg/dL | 0.593ns   |
| PCV (%)                    | 29.49 ± 8.85      | 29.33 ± 5.17        | 25%           | 0.959ns   |
| Hb (gm/dL)                 | 10.24 ± 7.62      | 9.99 ± 1.84         | 10 – 13 gm/Dl | 0.921ns   |
| WBC(mcl)                   | 8527.74 ± 2574.23 | 7355.58 ± 4578.51   | 4,500 – 10,000mcl| 0.208ns   |

ns = No significant difference (p >0.05). * = significant difference (p <0.05). AIP - Alkaline Phosphatase, SGOT - Serum Glutamate Oxaloacetate Transaminase and SGPT - Serum Glutamate Pyruvate Transaminase, Na⁺ - Sodium ion, PCV – Packed cell volume, Hb – Haemoglobin concentration

Table 2. Comparison of the biochemical and haematological indices, and the parasite density of infected children from communities and hospital in Anambra State, Nigeria

| Variables                  | Hospital infection | Community infection | Standard range | P value |
|----------------------------|--------------------|---------------------|----------------|---------|
|                            | (n = 157)          | (n =38)             |                |         |
| ALP (iu/L)                 | 475.23 ± 5110.77   | 53.03 ± 12.55       | 20 – 140 iu/L  | 0.61ns  |
| SGOT (u/L)                 | 29.35 ± 28.27      | 6.67 ± 6.81         | 8 - 40 µL      | 0.00*   |
| SGPT (u/L)                 | 18.79 ± 46.52      | 17.93 ± 17.54       | 5 - 35 µL      | 0.04*   |
| Na⁺ (mmol/L)               | 249.33 ± 83.50     | 153.80 ± 52.12      | 133 – 155 mmol/L| 0.00*   |
| Total protein (g/dL)       | 8.92 ± 10.99       | 24.29 ±109.25       | 6.0 – 8.0 g/dL | 0.08ns  |
| Total iron (µg/dL)         | 220.86 ± 147.82    | 389.63 ± 484.09     | 6 – 150 µg/dL | 0.04*   |
| Bilirubin (mg/dL)          | 1.64 ± 10.16       | 0.51 ± 0.25         | 0.02 – 10 mg/dL| 0.49ns  |
| PCV (%)                    | 29.49 ± 8.85       | 30.26 ± 4.54        | 25%            | 0.60ns  |
| Hb (gm/dL)                 | 10.24 ± 7.62       | 10.21 ± 1.60        | 10 – 13 gm/Dl | 0.45ns  |
| WBC (mcl)                  | 8527.74 ± 2574.23  | 5131.58 ±1129.00    | 4,500 – 10,000mcl| 0.00*   |
| Parasite density           | 93067.87 ± 173752.93 | 70.55 ± 69.22      |                | 0.00*   |
Table 3. Comparison of the biochemical and haematological indices, and the parasite density of uninfected children from hospitals and communities in Anambra State, Nigeria

| Variables       | Hospital (n = 9) | Communities (n = 44) | Standard range | P value |
|-----------------|-----------------|----------------------|----------------|---------|
| ALP (iu/L)      | 75.78 ± 39.29   | 55.18 ± 12.31        | 20 – 140       | 0.01*   |
| SGOT (µ/L)      | 23.44 ± 20.71   | 6.28 ± 9.55          | 8 – 40         | 0.04*   |
| SGPT (µ/L)      | 17.93 ± 17.54   | 5.09 ± 2.31          | 5 – 35         | 0.06ns  |
| Na⁺ (mmol/L)    | 222.56 ± 90.34  | 145.41 ± 25.60       | 133 – 155      | 0.03*   |
| Total protein (g/dL) | 12.70 ± 17.49 | 9.23 ± 11.27        | 6.0 – 8.0      | 0.45ns  |
| Bilirubin (mg/dL) | 1.14 ± 0.74    | 0.45 ± 0.16          | 0.02 – 10      | 0.02*   |
| Total iron (µg/dL) | 284.76 ± 150.14 | 292.43 ± 70.79       | 6 – 150        | 0.18ns  |
| PCV (%)         | 29.33 ± 5.17    | 30.14 ± 4.10         | 25             | 0.66ns  |
| Hb (mg/dL)      | 9.99 ± 1.84     | 10.33 ± 1.56         | 10 – 13        | 0.56ns  |
| WBC (mcl)       | 7355.56 ± 4578.51 | 4551.14 ± 1005.65  | 4,500 – 10,000 | 0.10ns  |
| Parasite density| 0.00 ± 0.00     | 0.00 ± 0.00          |                | 0.00*   |

4. DISCUSSION

Current detection and diagnosis of parasite infections still rely heavily on laboratory methods and/or clinical history, which in most cases is not sensitive. The World Health Organization in the year 2018 recommended that all persons of all ages in all epidemiological settings with suspected malaria should receive a parasitological confirmation of diagnosis. Alternations in haematological and biochemical parameters in malaria infection have been investigated and reported by many researchers [8, 9, 6] but their diagnostic relevance is not completely reliable. As shown in the study, there was no significant difference in some of the biochemical and haematological presentation between infected and uninfected children. However, an elevation of sodium ion above the normal range (hypernatremia) was observed in the malaria infected children. This is in agreement with [7] who confirmed that haematological changes are frequent in *Plasmodium* infection, even if asymptomatic. According to them, an analysis of the obtained data obtained from a rural area of Burkina Faso revealed no statistically significant differences between the asymptomatic infected children and uninfected children in terms of leucocyte, neutrophil, eosinophil or basophil count. WBC count in the body can vary during the different stages of malaria infection. Leucopenia (reduction in WBC) may be common during acute malaria, whereas leucocytosis (increase in WBCs) can occur during severe malaria. Alteration in WBC count has been associated with severity of infection, concurrent infections and response to treatment [13]. However, [6] observed that the mean values of Hgb, Hct and WBC were significantly lower in malaria patients than malaria negatives. Also, Francis et al. [14] reported that haematological parameters of malaria infected patients differed significantly from the uninfected in a study carried out at the University of Calabar Teaching Hospital, Calabar, Nigeria.

Drugs used in the treatment of malaria infection can cause slight elevation or reduction in the serum levels of some biochemical parameters, for example the use of chlorpromazine can cause rise in the level of serum bilirubin [15]; chloramphenicol and fluorides can affect the levels of serum iron (aplastic anemia) [16]. Many drugs such as aspirin, ibuprofen and antibiotics can also affect the levels of serum ALP [17]. Therefore, the type of drugs used at home should be taken into consideration before the determination of the serum levels of these parameters.

Comparison of haematological and biochemical parameters between infected (smear-positive) and uninfected (smear negative) children from homes and hospitals show that there was a significant difference in their WBC. Na⁺, SGOT, SGPT and total iron. To evaluate the diagnostic relevance of these haematological and biochemical parameters in predicting malaria, the same comparison was done on the control group. The non-significant difference in the WBC of the control group in relation to the significant difference in the infected group suggested that changes in the WBC may be as a result of malaria infection. Similarly, Jairajpuri et al. [18] reported a statistical reduction in haemoglobin and total leucocyte count in patient with malaria compared to those without the disease. However, [19] stated that *P. falciparum* uncomplicated malaria does not produce...
significant changes in the total WBC count. Leukocyte changes in malaria are variable and depend on many factors such as acuteness of infection, parasitaemia, disease severity, state of the host immunity to malaria, and concurrent infections, haemoglobinopathy, nutritional status and demographic factors [20-22].

The significant differences in the serum level of Na⁺, SGOT and SGPT in both the infected and uninfected children in hospitals and communities suggested that they may not have been as a result of malaria infection which is in line with [19]. On the contrary, Ali-Salahy et al. [9] stated that there were some significant increases in activities of enzymes aspartate transaminase (AST), alanine transaminase (ALT), and alkaline phosphatase (ALP) among patients with *P. falciparum* malaria which they said are the biomarkers of liver disorders. Although the diagnostic implications of the changes in haematological and biochemical parameters of children with severe malaria infection has been clearly mentioned from prior studies, there is still a lack of complete evidence regarding their diagnostic relevance. Haematological alterations that are thought to characterize malaria may be related to the overt biochemical changes that occur during the asexual stage of the life cycle of the malaria parasite [23]. The result from this study shows that haematological parameters of children with malaria infection, (except WBC) are unreliable indicators for malaria. Adamu and Jigam [24], reported that some of the parameters are useful in the monitoring and management of malaria. The changes observed may be dependent on the parasite species, disease severity (complicated versus uncomplicated malaria), the immune status of an individual (person living in a malaria-endemic region versus person living in a non-malaria-endemic region), and therefore were found to vary from one person to another or from one region to another [19].

5. CONCLUSION

In conclusion, the significant difference in the serum level of Na⁺, SGOT, ALP and SGPT of malaria-infected children from communities and hospitals may not be associated with malaria infection. However, mild changes were observed in the levels of Na⁺, although the differences were not diagnostically relevant because of very low sensitivities. These observations could possibly be explained by a milder biochemical reaction (lower cytokines, mild activation of endothelial cells, and coagulation cascade) that is thought to characterize uncomplicated malaria. Special attention should be applied when interpreting biochemical and haematological parameters in relation to the severity of malaria infection in both asymptomatic and symptomatic infected children living in malaria-endemic areas.

ETHICAL APPROVAL AND CONSENT

Ethical principles according to Belmont report [25] were applied during the survey. Ethical clearance was obtained from the University of Nigeria Teaching Hospital Ituku - Ozalla in Enugu State. Permission was obtained from the management of the selected General Hospitals and informed consent was obtained from the mothers/caregivers before the collection of blood samples. Children who have sickle cell haemoglobin were excluded from the study because of the fact that malaria parasites do not grow well in sickle Hb cells. There were no ethical issues during the collection of samples.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Drugs A-ZPill

IdentifierSupplementsSymptom CheckerDiseasesDictionary

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