

EVALUATION OF TOTAL PROTEIN IN MALARIA PARASITAEMIA

Abstract

Malaria endemicity is most common in the tropical region, continuous transmission still occurs in about 85 countries and regions. The liver is very essential in overall body physiology and it plays a very important role in the life cycle of malaria and its function may be altered due to malaria parasitaemia. This cross sectional study was aimed at evaluating the level of total protein in malaria parasitaemia among children attending Braithwaite Memorial Specialist Hospital, Palmars, Omega Children Hospital, Early Breed Group of Schools, St Francis Nursery and Primary school and Staff Nursery and Primary school in Port Harcourt, Rivers state Nigeria. Of the 1000 subjects within 1-10 years, 694 subjects had malaria parasitaemia while 306 subjects had no malaria and thus made up the control group. Venipuncture technique was used to collect samples for estimation of malaria parasite density and total protein (TP) using biuret method. The results showed low (873.8 ± 30.44), moderate (3248 ± 109.31) and high malaria (24813.8 ± 877.22) densities with TP values of 42.70 ± 0.50 g/l, 43.21 ± 0.60 g/l and 39.64 ± 0.60 g/l respectively. There was a significant difference (P -value < 0.05) in total protein (TP) levels among various groups of malaria densities. This study has shown that the impact of malaria on the liver health vary depending on the level of the infestation.

Key words: Evaluation, Total Protein, Malaria, Parasitaemia

INTRODUCTION

Malaria is endemic throughout most of the tropical region and continuous transmission still occurs in approximately 85 countries and regions [1]. The World Health Organization (WHO) reported about 241 million cases and 627 thousand deaths from malaria in 2020 (WHO, Feb 2022). Malaria cases have increased from an estimated 227 million cases and 558 thousand deaths recorded in 2019. Malaria as a global disease burden has increased from 4.8 percent (previously reported by WHO) to approximately 7.8 percent [1].

Malaria parasite is usually transmitted through the bite of a female *Anopheles* specie mosquito, the bite occurs mainly between nightfall and dawn [2]. There other rare mechanisms for malaria transmission they include congenitally acquired disease, blood transfusion, sharing of contaminated needles, organ transplantation, and nosocomial transmission [3]. Malaria affects both sexes and all ages. More than 95 percent of the disease burden occurs in the African region, 2 percent each occurs in the South East Asian and Eastern Mediterranean regions, the remaining 3 percent occurs in the American and Western Pacific regions [4]. Malarial transmission to the human host is established by sporozoites infection to the liver, sporozoites develops to merozoite which then move into circulation [5]. The symptoms of malaria include Malaria can cause symptoms that include fever, tiredness (fatigue), nausea and headaches. In some severe cases, it can progress to severe anaemia or cerebral malaria, yellow skin as a result of jaundice, seizures, coma or death [6].

The liver is bilobed, the largest organ in the human body, and its situated at the upper part of abdominal cavity. It comprises 2% of an adult's body weight, it has dual blood supply from the portal vein (approximately 75%) and the hepatic artery (approximately 25%) [7]. The liver is very important and has Significant functions which include; metabolism of fat, carbohydrate, protein, iron, hormones and drugs. The liver is helps in storage, blood filtration and synthesis of blood coagulation factors (vascular function). The liver forms and secretes bile, excrete particularly conjugated bilirubin which helps to arrest disease situation. After the liver has broken down harmful substances, the by-products are excreted into the bile or blood. Bile by-products enter the intestine and leave the body in the form of feces. Blood by-products are filtered out by the kidneys, and leave the body in the form of urine. The liver also protects the body from invasion of foreign particles [8]. The liver is very strategic in overall body physiology and any harmful effect will impair its function

The liver plays a very important role in the life cycle of malaria, (pre-erythrocytic and erythrocytic stage) affects the function of the liver, Liver enzymes are normally increased in malaria parasitemia, though the level is dependent on the degree of parasitemia. There's congestion of the liver when malaria parasite invades the liver cells, sinusoidal blockage can also present as the parasite invades the liver cells, destruction of liver cells and cellular inflammation can also occur [9]. As these process happen, the parenchyma aminotransferases, membranous alkaline phosphatase and gamma glutamyl transpeptidase enzymes of the liver starts leaking into circulation gradually, resulting to elevated enzyme activities [10].

Human serum protein have been used widely as a diagnostic tool to monitor the status of infection and diseases, it is made up of albumin and globulin [11]. Albumin is a 66.5 kDa protein, it's has the highest protein concentration in human plasma of approximately 35–52 g/L. The physiological function of albumin is to transport endogenous (bilirubin, hormones) and exogenous (drugs, metals) compounds. Albumin binds the compound, transports the compound into plasma, and then releases it at the target tissue [12]. Albumin is very relevant to liver disease, and can act as biomarker in some clinical issues [13].

The inclusion of liver in malaria cycle puts the liver in the position of liver damage with increased malaria parasitaemia. This can be clinically manifested as jaundice and raised liver transaminases in the laboratory [14].

Studies have demonstrated notable relationship between malaria parasitaemia and liver enzymes leakage, isoenzymes and bilirubin extensively overtime. However, detailed work on total protein levels in malaria parasitaemia is limited, hence the need for this study. Additionally, the aim of this study is to evaluate differences in proteins and albumin level in malaria parasitaemia, and its corresponding liver function.

2.0 MATERIALS AND METHOD

2.1 Study Area

This research is a cross sectional study carried out among children attending Braithwaite Memorial Specialist Hospital, Palmars, Omega Children Hospital and schools (St Francis Nursery and Primary school, Early Breed Group of Schools and Staff Nursery and Primary school) in Port Harcourt, Rivers state Nigeria. Rivers state is located in the south –south geopolitical zone of Nigeria with significant population strength and economic activities.

2.2 StudyPopulation

The study was conducted among 694 children suffering from malaria. They were grouped into three based on the level of malaria parasitaemia; low density parasitaemia, moderate density parasitaemia and high density parasitaemia.

2.3 Eligibility criteria

Inclusion Criteria

Children within 1-10 years who had malaria parasitaemia with no history of liver disease. Control group included children who had no malaria infection and did not have history of any liver disease. Only children whose parents gave informed consent and were attending Braithwaite Memorial Specialist Hospital, Palmars, Omega Children Hospital, St Francis Nursery and Primary school, Early Breed Group of Schools and Staff Nursery and Primary school were included in the study.

Exclusion Criteria

Individuals above 10 years old and children with history of liver disease, children not attending the hospital and schools used for this study and children whose parents didn't give informed consent were all excluded.

2.4 SamplingMethod

Simple random sampling method [15] was used in this cross sectional study among subjects attending Braithwaite Memorial Specialist Hospital, Palmars, Omega Children Hospital, Early St Francis Nursery and Primary school, Breed Group of Schools and Staff Nursery and Primary school. Subjects who met the criteria for inclusion and willingly offered consent were recruited. The recruitment continued until the expected sample size was reached.

2.6 Sample Collection and Analysis:

About 2 ml of venous blood samples were collected using venipuncture technique and was dispensed into heparin bottle meanwhile few drops from the EDTA were used for making thin

and thick films for malaria test and estimating parasitic densities using quantitative buffy coat [16-18]. The blood collected into heparin bottles were centrifuged to obtain plasma needed for total protein estimation using biuret method as described by Okolonkwo et al. (2022a) and Okolonkwo et al. (2022b) [19-20].

2.7 Statistical Analysis

Data collected were analyzed using Statistical Package for Social Science (SPSS) version 24 for ANOVA and Post-hoc. The test was considered significant at p-value less than 0.05.

3.0 Results

Table 1. shows the mean±SD values of TP among the parasitic densities. TP levels were 42.70±0.05 g/l, 43.21±0.60 g/l and 39.64±0.60 g/l in low, moderate and high malaria densities respectively. There as a significant difference (p<0.05) in TP level among the groups.

Table 1.: Mean Parasite Density/μl of blood and the Corresponding Liver Function Parameters

Parameter	Density of Parasite Less than 1000/μl (873.8 ± 30.44)	Density of Parasite >1000 ≤ 9999/μl (3248 ± 109.31)	Density of parasite Greater than 10000/μl (24813.8 ± 877.22)	P-value
TP (g/l)	42.70 ± 0.50	43.21 ± 0.60	39.64 ± 0.60	<0.05

Statistical significance: P < 0.05.

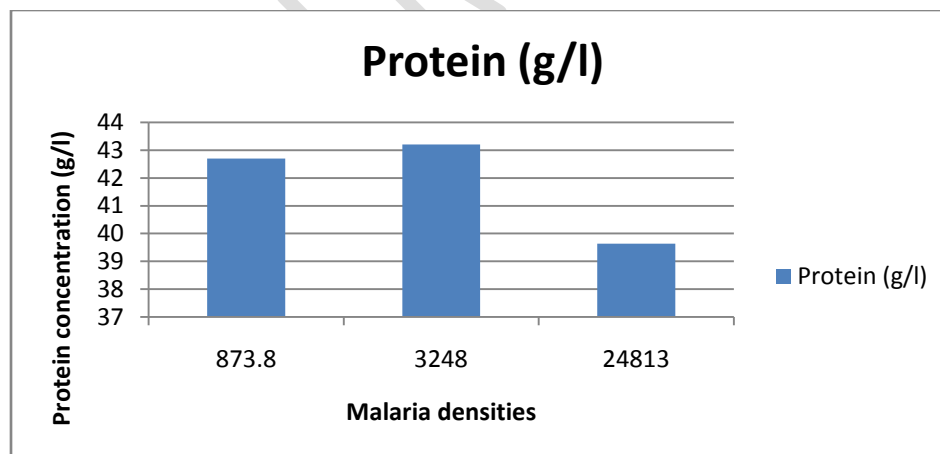


Fig 1 shows the graphical presentation of protein levels in various levels of malarial parasitic densities.

Table 2. shows that there was a significant decrease ($p < 0.05$) in TP levels in high density malaria parasitaemia when compared with moderate and low malaria parasitaemia. The also result shows no notable difference ($p > 0.05$) in TP levels in low as well as moderate malaria parasitaemia densities.

Table 2.: Comparing the Levels of Proteins among the Malaria Densities

Parameters	1+ vs 2+		1+ vs 3+	2+ vs 3+
	(P-value)		(P-value)	(P-value)
Protein (g/l)	>0.05	<0.05	< 0.05	

1+ represents low parasite-density

2+ represents moderate parasite-density

3+ represents high parasite-density

4.0 DISCUSSION

Serum protein levels in malaria parasitaemia were investigated in this study and a comparative analysis was performed to determine if the level of malaria parasitaemia has effect on total protein function since the liver is responsible for protein synthesis and as well as a key component in malaria cycle in human. This study showed that the function of liver can be altered depending on the severity of malaria parasitaemia.

The mean values of protein (g/l) in low, moderate and high parasitaemia was 42.70 ± 0.50 g/l, 43.21 ± 0.60 g/l, 39.64 ± 0.60 g/l respectively. It was observed that parasite density affected the level of subject's total protein. Subjects with high parasite density of $>10000/\mu\text{l}$ had very low level of protein compared to low ($<1000/\mu\text{l}$) and moderate ($>1000 \leq 9999/\mu\text{l}$) parasite density. This result is similar to that reported significant decrease in the level of total proteins [21].

Conversely, this study contradicts the research carried other researchers that reported normal level of protein for low, moderate and high parasite density, describing that it may be as a result of an increase in globulins fraction due to the production of antibodies (IgG, IgM, IgA) against sporozoites, sexual and asexual forms of malaria [22].

During the study, comparison of low density parasitaemia between moderate density parasitaemia of protein level was statistically not significant. However, there's significant difference between low density parasitaemia and high. The result also showed significant difference between moderate parasite density and high parasite density of protein level.

Impairment of hepatic function associated with severe malaria may be responsible for the hypoproteinemia reported in this study [23].

CONCLUSION

This study evaluated total protein levels in malaria parasitaemia. This study has shown that parasite density affected protein level. There was significant difference in total protein levels between low density parasitaemia and high. The result also showed significant difference between moderate parasite density and high parasite density of protein level.

RECOMMENDATION

The parameters assayed in this study are part of the liver function test, however they are not enough to ascertain the function of liver in malaria parasitaemia. The study suggest that a work should be done on the whole liver function test in malaria parasitaemia.

ETHICAL APPROVAL

Before this study commenced, ethical clearance was obtained from the Rivers State Health and Ethics Committee.

Consent

As per international standards, parental written consent has been collected and preserved by the author(s).

REFERENCES

1. World Health Organization, 2021 World malaria report. Accessed on March 20, 2022, from <https://www.who.int/teams/global-malaria-programme/reports/world-malaria-report-2021>.
2. Rhoda Nwalozie , Orevaoghene Evelyn Onosakponome , Brenda AnyakweNnokam and Tamunonengiye-Ofori Lenox-Prince (2022). Evaluation of Malaria Parasitaemia among COVID-19 Patients in Rivers State, Nigeria. *Journal of Applied Life Sciences International* 25(4): 12-18.
3. World Health Organization. 2021 World malaria report. Accessed on March 19, 2022, from <https://www.who.int/teams/global-malaria-programme/reports/world-malaria-report>.
4. Whitten, R., Milner, D.A., Yeh, M.M., Kamiza, S., Molyneux, M.E., Taylor, T. E. (2011). Liver pathology in Malawian children with fatal encephalopathy. *Human Pathology*. 42, 1230-1239.
5. Caraballo, H., King, K. (2014). Emergency department management of mosquito-borne illness: malaria, dengue, and West Nile virus. *Emergency Medicine Practice*. 16 (5): 1–23, 23–4.

6. Kalra, A., Yetiskul, E., Wehrle, C. J. et al. (2022). Physiology of Liver. *TreasureIsland* (FL), Retrieved from: <https://www.ncbi.nlm.nih.gov/books/NBK535438/>
7. Oyebola, D. O. (2002). In *Essential Physiology. National Institute of Horticultural Research*. 1,80-85. Google Scholar
8. Onyesom, I. (2012). Activities of some liver enzymes in serum of *P. falciparum* malarial infected humans receiving artemisinin and non-artemisinin-based combination therapy, *Annals of Biological Research*, 3, (7) 3097–3100.
9. Burtis, C. E., Ashwood, A. & Border, B. (2001). *Tietz Fundamentals of Clinical Chemistry 5th Edition*. Philadelphia, Saunders Company. 748-770.
10. Vavricka, S. R., Burri E, Beglinger, C., Degen L. (2009). Serum protein electrophoresis. An underused but very useful test. *Digestion*. 79(4):203–210.
11. Metzger, A., Gelasius, M., Schankar, A., Ndeezi, G., Melikiang, G., Semba, R., (2001). Antioxidant status and acute malaria in children in Kampala, Uganda. *American Journal of Tropical Medicine and Hygiene* 65 (2), 15-119.
12. Naldi, M., Baldassarre, M., Domenicali, M., Bartolini, M. & Caraceni, P. (2017). Structural and functional integrity of human serum albumin Analytical approaches and clinical relevance in patients with liver cirrhosis. *Journal of Pharmaceutical and Biomedical Analysis*. 144, 138–153.
13. Baheti, R., Laddha, P., & Gehlot, R. S. (2003). Liver Involvement in falciparum malaria histopathological analysis,” *Journal, Indian Academy of Clinical Medicine*, 4(1), 34–38.
14. World Health Organization. (2010) WHO guidelines on drawing blood: best practices in phlebotomy. *World Health Organization*. <https://apps.who.int/iris/handle/10665/44294>.
15. Onosakponome, Evelyn Orevaoghene , Nyenke, Clement Ugochukwu , Abah, Austin Edache and Okafor Roseanne Adah (2022) Prevalence of Plasmodium falciparum Malaria among Children Residing in Urban and Peri-urban Settlements in Rivers State. *Journal of Advances in Microbiology*, 22(4): 1-7
16. Monica Cheesbrough. (2010) *District Laboratory practice in Tropical Countries part 2* edition. Cambridge University Press, New York pp 320- 329.
17. Michael Wogu and Evelyn O Onosakponome (2021): Evaluating Prevalence and Misdiagnosis of Plasmodium Using Microscopy Compared With Polymerase Chain Reaction Technique in Two Tertiary Care Hospitals in Rivers State, *Nigeria International journal of infection*, 8(1):e109411.

18. Okafor, R.A., Amadi, F. C., Okolonkwo, B. N., Nyenke, C. U. and Okeke, C. U. (2022). Implication of Malaria on Liver Health. *Merit Journal of Microbiology and Biological Sciences*, 10(4), 40-45
19. Okolonkwo, B. N., Chukwubike, U. O. Amadi, C. F. and Nyenke, C. U. (2022a). Therapeutic Effects of Vitamin E on Paraquat Induced Liver Toxicity in Male Albino Rats (*Rattus Norvegicus*). *Journal of Advances in Medicine and Medical Research*, 34(17),1-7
20. Okolonkwo, B. N., Amadi, C. F., Chikwubike, U. O. and Nyenke, C. U. (2022b). The Comparative Effect of Vitamin E + C on the Chronic Toxicity of Paraquat in Albino Rats (*Rattus norvegicus*). *European Journal of Medicinal Plants*, 33(6), 7-13
21. Burtis CA, Ashwood ER, editors. Tietz Textbook of Clinical Chemistry, 2nd ed. Philadelphia, PA: WB Saunders; 1999:2204–5.
22. Mohammed, H.T., AbdElkarim, A., Abdrabo, Adham, A. A, Ammar, O.M. (2019). Evaluation of Liver Function Tests among Sudanese Malaria Patients. *Sudan Medical Laboratory Journal*, 6, 1858-6147
23. Nsonwu-Anyanwu, A. C., Edmund, R. E., Opara Osuoha, U. O., Inyang-Etoh, P. C., Offor,S. J., Usoro, A.O.C. (2017). Falciparum malaria associated changes in biochemical indices in children. *Journal of Medical and Allied Sciences* ,7(1):29-33. <https://doi.org/10.5455/jmas.253029>