Serum C-Reactive Protein And Serum Uric Acid As Prognostic Markers In Malaria At Western Odisha

Purna Chandra Karua[1], Manoj Kishan [2]

[*] M.D, Medicine, Associate professor, Department of General Medicine, VSS Institute of Medical Science And Research (VIMSAR), Burla (Odisha).
[**] Assistant Professor, SLN Medical College & Hospital, Koraput, Mob.9437660237

Abstract- **Background:** Malaria causes high mortality and morbidity in various tropical regions which suggest us to identify severity of this disease as early as possible so as to institute timely therapy and avoid the complications. As per world malaria report 2019, an estimated 228 million malaria cases were reported across the world in 2018. The number of malaria deaths in India also decreased by 40 percent in 2018.

Objective: To find relation of Serum CRP & Serum Uric Acid level with prognosis of malaria.

Methods: The study included patients suffering from malaria (P. vivax and/or P. falciparum) admitted to General Medicine Ward of VIMSAR, Burla. Inclusion criteria of the study is slide microscopy or QBC or ICT and patients aged $\geq$ 15 years of age of both sexes. Laboratory diagnosis done: Peripheral blood smear examination, Quantitative Buffy Coat (QBC), Non-Microscopic methods: Immunological methods, Serum Uric Acid estimation and Serum C-reactive protein estimation with other routine examination.

Result : In the present study total 55 patients were included, out of which male to female ratio is 1.6:1. Out of which 55% patients were complicated malaria cases and 45% patients were uncomplicated malaria cases. The more number of complicated malaria is contributed by the illiteracy and lack of awareness of the people about the disease and its complications. Uric Acid derived from Plasmodium hypoxanthine would promote crystallization within the local environment and inflammation plasmodium falciparum-derived UA also contributes to the secretion of inflammatory cytokines, such as TNF-α, IL-1β, IL-6 by peripheral blood mononuclear cells which increases the inflammation. Thus, measurement of CRP and Uric Acid can be useful in understanding the pathogenesis of severe malaria.

Conclusion: CRP and UA can be considered a new, cost-effective, and reliable tool in assessment of prognosis of malaria.

Index Terms- malaria, CRP, uric acid, morbidity, mortality

I. INTRODUCTION

Malaria is a protozoan diseases caused by infection with plasmodium parasite which is transmitted by the bite of infected female anopheles mosquitoes. It is transmitted in 108 countries containing 3 billion people and causes nearly 1 million deaths each year, plasmodium flaciparum being the most deadly parasite species infecting humans.[1] It remains a major cause of morbidity and mortality worldwide. Malaria continues to pose major public health problem is state of Odisha. It has only 4% of land area and 3% of population of India. In 2010, Odisha contributed 20% of cases and 17% of deaths due to malaria to the country's burden. Around 85% of the cases reported from the State are due to P.falciparum malaria. Female Anopheles mosquito is the vector of malaria. Five species of Plasmodium parasites are there. They are Plasmodium falsmodium vivax, Plasmodium ovale, Plasmodium malariae, and Plasmodium knowlesi.[1]

The pathogenesis of P. falciparum malaria is complex, involving multiple parasite and human factors that, in combination, produce varying levels of immune stimulation and microvascular inflammation. While the degree of inflammation generally correlates with the severity of a malaria episode, the parasite factors that elevate host inflammatory responses from beneficial to pathological levels are not well characterized.

Uric acid (UA) is produced in humans and higher primates as the final product of purine metabolism. Its biosynthesis is catalyzed by xanthine oxidase, which produces reactive oxygen species (ROS) as by-products. Plasmodium elicits host inflammatory responses that causes the symptoms and severe manifestations of malaria. C-Reactive Protein is an acute phase protein that is involved in the activation of complements.[2] Its secretion is induced by pro-inflammatory cytokines that are secreted by host mononuclear cells in malaria.[3,4] It binds to the infected RBCs and help in their clearance. This immune activation toward infected RBCs also result in various deleterious effect.[5,6] Thus, measurement of CRP & Uric acid level can be useful in understanding their role in malaria. In VIMSAR, Burla, in daily duties we encounter a number of cases of malaria and thus, we undertake a prospective observational study among the patients admitted with malaria.

II. MATERIALS & METHODS

The patients having fever with or without features of complications of malaria, like altered sensorium, coma, convulsion, oliguria, icterus, bleeding, acidotic breathing, shock etc. are taken in to consideration and screened for presence of P.falciparum and/or P. vivax by above mentioned tests. Once malaria is confirmed, then other diseases are excluded by proper history taking, clinical examination, and investigations. And after exclusion of other diseases, the cases are included in the study. Laboratory Diagnosis of Malaria :

1. Peripheral blood smear examination

Blood films prepared directly from capillary blood. Best time for collection of blood sample is the midway between paroxysms of chills and fever, when the greatest numbers of intracellular organisms are present. It may be necessary to take repeated films.[7] two types of blood films are used : (a) Thin smears (b) Thick smears.

Thin Smear :

One end of the slide is allowed to touch the top of the blood drop on the patients' finger. The spreader is held at 45 degrees in contact with the drop of blood and the smear is prepared with quick uniform movement such that the margins of the film do not extend to the sides of the slide, and the tail ends near the center of the slide. The film is dried in air.[7]

Thick smear :

Usually 2 to 3 drops of capillary blood from finger prick are directly placed on a clean glass slide, and spreaded with a needle or with the corner of another slide to form an area of about 1 cm^2. The film is dried in air. The thick film is dehemoglobinised by placing it vertically in distilled water in a glass cylinder for 5-10 minutes, and then dried in air in upright position.[8]

Staining :

Leishman's stain is used for staining of the smear.[9]

2. Quantitative Buffy Coat (QBC):[10]

This technique works on the principle of differential centrifugation of blood. Infected red cells appear to be less dense than unifected ones, and concentrate within a small 1-2mm region near the top of the RBC column, i.e. the buffy layer.

The QBC tube is a specially prepared glass hematocrit tube, pre-coated internally with acridine orange and anti-coagulant potassium oxalate. It has a separating plastic float, which occupies 90% of the interior of the lumen of the tube.[10] The parasites present in the buffy layer are held close to the wall by the plastic float which has the same specific gravity as that of the buffy layer.

Upon centrifugation, whole blood separates into plasma, buffy coat and packed red cell layer. The float gets buoyed by the packed blood cells and is automatically positioned within the buffy coat layer.

Blood cells in the buffy coat layer separate according to their densities, forming discrete bands. Platelets remain at the top, lymphocytes and monocytes in the middle, and granulocytes in the bottom, within the buffy coat. Acridine Orange stains the malaria parasite green (nucleus) and orange (cytoplasm).[11]

The advantages of this method are :

1. Examination time is short, hence can be used to screen large number of blood samples.
2. It is technically easy to perform.
3. The sensitivity is very high. Low levels of parasitemia can be easily detected, as more blood is being used per sample (55μl).[12]
4. It does not require immunological reagents.[13]
5. It can also detect other parasites in blood (Filaria).[14]

Serum Uric Acid Esimation : [15]

An automated colorimetric procedure is used for the determination of serum uric acid with improved specificity that is by enzymatic method (uricase peroxidase) with normal value in the range 3-7.9 mg/dl.

Serum C-Reactive Protein Estimation : [16]

Semi-quantitative estimation of CRP was done by agglutionation method with commercially available CRP latex test kit with normal value ≤ 6mg/L. fresh serum obtained by centrifugation of clotted blood is preferred. The sample may be stored at 2-8 degree celcius for 48 hours before performing the test. For longer periods of time the serum must be frozen. Haematic, lipaemic or contaminated serum must be discarded. Other routine investigations: Hb%, TLC, DC, TPC, Blood Urea Nitrogen, S. Creatinine, S. Billirubin, Serum Transaminases, Urine etc done.

Statistical Analysis:

Standard statistical methods were applied, and the analysis performed in Microsoft Excel. Chi square test was applied for qualitative data, and Unpaired student's t-test was applied for numeric data. Level of statistical significance was taken as $p<0.05$.

III. RESULT & ANALYSIS:

C-reactive protein is thought to have pathogenic role in malaria and it correlates with the complications in malaria. It binds to plasmodium infected erythrocytes and helps in their clearance by both humoral and celluar immune mechanisms. Also, CRP activates complement pathway and platelet activation, and results in various untowards effects.

Similarly, Uric Acid has recently emerged as an important mediator of malaria-induced inflammation. Plasmodium infected erythrocytes accumulate hypoxanthine, a precursor of UA. Imported hypoxanthine is not degraded in to UA within the erythrocyte, since xanthine dehydrogenase activity, which converts hypoxanthine in to UA, has not been detected in this cell type or in the Plasmodium parasite. However, upon erythrocyte rupture and which is normally present in the blood, crystal and whose expression is increased during Plasmodium infection, will efficiently degrade in to UA.

In this study total 55 patients were included, out of which male to female ratio is 1:6:1. Out of which 30 patients were complicated malaria cases and 25 patients were uncomplicated malaria cases. 13 patient died out of 55 malaria patient that accounts for 23% mortality. (Table-1) And out of 13 patients died 11 died of complicated malaria with multiorgan failure.

**Table – 1: Demographic and clinical profile of the patients**

Parameters	Uncomplicated malaria	Complicated malaria	Total
Male	14	20	34 (61.8%)
Female	11	10	21 (38.2%)
No. of patients survived	23	19	42 (76.4%)

No. of patients died	02	11	13 (23.6%)

Out of 55 patients with uncomplicated and complicated malaria 30 patients (54%) had raised CRP, among which 22 patients (73.%) were complicated malaria and 8 patients (27%) were uncomplicated malaria. So, in this observational prospective study, we found significantly high CRP levels in malaria patients. The CRP level is more in complicated malaria as compared to those in uncomplicated malaria cases. Similarly out of 17 patients who had raised uric acid level, 14 patients (82.4%) were complicated malaria and 03 patients (17.6%) were uncomplicated that is Uric Acid is raised in more number of patients of complicated malaria in which p value is <0.00014 (highly significant).

Out of 13 death patients, 11 patients (85%) had raised CRP level and among them 10 patients (91%) were complicated malaria. So from the above sentence we find significantly high level of CRP in patients who died as a result of malaria, compared to those who survived. This finding is in agreement with previous studies done at West Bengal, India.[5] Similarly, Fig. 1 shows the serum uric acid level is found to be significantly high in complicated malaria compared to uncomplicated malaria. This is in agreement with study done by Tatiana M. Lopera-Mea et al on Malian children.[17] In our study, we found that uric acid level is also high in patients who died of malaria, compared to those who survived.

Fig. 1: CRP and Uric Acid level in relation to malaria
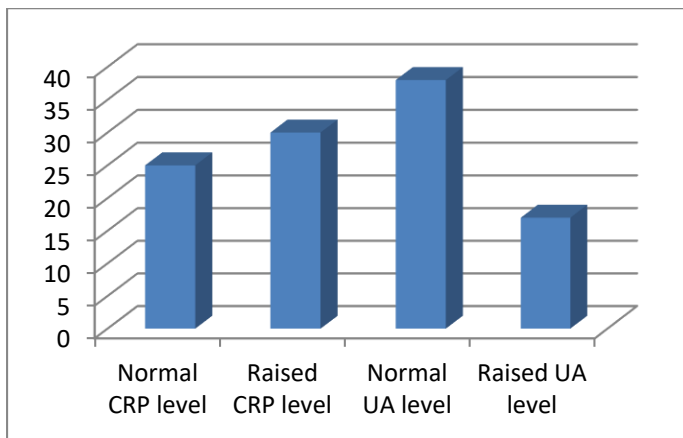
From table 2, we found mean level of UA in complicated malaria to be 7.61 mg/dl and uncomplicated malaria to be 5.21 mg/dl. A similar study conducted by Tatiana M. Lopera-Mesa also found similar result but in their study they found comparatively lower mean value as compared to our study, that is, in complicated malaria they found mean serum UA level as 5.69 mg/dl and in Uncomplicated malaria as 4.60mg/dl.[17]

Table-2: UA mean value in complicated and uncomplicated malaria

Parameters	Uncomplicated malaria mean (mg/dl)	Complicated malaria mean (mg/dl)
Males	6.65	7.43
Female	4.73	7.97
Total	5.21	7.61

From table 3, we found mean level of CRP in complicated malaria to be 23.71 mg/dL and uncomplicated malaria to be 10.77mg/L. That means it is more raised in complicated malaria as compared to that in uncomplicated malaria (p<0.0423).

Table 3 : CRP mean value in complicated and uncomplicated malaria

Parameters	Uncomplicated malaria mean (mg/dl)	Complicated malaria mean (mg/dl)
Males	5.42	25.84
Female	17.59	19.46
Total	10.77	23.71

Similar studies done by Bhavita Patel et al.[6] and Vandana Agrawal et al.[3] also found similar result but they found higher level of Serum CRP as compared to our study in both complicated and uncomplicated malaria.

IV. SUMMARY & CONCLUSION

To summarize, the serum level of CRP and UA are increased in malaria patients. And their value was more in complicated malaria as compared to uncomplicated malaria which indicates that it correlates with the severity of the disease. Hence, early estimation of serum CRP and UA can be done to know the prognosis of malaria.

Case fatality rate in this study was 23.6%. Incidence was higher in males than in females. The male to female ratio was 1.6:1. Out of 55 patients 30(55%) patients have raised serum CRP level from which 22 (73%) were complicated malaria and 8 (27%) were uncomplicated malaria. Serum UA level were increased in 17 (31%) patients out of 55 patients, from which 14 (82%) were complicated malaria and 3 (18%) were uncomplicated malaria. Out of 13 death patients 11 patients (84.6%) had raised CRP level and among them 10 patients (91%) were complicated malaria. Out of 13 death patients 8 patients (61.5%) had raised UA level and among them 7 patients (87.5%) were complicated malaria. The mean value of UA in complicated malaria is 7.61 mg/dl and uncomplicated malaria is 5.21 mg/dl. The mean value of CRP in complicated malaria is 23.71 mg/L and uncomplicated malaria is 10.77 mg/L.

Malaria is a protozoan disease whose incidence is high worldwide including India causing high mortality. We analyzed how serum CRP and serum UA levels correlates with the severity of the disease. The higher levels of serum CRP and UA in complicated and uncomplicated malaria suggest their definite relation with the disease. But whether this high levels of serum CRP and UA is contributed by the disease or they are giving rise to the severity of the disease need to be ascertained. That is, the role of serum CRP and UA in the pathogenesis of malaria is to be determined which require further studies.

A larger study, involving more inflammatory biomarkers and including serial measurement of these parameters would better clarify their role, either singly, or in combination, in malaria.

REFERENCES

[1] Fauci AS, Kasper DL, Longo DL, Braundwald E, Hauser SL, Jameson JL, et al. Malaria in: Osler W, editor. Harrison's Principles of Internal Medicine. 19th Ed.

[2] McGuire W et al. C-reactive protein and haptoglobin in the evaluation of a community-based malaria control programme. Trans R Soc Trop Med Hyg 1996;90:10-14.

[3] Agrawal Vandana, Jain Vaishali, Biswas Shubho. Evaluation of C-relative protein as a biochemical marker for assessing disease severity in Malaria. IOSR Journal of Dental and Medical sciences (IOSR-JDMS).2013;8(2):23-26.

[4] Harpaz R, Edelman R, Wasserman SS, Levine MM, Davis JR, Sztein MB 1992; Serum cytokine profiles in experimental human malaria. Relation to protection and disease course after challenge. J Clin Invest 90:515-523.

[5] Paul R, Sinha PK, Bhattacharya R, Banerjee AK, Raychaudhuri P, Mondal J. Study of C-reactive protein as a prognostic marker in malaria from eastern India. Adv Biomed Res. 2012;1:41.

[6] Chhatriwala M, Patel B, Shah R, Seikh N, Gokani R, Nilawar A.; Prognostic value of serum C-reactive protein in Malaria. ISSN:2229-3809 (online).

[7] Chakraborthy P. Plasmodium and Babesis. In: textbook of Medical Parasitology. 2nd ed., 2010, Pg. 83-104.

[8] Bain BJ, Lewis SM, Bates I. Basic Hematological Techniques. In: Lewis SM, BB, Bates I, editors. Dacie and Lewis Practical Hematology, 10th ed., New York: Churchill Livingstone; 2006. P. 25-8.

[9] Thakor HG. Laboratory Diagnosis of Malaria. J Ind Med Assoc 2000;98(10): 623-7.

[10] Estacio RH, Edwin ER, Cresswell S, Coronel RF, Alora AT; The Quantitative Buffy Coat (QBC) Technique in Early Diagnosis of Malaria: The Santo Tomas University Hospital Experience. Phil J Microbial Infect Dis 1993;22(2):56-9.

[11] Kakkilya BS. Malaria Site- All about malaria. (Monograph on the internet) India; 2009: Available from : URL:_http://www.malariasite.com/

[12] Solanki RL, Kumawat BL, et al; Pathology of malaria with special references to hematological changes in malaria: Indian J Pathol Microbial. 1996, Dec;39(5): 391-414.

[13] Rickman LS, Long GW, Oberst R, Cabanban A, Sangalang R. et.al. Rapid diagnosis of malaria by acridine orange staining of centrifuged parasites: Lancet Jan 1989;1(8629):68-71

[14] Moody A, Rapid diagnostic tests for malaria parasites. Clin Microbial Rev, Jan 2002;15(1):66-78.

[15] Nathan Gochman and Joan M. Schmitz; Automated Determination of Uric Acid, with use of a Uricase-Peroxidase system.

[16] Hayashi, H., and Loggrippo, G.A., H. Ford Hosp. Med. J 20:90 (1972).

[17] Tatiana M. Lopera-Mesa, Neida K. Mita-Mendoza, Diana L. van de Hoef, Saibou Doumbia, Drissa Konate, Mory Doumbouya, Wenjaun Gu, Karim Traore, Seidina A,S Diakite, Alan T. Remaley, Jennifer M. Anderson, Ana Rodriguez, Michael P. Fey, Carole A.Long, Mohamadou Diakite, and Risk M.Fairhurst. Plasma Uric Acid Levels Correlate with inflammation and disease severity in Malian children with Plasmodium Falciparum Malaria. Plos One. 2012;7(10)

AUTHORS

First Author – Purna Chandra Karua, M.D, Medicine, Associate professor, Department of General Medicine, VSS Institute of Medical Science And Research (VIMSAR), Burla (Odisha).
Second Author – Manoj Kishan, Assistant Professor, SLN Medical College & Hospital, Koraput, Mob.9437660237

Correspondence Author – Purna Chandra Karua Mob.no.9437202869.e-mail-karuapcvss@gmail.com.