Original Article

DOI: 10.7860/JCDR/2021/47183.14636

Clinical Significance of Hypocalcaemia in Malaria: An Experience from Coastal Karnataka, India

Internal Medicine Section

AVINASH N SHETTY[1], AKSHATHA RAO AROOR[2], ARCHANA BHAT[3]

ABSTRACT

Introduction: Malaria is a major public health problem leading to significant morbidity and mortality in endemic regions. Malaria is a major public health problem in India, which contributes significantly to the overall malaria burden in Southeast Asia. Data on significance of hypocalcaemia in malaria is scarce.

Aim: To correlate hypocalcaemia with the complications in malaria.

Materials and Methods: This cross-sectional, observational study was conducted at Father Muller Medical College in Coastal Karnataka between January 2018 to June 2019. Patients diagnosed as malaria by peripheral smear were included. Serum calcium level was measured in all the patients and calcium levels <8.4 mg/dL was considered as hypocalcaemia. This was correlated with the complications such as jaundice, acute kidney injury, cerebral malaria, shock, multiorgan dysfunction and Acute Respiratory Distress Syndrome (ARDS) in malaria. Data were analysed using frequency, percentages, Chi-square test, student t-test, relative risk estimation and Receiver operating Characteristic (ROC) analysis.

Results: A total of seventy five patients with malaria were included in the study. Majority were in the age group of 21-30 years (30.6%), with a male predominance (53 patients, 70.67%). Among these patients, complications were documented in 18 (24%) of them. Hypocalcaemia was seen in 25 patients (33.3%) among whom 13 (52%) had complications. The association of hypocalcaemia with the complications was statistically highly significant (p=0.001). Patients with hypocalcaemia had a relative risk of 9.75% (2.901-32.766, 95% CI) for developing complications. With ROC analysis, the calcium value cut-off for complications was 8.25 with a sensitivity of 72% and specificity of 86%. Calcium reverted to normal in all patients after recovery from complications.

Conclusion: Hypocalcaemia had a significant association with high specificity for complications in malaria.

Keywords: Calcium, Falicparum, QT prolongation, South India

INTRODUCTION

Malaria is a major global public health problem in tropical countries [1]. It accounts for an estimated 216 million infected cases and approximately 445,000 deaths in the year 2016 [2]. Malaria morbidity blunts the development of a country as a result of shortage of manpower [3,4]. India, being the hub of many endemic regions for malaria accounts for nearly 70% of the total reported cases in Southeast Asia [5-7]. Mangaluru city, situated along the coastal area of Arabian Sea in Karnataka state, South India, is endemic to malaria. The city has a warm and humid climate with high rainfall during monsoon seasons. This region has high vector density and is disposed to efficient malaria transmission. The relative proportions of Plasmodium vivax and *Plasmodium falciparum* infections in this region are ~80% and ~20%, respectively [8].

Malaria is a highly complex disease with protean manifestations. Prolonged infections can lead to severe anaemia, metabolic acidosis, haemoglobinuria, splenomegaly, hepatomegaly, and other severe illnesses. P.falciparum infection is known to cause single and multiorgan related fatal conditions, including cerebral malaria, renal failure, hepatic dysfunction and failure, and ARDS [9].

Hypocalcaemia has established itself to correlate with the complications in this parasitic disease [10]. The mechanism of hypocalcaemia in malarial disease has not been explained, but various theories have been postulated. The plasmodium is found to use calcium signalling pathway and it increases the RBC's permeability to calcium. This then increases the calcium influx and thus reduces calcium levels [11]. There is inadequate data supporting the role of hypocalcaemia in evaluating the severity of the disease [12].

Due to the high incidence of this parasitic disease in Mangaluru and the scarce data on the clinical significance of hypocalcaemia in malaria, this study was done to correlate hypocalcaemia with the complications in malaria.

MATERIALS AND METHODS

This was a cross-sectional, observational study conducted in a tertiary care centre from January 2018 to June 2019. The study was approved by Father Muller Institutional Ethics Committee (FMMCIEC/CCM/09/2018). A written informed consent was obtained from all the patients participating in the study. All adult patients ≥18 years of age, presenting with acute fever (oral body temperature >38°C) diagnosed to have malaria by peripheral smear and admitted to the medical wards and intensive care unit were included in the study.

The study excluded patients on calcium supplementation, drugs causing hypocalcaemia and those having pre-existing kidney or liver disease. Sample size was calculated using the formula

$$\frac{Z\alpha^2\, p(1-p)}{e^2}$$

Zα=1.96 at 95% Confidence Interval; p=17/60=28% [10]; e=10%. Hence, a sample size of 75 was considered.

A detailed history with a special importance to the complications was taken followed by a thorough general physical examination and a systemic examination to document the findings in a structured proforma, which included the symptomatology, type of malaria, various complications, necessary laboratory data such as haemoglobin, platelet count, liver and renal functions serum electrolytes, chest X-ray and arterial blod gas analysis.

Patients were classified as having complicated malaria if they met atleast one of the following criteria as per World Health Organisation (WHO): Hypoglycaemia (<40 mg/dL), Metabolic acidosis (HCO_3 <15 mmol/L or Lactate >5 mmol/L with rapid, deep and labored breathing), Haemoglobin <7 g/dL or Haematocrit <20%, significant

bleeding including recurrent or prolonged bleeding from nose, gums or venepuncture sites; hematemesis or melaena, prostration (Generalised weakness so that a person is unable to sit, stand, or walk without assistance). Jaundice: Serum bilirubin >3 mg/dL, Renal failure (S. Creatinine >3 mg/dL or S.Urea >60 mg/dL), Pulmonary oedema (Radiological, oxygen saturation of <92% on room air, respiratory rate >30/min, severe chest indrawing and crepitations on auscultation), Shock (Systolic BP <80 mm of Hg with cold peripheries and prolonged capillary refill time) and impaired consciousness with Glasgow coma scale <11 [13].

All the patients underwent confirmatory test for malaria using peripheral smear. Other routine tests included complete blood count, renal function tests, blood sugar levels, liver function tests, chest X-ray and standard 12 lead electrocardiogram.

Serum calcium level was estimated using NM-BAPTA technique [14] and blood samples were obtained without using tourniquet. Patient were classified as having hypocalcaemia if serum calcium level is <8.4 mg/dL (The normal range of serum calcium considered as 8.4-10.2 mg/dL) [15]. Serum albumin was estimated and corrected calcium was calculated using the formula S.Ca+{(4 -S.Albumin) × 0.8}. Repeat serum calcium was done in patients who recovered from complications and was correlated accordingly. QTc prolongation was calculated using the formula (QT/√RR). The patients diagnosed with malaria were grouped into complicated or uncomplicated, and the calcium levels were compared among the two groups.

STATISTICAL ANALYSIS

Descriptive statistics were used to define characteristics of the study variables by showing mean, dispersion (standard deviation) and distribution by showing counts and percentages. Chi-square test and student t-test were used to establish the relationship between calcium levels, QTc prolongation and complications. A conventional p-value <0.05 was the rejection criteria for the null hypothesis and was considered significant. Sensitivity/specificity and Receiver Operating Characteristic (ROC) curve analysis was done to determine the relationship between calcium levels and complications. This study was analysed using SPSS version 23.

RESULTS

During the study period, a total of 75 patients who met inclusion criteria were included in the study. In this study, majority of the patients were young (21-30 years) with a mean age of 38.4±8.2 years [Table/Fig-1]. In the present study 53 cases (70.7%) were males and 22 cases (29.3%) were females, with a M:F ratio of 2.4:1. Among the patients, majority had vivax malaria (41 patients, 54.6%) followed by falciparum (30 patients, 40%) and mixed (4 patients, 5.3%).

Age distribution (Years)	Number of patients (%)
18-20	16 (21.33%)
21-30	23 (30.67%)
31-40	6 (8.00%)
41-50	12 (16.00%)
51-60	10 (13.33%)
61-70	5 (6.67%)
71-80	3 (4.00%)
Total	75

[Table/Fig-1]: Age distribution of the patients.

In the present study, 37 patients (49.3%) had low serum calcium levels on admission. After calculating the corrected calcium, hypocalcaemia was present among 25 patients (33.3%). The association of age and hypocalcaemia was not statistically significant with a p-value of 0.691 by Chi-square test. Among the patients with hypocalcaemia, majority of them had *P.vivax* malaria. However, this was not statistically significant with a p-value of 0.320 [Table/Fig-2]. The laboratory profile of patients is shown in [Table/Fig-3].

Malaria parasite smear	Ca level				Total	
	<8.4 (mg/dL)		8.4-10.2 (mg/dL)			
	Count	%	Count	%	Count	%
Falciparum	11	37.9	18	62.1	29	100
Mixed	0	0	4	100	4	100
Vivax	14	33.3	28	66.7	42	100
Total	25	33.3	50	66.7	75	100

[Table/Fig-2]: Serum calcium levels in different types of malaria.
Chi square test p=0.320, Not significant

Parameter	Number of patients (N=75)
Haemoglobin	
<7 gm/dL	0 (0%)
7-10 gm/dL	10 (13.3%)
10-12 gm/dL	35 (46.6%)
>12 gm/dL	30 (40%)
Platelet count	
<1,00,000/mm^3	40 (53.3%)
1,00,000-1,50,000/mm^3	22 (29.3%)
>1,50,000/mm^3	13 (17.3%)
Serum creatinine	
>3 mg/dL	1 (1.3 mg/dL)
1.3-3 mg/dL	6 (8%)
<1.3 mg/dL	68 (90.6%)
Serum bilirubin	
>3 mg/dL	8 (10.6%)
≤3 mg/dL	67 (89.3%)
AST	
>45 U	20 (26.6%)
10-45 U	55 (73.3%)
ALT	
>45 U	22 (29.3%)
10-45 U	53 (70.6%)

[Table/Fig-3]: Laboratory profile of study participants.

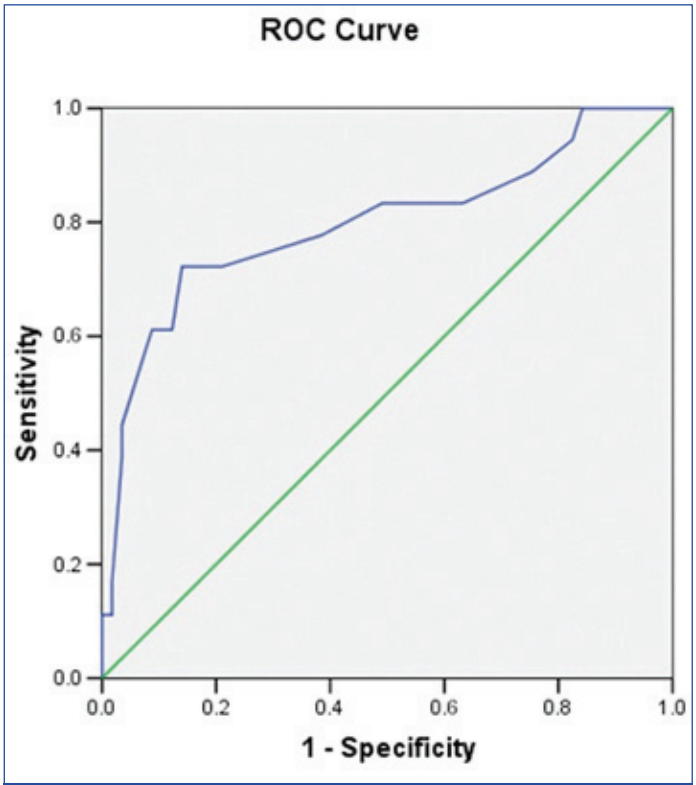
In the present study, 18 (24%) of the cases had complications. Among the 25 patients who had hypocalcaemia, 13 (52%) of them had complications during their illness [Table/Fig-4]. It was statistically significant with a p-value of 0.001 by fisher's-exact test. Hypocalcaemia had a relative risk estimate of 9.75% (CI:2.901%-32.766%) for developing complications. With ROC analysis, the calcium value cut-off for complications was 8.25 with a sensitivity of 72%, specificity of 86%, Positive Predictive Value (PPV) of 61.9% (CI:41.1%-82.7%), Negative Predictive Value (NPV) of 90.7% (CI:83%-98.5%), likelihood ratio of 5.146 (CI:2.546-10.399) and accuracy of 82.7% (CI:74.1%-91.2%) with AUC of 0.797 [Table/Fig-5].

Complications	Calcium <8.4 mg/dL (N=25)	Calcium 8.4-10.2 mg/dL (N=50)
No complications	12 (48%)	45 (90%)
Acute kidney injury	1 (4%)	0 (0%)
Acute respiratory distress syndrome	1 (4%)	0 (0%)
Cerebral malaria	1 (4%)	0 (0%)
Haematuria	1 (4%)	0 (0%)
Jaundice	3 (12%)	5 (10%)
Multiorgan failure	1 (4%)	0 (0%)
Shock	5 (20%)	0 (0%)

[Table/Fig-4]: Association of serum calcium levels with complications.
Fishers exact test p=0.001, Highly significant

In the present study, only a small subset of 4 cases (5.3%) infected with faciparum had abnormal ECG changes in the form of QTc

prolongation. All these patients also had hypocalcaemia. The association of hypocalcaemia and QT prolongation was highly significant with a p-value of 0.001 by t-test. All the patients with QT prolongation had complications and this was statistically significant with a p-value of 0.002 by Fisher's-exact test. Patients with QT prolongation had a relative risk of 5.071 (3.172-8.109, 95% CI) for developing complications. With ROC analysis, the calcium value cut off for QT prolongation was 7.85 with a sensitivity of 100% and specificity of 99.9% and AUC 0.986.



[Table/Fig-5]: ROC curve for hypocalcaemia and complications.

Among the hypocalcaemic patients having complicated malaria, the serum calcium was repeated after the patient recovered from the complications. The calcium levels reverted to normal in all the patients. However, this was not statistically significant (p-value=0.185).

DISCUSSION

The resurgence of malaria is a serious public health problem in many parts of the world. It is therefore prudent to identify the factors which contribute to susceptibility of hosts. This parasitic disease is a major public health problem in developing countries, especially in India, which contributes drastically to the parasitic disease burden on the whole in Southeast Asia countries [16,17]. India is one of the major contributors to malaria mortality and morbidity in South and Southeast Asia region [17].

In the present study, majority belonged to the young age group which is in comparison with various other studies [18-20]. The age distribution depends on the endemicity of malaria in the geographical area. The comparison studies were also conducted in malaria endemic areas.

In our study, there was a male preponderance which is in comparison with various other studies [21,22]. The male predominance could be due to prolonged exposure of men to infected mosquitoes during outdoor working hours.

In the present study, majority of the patients had vivax, which is comparable to a recent large population study [23]. This is in contrast to another Indian study where majority of the patients had falciparum malaria [24]. The type of malaria depends on the geographical location and as vivax predominates over the other types of malaria in our area, this could explain the difference [23].

In the present study, hypocalcaemia was present in nearly one-third of the patients, among whom half of them had complications. Our observations were comparable to study done by Petithory JC et al., where hypocalcaemia was evident in 33% of the patients with 60% of them having complications [25]. However, another study reported a higher percentage (42%) of hypocalcaemia as compared to our study. This could be due to larger sample size in the former study [26]. Similarly, in a study done in South India where the sample size was comparable, hypocalcaemia was present in nearly half of the study subjects (45%) [10]. Hypocalcaemia was documented in a strikingly large number of complicated malaria cases (88.2%) in their study. This percentage was marginally higher as compared to the present study. The reason could be due to slightly higher percentage of the complications in the former study. The comparison study was conducted nearly two decades ago, since which time there has been a significant improvement in hospital care, patient awareness about the disease with early detection and timely treatment thus lessening the complications and mortality.

In a study done by Maitland K et al, hypercalcaemia was found to be more common in malaria [27]. This result was not comparable to our study as we did not have any patients with hypercalcaemia. This could not be explained and was deemed as incidental. Further studies need to be done to validate the same.

In the present study, normalisation of serum calcium level was seen in majority of hypocalcaemic patients after they recovered from the complications. Similar finding was documented in a study which showed that return of calcium levels to normal coincided with clinical recovery and parasite clearance [10]. Although there is a mention of hypocalcaemia as a feature of complicated malaria, there is no literature on its prevalence and clinical implications. Severity of hypocalcaemia in malaria was found to correlate with heavy parasitaemia and complications. Hence, hypocalcaemia in malaria may be a biochemical marker for complications. Hypocalcaemia secondary to parathormone suppression is another entity which was not studied and can possibly explain the reason behind this abnormality [10,28]. Recovery of hypocalcaemia correlates with improvement of parathyroid glandular function when parasitaemia gets cleared. Other proposed hypothesis for hypocalcaemia in malaria includes hypophosphatemia and hypomagnesemia [10].

In the present study, QTc prolongation was observed in a small subset of patients with falciparum malaria who had hypocalcaemia and complications. This was slightly low as compared to study done by Mananje SR et al., where 13% of the patients had QTc prolongation [18]. The difference could be due to larger number of study subjects in the comparison study. However, majority of the patients with QTc prolongation had complications similar to our study. QTc prolongation may be due to sequestration of the parasite in the myocardium.

In a prospective study, QTc prolongation was seen in nearly half of the cases of complicated malaria (53.8%) [24]. This is higher compared to our study where 22.2% of the complicated malaria had QTc prolongation. The reason for this difference is probably due to larger study participants and higher percentage of complications in the comparison study as compared to our study.

An Indian study showed that out of the 27 patients with hypocalcaemia, QTc prolongation was seen in 11 patients (40%) [10]. This is higher as compared to our study where 16% of hypocalcaemic patients had QTc prolongation. One of the possible explanations for the difference could be due to the judicious use of newer antimalarials for treatment of malaria and reduced use of quinine which can cause QTc prolongation.

Limitation(s)

The study was limited by a small sample size. The exact cause of hypocalcaemia in the study could not be ascertained.

CONCLUSION(S)

Hypocalcaemia had a significant correlation with the complications in malaria. Serum calcium level should be estimated early in the course of the illness to predict the future complications in malaria.

Acknowledgement

The authors would like to acknowledge Dr. Sucharitha Suresh for rendering her helping hand towards statistical analysis.

REFERENCES

[1] Snow RW, Guerra CA, Noor AM, Myint HY, Hay SI. The global distribution of clinical episodes of *Plasmodium falciparum* malaria. Nature. 2005;434:214-17. https://doi.org/10.1038/nature03342.

[2] World Health Organisation (WHO). "World Malaria Report 2017". Geneva, Switzerland: WHO; 2017 [Accessed 29- November-2017] Available from: http://www.who.int/malaria/publications/world-malaria-report-2017/en/.

[3] Guerra CA, Snow RW, Hay SI. Mapping the global extent of malaria in 2005. Trends Parasitol. 2006;22:353-58. https://doi.org/10.1016/ j.pt.2006.06.006.

[4] Kumari A, Kant R, Sharma PK. Estimating the Economic Burden of Malaria and Assessing Its Relationship with Socio-Economic Condition in Rohtak and Mewat Districts of Haryana, India. Journal of Advances in Medicine and Medical Research. 2015;7(878):654-61.

[5] Kumar A, Valecha N, Jain T, Dash AP. Burden of malaria in India: Retrospective and prospective view. Am J Trop Med Hyg. 2007;77:69-78.

[6] Dash AP, Valecha N, Anvikar AR, Kumar A. Malaria in India: Challenges and opportunities. J Biosci. 2008;33:583-92. https://doi.org/10.1007/s12038-008-0076-x.

[7] Jha P, Gajalakshmi V, Gupta PC, Kumar R, Mony P, Dhingra N, et al. Prospective study of one million deaths in India: Rationale, design, and validation results. PLoS Med. 2006;3:e18. https://doi.org/10.1371/journal.pmed.0030018.

[8] Dayanand KK, Punnath K, Chandrashekar V, Achur RN, Kakkilaya SB, Ghosh SK, et al. Malaria prevalence in Mangaluru city area in the southwestern coastal region of India. Malar J. 2017;16:492. https://doi.org/10.1186/s12936-017-2141-0.

[9] Beg MA, Khan R, Baig SM, Gulzar Z, Hussain R, Smego RA. Cerebral involvement in benign tertian malaria. Am J Trop Med Hyg. 2002;67:230-32. https://doi.org/10.4269/ajtmh.2002.67.230.

[10] Prabha MR, Pereira P, Chowta N, Hegde BM. Clinical implications of hypocalcaemia in malaria. Indian J Med Res. 1998;108:62-65.

[11] Weir EG, King KE, Ness PM, Eshleman SH. Automated RBC exchange transfusion: Treatment for cerebral malaria. Transfusion. 2000;40:702-07. https://doi.org/10.1046/j.1537-2995.2000.40060702.x.

[12] Chotsiri P, Wattanakul T, Hoglund RM, Hanboonkunupakarn B, Pukrittayakamee S, Blessborn D, et al. Population pharmacokinetics and electrocardiographic effects of dihydroartemisinin-piperaquine in healthy volunteers. British Journal of Clinical Pharmacology. 2017;83(12):2752-66. https://doi.org/10.1111/bcp.13372.

[13] WHO. Severe malaria. Tropical Medicine and International Health. 2014;19:7-131. DOI: 10.1111/tmi.12313.

[14] Davies SL, Hill C, Bailey LM, Davison AS, Milan AM. The impact of calcium assay change on a local adjusted calcium equation. Ann Clin Biochem. 2016;53(Pt2):292-94. DOI:10.1177/0004563215583699.

[15] Zagzag J, Hu MI, Fisher SB, Perrier ND. Hypercalcaemia and cancer: Differential diagnosis and treatment. CA: A Cancer Journal for Clinicians. 2018;68(5):377-86.

[16] Wangdi K, Gatton ML, Kelly GC, Banwell C, Dev V, Clements ACA. Malaria elimination in India and regional implications. Lancet Infect Dis. 2016;16:e214-24. https://doi.org/10.1016/s1473-3099(16)30123-2.

[17] Murray CJ, Ortblad KF, Guinovart C, Lim SS, Wolock TM, Roberts DA, et al. Global, regional, and national incidence and mortality for HIV, tuberculosis, and malaria during 1990-2013: A systematic analysis for the Global Burden of Disease Study 2013. Lancet. 2014;384:1005-70. https://doi.org/10.1016/SO140-6736(14)60844-8.

[18] Mananje SR, Kabekkodu SP, Sharma A, Saya RP. QT-prolongation as an indicator of complications in malaria. Medical Journal of Dr. DY Patil University. 2017;10(6):522-25. https://doi.org/10.4103/MJDRDYPU.MJDRDYPU_95_17.

[19] Gupta I, Chowdhury S. Economic burden of malaria in India: The need for effective spending. WHO South East Asia J Public Health. 2014;3:95-102. https://doi.org/10.4103/2224-3151.206894.

[20] Dondorp AM, Nosten F, Yi P, Das D, Phyo AP, Tarning J, et al. Artemisinin resistance in *Plasmodium falciparum* malaria. N Engl J Med. 2009;361:455-67. https://doi.org/10.1056/NEJMoa0808859.

[21] Panda AK, Panda SK, Sahu AN, Tripathy R, Ravindran B, Das BK. Association of ABO blood group with severe falciparum malaria in adults: Case control study and meta-analysis. Malar J. 2011;10:309. https://doi.org/10.1186/1475-2875-10-309.

[22] Zerihun T, Degarege A, Erko B. Association of ABO blood group and *Plasmodium falciparum* malaria in Dore Bafeno Area, Southern Ethiopia. Asian Pac J Trop Biomed. 2011;1:289-94. https://doi.org/10.1016/S2221-1691(11)60045-2.

[23] Dayanand KK, Kishore P, Chandrashekar V, Achur RN, Ghosh SK, Kakkilaya SB, et al. Malaria severity in Mangaluru city in the Southwestern coastal region of India. Am J Trop Med Hyg. 2019;100:275-79. https://doi.org/10.4269/ajtmh.18-0005.

[24] Soni CL, Kumhar MR, Gupta BK, Singh VB, Srimali L, Nayak KC, et al. Prognostic implication of hypocalcaemia and QTc interval in malaria. Indian J Malariol. 2000;37:61-67.

[25] Petithory JC, Lebeau G, Galeazzi G, Chauty A. Hypocalcaemia in malaria. Study of correlations with other parameters. Bull Soc Pathol Exot Filiales. 1983;76:455-62.

[26] Rani A, Akhtar S, Nawaz SK, Irfan S, Azam S, Arshad M. Electrolyte Disturbance and the Type of Malarial Infection. Iran J Public Health. 2015;44:1492-97.

[27] Maitland K, Pamba A, Fegan G, Njuguna P, Nadel S, Newton CR, et al. Perturbations in electrolyte levels in kenyan children with severe malaria complicated by acidosis. Clin Infect Dis. 2005;40:9-16. https://doi.org/10.1086/426022.

[28] Yasri S, Wiwanitkit V. Abnormal parathyroid in some important tropical parasitic infections: A new trend. J Parathyr Dis. 2018;6(2):42-43. https://doi.org/10.15171/jpd.2018.16.

PARTICULARS OF CONTRIBUTORS:

1. Consultant, Department of General Medicine, Hemalatha Hospital, Bengaluru, Karnataka, India.
2. Associate Professor, Department of General Medicine, Father Muller Medical College, Mangaluru, Karnataka, India.
3. Associate Professor, Department of General Medicine, Father Muller Medical College, Mangaluru, Karnataka, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Akshatha Rao Aroor,
101, Saraswathi Residency, Bejai Kapikad 4th Cross,
Mangaluru-575004, Karnataka, India.
E-mail: akshathaaroor55@gmail.com

PLAGIARISM CHECKING METHODS: [Jain H et al.]

- Plagiarism X-checker: Oct 14, 2020
- Manual Googling: Dec 04, 2020
- iThenticate Software: Dec 23, 2020 (11%)

ETYMOLOGY: Author Origin

AUTHOR DECLARATION:

- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. NA

Date of Submission: **Oct 13, 2020**
Date of Peer Review: **Nov 05, 2020**
Date of Acceptance: **Dec 06, 2020**
Date of Publishing: **Mar 01, 2021**