The Pharma Innovation

ISSN: 2277- 7695
CODEN Code: PIHNBQ
ZDB-Number: 2663038-2
IC Journal No: 7725

Received: 22-01-2013
Accepted: 29-03-2013

Vol. 2 No. 2 2013
Online Available at www.thepharmajournal.com

THE PHARMA INNOVATION - JOURNAL

Malaria-Precipitated Hypocalcaemia and related complications

Dr Seema Mishra[1*]

1. Prof, Deptt of Clinical Nutrition, Govt Bilasa Girl's College, Bilaspur, Chhattisgarh, India [E-mail: drseema.mishra@gmail.com]

70 confirmed cases of malaria were included in the present study to determine the clinical and prognostic implications of hypocalcemia and correlated QT interval (QTc) prolongation in malaria. Peripheral blood smear examination was done to determine the parasite species and the parasite load. Serum calcium level and QTc measurements in electrocardiogram were done for each patient. Thirty patients were of P. falciparum malaria (18 complicated and 12 uncomplicated), 30 of vivax malaria and 10 patients were having mixed (P. falciparum and P. vivax) infection. Hypocalcemia was found in 44 cases in which QTc was prolonged. Fourteen patients who had convulsions, all of them were having QTc prolongation and Ten had hypocalcemia. A total number of eight patients had muscle spasm, of which six had QTc prolongation and five had hypocalcemia. There were 54 cases of cerebral malaria, of which 23 had hypocalcemia as well as QTc prolongation, 15 of them developed renal failure and 14 had high parasitaemia. Two patients died who had hypocalcemia and QTc prolongation due to hepatorenal syndrome. The mean parasite load, QTc interval and serum calcium were 3.07 +/- 1.2, 0.411 +/- 0.038 sec and 7.06 +/- 0.77 mg/dl respectively in complicated falciparum malaria; 1.9 +/- 0.53, 0.512 +/- 0.033 sec and 7.02 +/- 0.49 mg/dl in complicated mixed (Pf + Pv) infection. 1.04 +/- 0.61, 0.435 +/- 0.035 sec and 8.07 +/- 1.07 mg/dl in uncomplicated falciparum malaria and 1.43 +/- 0.58, 0.403 +/- 0.019 sec and 8.68 +/- 0.03 mg/dl in vivax malaria. The difference was significant between complicated falciparum and Vivex (Pf + Pv) infection when compared to uncomplicated falciparum and vivax malaria ($p < 0.05$).

Keyword: Calcium, hypocalcemia, malaria - Q-Tc prolongation, quinine, Clotting-bleeding time, Parathyroid Hormone.

1. Introduction

Malaria is very common and still a fatal communicable disease. According to the "World Bank Health Sectoral Priorities Review" malaria will be most dangerous epidemic of this century. It will contribute much in future as a cause of mortality. Though approximately 95% of the country's population lives in malaria-endemic areas, 80% of malaria occurs among 20% of the population who are classified as 'high risk populations'. The geographical areas where 'high-risk populations' reside are in Andhra Pradesh, Chhattisgarh, Gujarat, Jharkhand, Madhya Pradesh, Maharashtra, Orissa, Rajasthan, the seven north-eastern states and Sikkim. In these states, some 200 districts contribute most to the burden of the disease. Retrospective analysis of reported cases and deaths in Chhattisgarh State revealed a mean incidence of 12.2 cases per 1,000 which was nine times greater than that officially

reported (1.7 cases per 1,000 annually). In the same study, annual malaria-attributable mortality detected was 22 deaths per million which was far greater than the officially reported (0.3 deaths per million) (Yadav *et al.*, 2003). Sixty percent of the total malaria deaths are believed to occur in the forested areas of the North-eastern plateau (the whole of Orissa, eastern Madhya Pradesh and North-east of Andhra Pradesh). In these areas a high degree of malaria transmission is maintained by two efficient mosquito vectors (*An. culicifacies* & *An.fluviatilis).*Malaria and six other diseases viz. diarrhea, HIV/AIDS, tuberculosis, measles, hepatitis B and pneumonia account for 85% of Global infectious disease burden (Murray and Lopez 1996, 1997). Malaria afflicts 36% of the world population i.e. 2020 million in 107 countries and territories situated in the tropical and subtropical regions. In the South East Asian Region of WHO, out of about 1.4 billion people living in 11 countries, 1.2 billion (85.7%) are exposed to the risk of malaria and most of whom live in India (Kondrachine 1992). Of the 2.5 million reported cases in the South East Asia, India alone contributes about 70% of the total cases. Currently, 80.5% of the 109 billion population of India live in malaria risk areas.

"Estimated & Projected Mortality Rates (Per 100000) By Sex, For Major Causes Of Death In India"

Causes	Years					
	1985		2000		2015	
	M	F	M	F	M	F
All causes	1158	1165	879	879	846	745
Infections	478	476	215	239	152	175
Circulatory	145	156	253	204	295	239
Diabetes	239	293	280	285	311	301
Malaria	43	51	88	74	108	91

(Srinivasan K, Demographic & epidemiological transition in India, Institute of Population Sciences Deonar Mumbai)

Malaria not only disturbs the blood picture but it disturbs the whole biochemistry of the body. This work is based on identification of risk factor concept, as malaria is becoming fatal nowadays & we must identify all possible risk factors.

Trigger Mechanism: Many previous studies showed that in malaria calcium homeostasis gets disturbed. Many possible mechanisms have been suggested to explain the relation between malaria and hypocalcaemia. In "Falciparum " malaria this effect is found most severe –The nature of the mechanisms underlying Ca++ homeostasis in malarial parasites has puzzled the investigators for almost two decades. Some possible mechanisms are:

1. Plasmodium uses Ca^{++} based signaling pathway, this results in reduced calcium status, especially intracellular calcium but not usually millimolar concentration of Ca^{++} found in body fluids. This disturbed the environment of the host cell cytoplasm. But how the protozoa achieved the calcium homeostasis – was unanswered.
   [Celia GARCIA-"Paracitol Today", 2011 Dec,:15 (12):488-91
2. Plasmodium Falciparum infected red blood cells have increased permeability for calcium. The magnitude of the increase is greater than that normally require activating the Ca^{++} dependent K^{+} channel. This study found that due to Falciparum infection the influx of Ca^{++} is increased to over 1mmol which is much higher than the normal values. The pathway responsible for the enhanced influx was expressed at approximately 30 hrs post invasion.
   [J. Member biol 2009 Nov 1;172(!):13-24]
3. Increase in calcium dependent Transglutaminase activity is found as a cause of decreased level of calcium in some studies. This decrease is found simultaneous with maturation of the parasite. The effect was found maximum when the trophozites were 48 hrs old and at that time most of the calcium was shown to be located in the parasite.
   [Mem Inst Oswaldo Cruz 2000,Jan –Feb ;94(!) 95-100]
4. There is a decreased ionized calcium "set point for basal PTH secretion, but a normal

PTH response to acute hypocalcaemia in malaria skeletal resistance may attenuate the effect of the PTH response but patients with malaria appear relatively resistant to the calcium chelating effects of citrated blood products.

[Davis. T.M., Singh B.,Choo K E, 4: J Intern Med 1998 May : 243(5) ; 349-54

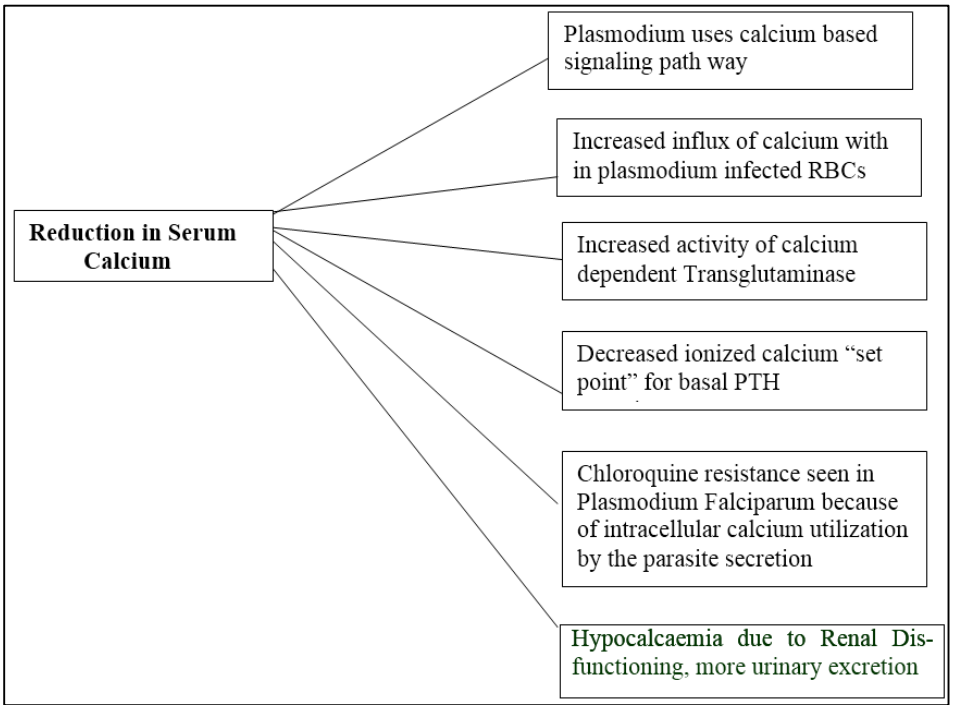
"Dynamic assessment of PTH function in malarial condition "

5. There is a study to show that serum calcium is utilized by the parasite and this creates drug resistance in parasites. Sometimes there is Chloroquine resistance seen in Plasmodium Falciparum because of intracellular calcium utilization. This results in decreased calcium status in patients as well as drug resistance. When the channels of calcium were blocked with Verapamil & Fantofarone, then the Chloroquine resistance is totally reversed. Verapamil appeared 2to 3 times more potent than Fantofarone in reversing the drug resistance.

[5; Biochem Pharmacol 1998,Feb 15;55(4): 433-440 By Adovalande J, Deleze J.]

6. Disturbed Parathyroid hormone profile has also contribution to the lowered Calcium Status.

Trigger Mechanism



Objectives of the Study

To find and understand the relation between malaria and serum calcium status, we plan –

1. To measure serum Ca^{++} status in malaria patients and compare it with healthy persons.
2. To compare the Ca^{++} status with the Falciparum malaria type with other types of Malaria.
3. To estimate the 'Serum Alkaline Phosphatase' level in patients and in healthy controls. This result will be assessed according to type of malaria.
4. The Serum Phosphate level of patients and controls will be monitored.
5. Total Blood Count will be estimated as type of infection and growth and severity of malarial parasites affected blood count of the patients.
6. All the estimations will be done in only those patients who are taking Quinine / Hydroxy Chloroquine as anti- malaria drug.
7. To estimate the correlation between Malarial parasitic load with the serum status of calcium in various Malaria Affected patients.

8. To assess the effect of lower status of calcium on the Cardiac profile of the Malarial Patients.
9. Effect on ECG, specially on Q-Tc prolongation will be observed.
10. Effect on serum Calcium level on blood pressure and Heart rate will be observed in Quinine treated malarial Patients.
11. Heart Rate of Malarial Patients was also estimated and it was correlated with the serum Calcium status of the patients.
12. The effect of Lowered Calcium Status in Malarial Patients on Clotting and Bleeding Time Profile of the patients.
13. Total Serum Bilirubin will be estimated as increased destruction of RBSc causes increase production of Bilirubin and it is related with the severity of the disease.
14. Serum level of Parathyroid hormone will be estimated and a correlation will be estimated between serum Parathyroid level and serum calcium status.
15. Estimation of serum Creatinin and Urea to assess the Renal Functioning Level, as malfunctioning of kidneys and hampered working capacity of kidneys precipitates hypocalcaemia due to increased excretion of this ion.

Methodology

The subjects for the study were selected randomly by contacting Malarial patients in clinics and personally, those healthy controls were selected who are demographically matched with the patients.

Subjects –Total 70

(Falciparum Malarial Patients-30, Vivex Malarial Patients-30, Mixed -10)

Study Area- Bilaspur City and outskirt area.

Study Time- September 2014-February 2015

Study Duration-6-7 months.

- **Collection of blood specimen:** About 5 mL of blood was used for analysis. Sample was collected from each subject by vein puncture technique using 21 gauge hypodermic needle and syringe. The blood sample was collected into plain tube and allowed to clot. It was centrifuged at 1200 g for 5 min at room temperature. Serum was separated into a bijou bottle using a pipette. The serum in the bijou bottle was stored frozen until required for analysis.

1. Estimation of serum calcium-was done by Method of 'Clark and Collip' modification by Kramer-Tisdall Method.
2. The Serum Phosphorus was measured calorimetrically by using molibadate at acidic pH to form Phosphomolibdate Complex, the absorbance was measured at 340 nm.
3. For the estimation of Serum Alkaline Phosphatase, enzymatic splitting of P-Nitrophenyl Phosphate was done and the color intensity was measured, which was proportional to the enzyme concentration.For this estimation Biochemical Autoanalyser Star 21 was used.
4. ECG was estimated by routine electrode method, Chest Leads were measured in subjects and normal.
5. Blood pressure was estimated by Auscultatory Method.
6. Clotting time was estimated by Capillary Glass tube method and Bleeding time was estimated by Ink Spot Method.
7. All these estimations were done in randomly selected adult patients & healthy controls,
8. Heart Rate was estimated by counting pulse.
9. CBC by using "Sysmex KX-21 Three Part Differential Automated Haematology Analyser".
10. Evaluation of serum billirubin and creatinin by using a semi-automated biochemistry analyser**.**
11. There are one acceptable methods of expressing the parasite load: as the percentage of infected erythrocytes as counted on a stained thin blood film (e.g. 1% parasitaemia) I had opted this method to assess parasitic load.
12. Serum Parathyroid level was estimated by using Mini Vidas-500 of Biomarix company.
13. Serum Creatinin and serum Urea were assessed by using enzymatic analysis method by using auto analyzer Star 100.

Observations

Table 1: Details of patients involves

P.FALCIPARUM n=30						
Age/ Gender	**5-10YEAR**	**6-12YEAR**	**13-20YAER**	**21-30YAER**	**30-50YAER**	**> 50 year**
Male	**3**	**2**	**4**	**5**	**3**	**3**
Female	**-**	**2**	**3**	**2**	**2**	**1**
P.VIVEX n=30						
Male	**-**	**2**	**4**	**3**	**2**	**2**
Female	**3**	**2**	**2**	**3**	**5**	**2**
Mixed n=10						
Male	**1**	**3**	**3**	**-**	**2**	**1**

Table 2: Status of Serum Ca Level In Subjects

Group	**Age [years]**	**Ca mg/ dL**	**Parasitic Load**	**Level of Correlation**	
				Age of Patient & serum Calcium Level of Patients	**Serum Ca Level of Patients & Parasitic Load**
Control	33.1 ± 4.22	9.87± .23	Nil	0.46532	- 0.7706
MAL	29.2 ±4.09	7.16± 4.77	≥ 20% RBCs		
MAL+ QIN	32.5± 2.16	6.07±1.62	≥ 11-15% RBCs		

Significant difference when compared with CTR at $p < 0.05$
** -Significant difference when compared with MAL at $p < 0.05$
CTR= Normal Control Subjects, CTR + QIN = Normal Subjects taking Quinine treatments
MAL= Malaria Infected patients without treatments, MAL+ QIN= Malaria patients taking quinine treatments.

Table-3: Cardio-toxic effect of Hypcalcaemic status in Malarial Patients

Group	**Age [years]**	**Ca mg/ dL**	**Q-T Interval Seconds**	**Level of Correlation Serum Ca level & Q-T Interval**
Control	33.1 ± 4.22	9.87± 1.23	0.368±0.019	-0.7944
MAL	29.2 ±4.09	7.16± 4.77	0.469±0.057	
MAL+ QIN	32.5± 2.16	6.07±1.62	0.483±0.043	

Table 4: Various Biochemical Parameters of the Studied Subjects

Sr No	**Factors**	**Falciparum**	**Vivex**	**Mixed**	**Difference T Value**
1.	Parasitic Load	9.1% RBC infected	4.3 % RBC Infected	2.04% RBC infected	1.146
2.	Serum Calcium	6.09±1.02	7.27±0.91	9.01±1.82	4.677
3.	Blood Pressure	63/99	81/111	87/124	0.4525
4.	Heart Rate	83	79	75	1.232
5.	Serum Bilirubin	4.06	3.68	2.19	8.033
6.	Serum Alkaline Phosphatase	267 IU/L	188 IL/L	67 IL/L	8.674
7.	Average Clotting Time	7.33min	4.12 min	2.55 min	7.07
8.	Average Bleeding Time	6.45 min	4.56 min	2.40 min	3.582
9.	RBCs	3.78 trillion cells/L	4.79 trillion cells/L	5.59 trillion cells/L	3.277
10.	Hemoglobin	8.77	9.48	11.22	3.496
11.	Serum Phospharus	2.13 mg/dL	3.04 mg/dL	3.5 mg/dL	3.326
12.	Serum Parathyroid Hormone	71 ng/ L	53ng/L	49 ng/L	8.241
13.	Creatinine	4.7 mg/dL	2.3mg/dL	0.9 mg/dL	3.044
14.	urea	36.7 mg/dL	22 mg/dL	11 mg/dL	1.678

Discussion

The serum Calcium level was observed significantly low in malarial patients, especially Falciparum infected patients, also were taking Quinine for treating malaria. This may be due to various trigger mechanisms as mentioned earlier, also perhaps due to functionally hampered kidneys in malarial patients, with increased excretion/ reduced reabsorption of Ca^{++} by kidneys. The malfunctioning in patients was indicated by elevated serum levels of creatinin and urea.

The parasitic lead, as indicated by infected RBCs in per 100 count was much higher in Falciparum infected patients. As serum Ca level was observed lower in patients, thus their blood pressure was also low, because ionic Ca helps for the tonic contraction of cardiac muscles and helps to maintain blood pressure within normal range. As blood pressure was lower, so the heart rate was higher in patients, due to Pulses Paradoxes. The serum Bilirubin was also higher in patients due to more production by increased destruction of RBCs and also by reduced excretion by kidneys. The total count of RBCs was prominently reduced in patients, especially in Falciparum patients. The hypocalcaemic state was reflected in prolonged bleeding and delayed clotting profile of the patients. Due to reduced Ca level in blood, the Parathyroid hormone was observed elevated in patients. This reduction of serum Ca was reflected in Cardiac functioning – the Q-T interval was prolonged in Falciparum patients, finally this could cause heart failure in malarial patients.

In conclusion, hypocalcemia is a feature of severe/ complicated malaria as it had a good correlation with parasite load and complications. It has a prognostic value in malaria, as recovery from malaria was associated with return of serum calcium levels to normal. Although there was a statistical correlation between hypocalcemia and Q - Tc prolongation further studies are warranted to rule out other causes of Q - Tc prolongation. Q - Tc prolongation in malaria could be a risk factor for quinine cardiotoxicity and controlled trials with reduced quinine dosage are warranted in patients with prolonged Q - Tc to confirm quinine dosage reduction in such patients.Hypocalcemia in malaria was found to correlate with heavy parasitemia, complications and Q-Tc prolongation. Hence hypocalcemia in malaria may be a biochemical marker for complications. It was found to return to normal with clinical recovery, parasite reduction and return of Q-Tc interval to normal. Hence serum calcium levels may be of prognostic value.

Recommendations-

–Malaria is found to be associated with hypocalcaemia, especially in Falciparum malaria. The patients using Hydroxychloroquine showed severe results in this concern. Until controlled research explores this interaction more thoroughly, patients taking Hydroxy-chloroquine might consider having their calcium status monitored by a health practitioner.

Reference-

1. Celia GARCIA- Paracitol Today. 1999; 15(12):488-9.
2. J Membr boil. 1999;172(!):13-24.
3. Mem Inst Oswaldo Cruz. 2000; 94(!)95-100.
4. Dynamic assessment of PTH function in malarial condition.
5. Adovalande J, Deleze J. Biochem Pharmacol. 1998; 55(4):433-440 By
6. Barre PE. Hydroxychloroquine treatment and hypocalcemia. Am J Med. 1987, 1259-62.
7. McCaryDJ.Antimalarial preparations. 1993, 1913:6.
8. Olin BR. Antimalaril preparations-Bioavailability of Vitamins. 1991, 23-35.
9. Sawev PN, Oswalt C. Antimalarial Preparations. 1999, 455-459.
10. Ollip AS. Am J Med Assessment of bone health. 1996, 223-231.
11. Snow RW, Guerra CA, Noor AM *et al*. The global distribution of clinical episodes of Plasmodium falciparum malaria. Nature 2005; 434:214-17.
12. Cross Ref Medline Web of Science
13. Bell D, Winstanley P. Current issues in the treatment of uncomplicated malaria in Africa. Br Med Bull. 2004; 71:29-43.

14. WHO. Severe falciparum malaria. World Health Organization, Communicable Diseases Cluster. Trans R Soc Trop Med Hyg. 2000; 94(Suppl. 1):S1-90.
15. Kochar DK, Saxena V, Singh N *et al*. Plasmodium vivax malaria. Emerg Infect Dis. 2005;11:132-4.
16. Newton CR, Kirkham FJ, Winstanley PA *et al*. Intracranial pressure in African children with cerebral malaria. Lancet. 1991; 337:573-6.
17. Silamut K, White NJ. Relation of the stage of parasite development in the peripheral blood to prognosis in severe falciparum malaria. Trans R Soc Trop Med Hyg. 1993; 87:436-43.
18. Nguyen PH, Day N, Pram TD *et al*. Intraleucocytic malaria pigment and prognosis in severe malaria. Trans R Soc Trop Med Hyg. 1995; 89:200-4.
19. Gachot B, Houze S, Le Bras J *et al*. Possible prognostic significance of a brief rise in parasitaemia following quinine treatment of severe Plasmodium falciparum malaria. Trans R Soc Trop Med Hyg. 1996; 90:388-90.
20. Craig MH, Bredenkamp BL, Williams CH *et al*. Field and laboratory comparative evaluation of ten rapid malaria diagnostic tests. Trans R Soc Trop Med Hyg. 2002; 96:258-65.
21. Pasvol G. Management of severe malaria: interventions and controversies.Infect Dis Clin North Am. 2005; 19:211-40.
22. Maitland K, Nadel S, Pollard AJ *et al*. Management of severe malaria in children: proposed guidelines for the United Kingdom. BMJ. 2005; 331:337-43.
23. Planche T, Krishna S. The relevance of malaria pathophysiology to strategies of clinical management. Curr Opin Infect Dis. 2005; 18:369-75.
24. Njuguna P, Newton C. Management of severe falciparum malaria. J Postgrad Med. 2010; 50:45-50.
25. Maitland K, Levin M, English M *et al*. Severe P. falciparum malaria in Kenyan children: evidence for hypovolaemia. QJM. 2009; 96:427-34.
26. Pasvol G, Newton CR, Winstanley PA *et al*. Quinine treatment of severe falciparum malaria in African children: a randomized comparison of three regimens. Am J Trop Med Hyg. 1999; 45:702-13.
27. White NJ, Looareesuwan S, Warrell DA *et al*. Quinine loading dose in cerebral malaria. Am J Trop Med Hyg. 1993; 32:1-5.