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Original Article

A Comparative Study of Serum Iron, Manganese and Zinc Levels in Falciparum Malaria Patients with Healthy Controls

Jamil Mohammad, R.K. Vyas, Yogita Soni, Dileep Nirwan, Gajanand Joshi
Department of Biochemistry, Sardar Patel Medical College, Bikaner, Rajasthan, INDIA.

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***Correspondence to:**
Jamil Mohammad
Department of Biochemistry, Sardar Patel Medical College, Bikaner, Rajasthan, INDIA.

ABSTRACT

Background: Patients with *Falciparum Malaria* may not be apparently seriously ill but may develop serious complications. The question of interaction between malaria infection and nutritional status of the host is always controversial and their relationship remains difficult to establish.

Aims: To evaluate the effects of the *falciparum* malaria infection on the concentration of trace elements (iron, manganese, zinc).

Design & Methods: Study was conducted in Sardar Patel Medical College and attached Hospital, Bikaner from the period of July 2014 to November 2015. Total 100 subjects were divided in two groups, 50 *falciparum* malaria patients and 50 healthy controls, between 5-45 years of age. Malaria diagnosis was based on clinical sign and symptoms and confirmed by laboratory test (thick and thin blood films). Serum iron, manganese and zinc concentrations were determined by atomic absorption spectrometry (AAS).

Results: The mean serum iron levels were significantly lower in subjects with *falciparum* malaria patients compared with control group of healthy subjects (0.983±0.155 mg/L versus 0.770±0.089 mg/L, $p<0.0001$). Similarly mean serum zinc levels were also significantly lower in *falciparum* malaria patients compared with control group (0.653±0.065 mg/L versus 0.561±0.061 mg/L, $p<0.0001$). However, there was no significant variation in manganese levels in *falciparum* malaria patients compared with control group (0.007±0.065 mg/L versus 0.007±0.061 mg/L, $p >0.05$).

Conclusion: *Falciparum* malaria has a damageable effect on the quantity of iron and zinc. The deficiency in trace elements like iron and zinc might be one of the causes of malaria *falciparum* due to decrease immunity.

KEYWORDS: *Falciparum* malaria, Malnutrition, Trace elements.

INTRODUCTION

In malaria-endemic areas, particularly in India, the fight against malaria is a growing concern and lies partly in the multi-resistance of parasite to various antimalarial drugs currently available. This resistance is sometimes the result of poor compliance to treatment, causing point mutations leading to repeated therapeutic failures, and often impedes the management of malaria. Thus, malaria remains a public health problem. In India, malaria and malnutrition coexist and forms a deadly combination. Malaria exacts a heavy burden on the poorest and most vulnerable communities.

Nutrition plays a major role in maintaining health, and malnutrition appears to generate vulnerability to a wide variety of diseases and general ill health.[1,2] The question of interaction between endemic malaria and nutritional status of the host is still controversial and their relationship remains difficult to establish.[3,4] Some studies show the protective effect of normal and adequate nutritional status.[5-10] On the other hand some studies in past argue that malnutrition protects against exacerbation of malaria and that nutritional supplementation would increase the host susceptibility to infection.[11,12] Furthermore we know that the nutritional requirements of the host for his welfare include some trace elements,[13-16]certain of these trace elements are also essential for metabolism of parasite.[17-19]

Infection and malnutrition have always been intricately linked. Malnutrition is the primary cause of immunodeficiency worldwide, and we are learning more and more about the pathogenesis of this interaction. The worldwide magnitude of parasite infection is enormous. It is understood that parasites may lead to malnutrition,

but the extent to which malnutrition causes increased parasite infestation is not known; thus, the conditions need to be addressed together.

The involvement of nutritional supplements and trace elements in the protection or exacerbation of malaria has been a subject of numerous studies. The immunomodulatory properties of zinc are known,[20-21] but its interest in the treatment of malaria is variously interpreted.[3,22] Some studies show that the nutritional supplementation during treatment of malaria improves the therapy and nutritional status.[8,10] Iron, manganese and zinc are antioxidants or antioxidant enzyme cofactors fighting against abnormal elevation of oxidative stress both from host and the parasite.[17-19,23] Iron deficiency weakens the immune system, increases the risk of anemia and infection.[18] However, iron-deficient among individuals may confer resistance to malaria.[24-25] Thus the interest to assess these micronutrients in the blood lies in the management of intake risk and optimization of the therapy of malaria.

The aim of this work was to assess serum titers of iron, manganese and zinc, which is an indicator of oxidative stress, in the course *Plasmodium falciparum* malaria patients.

MATERIALS AND METHODS

Study Place and Design

This study carried out in Sardar Patel Medical College and attached Hospital, Bikaner from the period of July 2014 to November 2015. It was observational cross-sectional study, which included 100 subjects. Out of 100 subjects, 50 subjects were patients of *falciparum* malaria and another 50 normal subjects were age and sex matched healthy volunteers as control group.

The eligibility criteria were- Age 5 to 45 years, Clinically suspicion of malaria (fever, chills, sweating, headache, polyalgia, infectious syndrome) during consultation, no intestinal parasite infection, no micronutrients supplementation (trace elements and vitamins and any herbal medicine) and without known associated pathology. Patients not received antimalarial treatment before enrolments were included in the study. The patients included in study were fasted of at least 8 hours before taking sample. The subjects who did not satisfy all these criteria were excluded from the study. Very sick patients and Patients having history of severe anemia were excluded. The subject consent was taken prior to study.

Physical Examination

All the subjects had undergone a complete physical examination. Socio-economic status of every individual was assessed by living standard and monetary status. The family history regarding anemia, obesity, diabetes, hypertension and coronary artery disease was carefully recorded by a questionnaire and subjects with any acute and chronic illness, severe anemia, liver, kidney, cardiovascular and endocrine disorders were excluded. Complete physical Examination and clinical history of subjects were also recorded.

Collection and Analysis of Samples:

After overnight fasting, venous blood of the subjects was drawn from anticubital vein using aseptic techniques. Samples were collected in plain vials for trace elements. The samples were left standing for one hour and serum were separated.

INVESTIGATIONS

Diagnosis of *P. falciparum*

Thick and thin blood smears for detection of *P. falciparum* made by niddle prick method. The absence of *P. falciparum* infection was noted when 200 fields were read negative.

Determination of Micronutrients

The Atomic Absorption spectrophotometry (SHIMADZU AA-7000) at flame air-acetylene was used for the determination of trace elements. The limit of detection was 0.001mg/L. The characteristic wavelengths were of 248, 214 and 279nm respectively for iron, zinc and manganese. For determining zinc and manganese, serum samples were digested according to ratio 1:9 (v/v) with 6% deionized water-n-butanol during 30min at 100ºC. The determination of iron included deprotenization of serum according to ratio 1:9 (v/v) with 5% deionized water-trichloro acetic acid. A multielement standard solution of 1000ppm (Merck), diluted just before use at 1/500 with deionized water-nitric acid (0.03M), was used to prepare calibration range (0.5, 1.0, 1.5, 2.0ppm). The measurements of concentrations were performed in triplicate and adjusted against the blank (deionized water).

Statistical Analysis

Results are expressed as Mean±SD. Data were analyzed with the help of Microsoft excel 2007, using student's t-test. A p-value < 0.05 was considered as statistically significant.

RESULTS

The mean serum Iron levels were significantly lower in *falciparum* malaria patients compared with control subjects (0.983±0.155 mg/L v/s 0.770±0.089 mg/L). The mean serum Zinc levels were significantly lower in *falciparum* malaria patients compared with control subjects (0.0653±0.065 mg/L v/s 0.561±0.061 mg/L). The mean serum Manganese levels were statistically not significant in *falciparum* malaria patients compared with control subjects (0.007±0.065 mg/L v/s 0.007±0.061 mg/L) (Table 1).

According to gender, the serum titers of trace elements showed no significant variation ($p>0.05$) during the infection. The mean of serum Iron was of 0.990±0.119 mg/L in control male and of 0.785±0.106 mg/L in *falciparum* malaria male patients. The mean of serum

Iron was of 0.977±0.178 mg/L in control female and of 0.750±0.057 mg/L in *falciparum* malaria female patients.

The mean of serum Zinc was of 0.662±0.075 mg/L in control male and of 0.572±0.066 mg/L in *falciparum* malaria male patients. The mean of serum Zinc was of 0.640±0.045 mg/L in control female and of 0.546±0.051 mg/L in *falciparum* malaria female patients. The mean of serum Manganese was of 0.008±0.002 mg/L in control m male and of 0.007±0.002 mg/L in *falciparum* malaria male patients. The mean of serum Manganese was of 0.007±0.002 mg/L in control female and of 0.006±0.002 mg/L in *falciparum* malaria female patients. (Table 2) It was the same with the age categories, the variations of trace elements studied were not significant.

Table 1: Mean ± SD of various trace elements of case & control group subjects

Parameters	Mean ± SD		p-value	Significance
	Control	Case		
Fe	0.983±0/155	0.77±0.089	<0.0001	HS
Zn	0.0653±0.065	0.561±0.061	<0.0001	HS
Mn	0.007±0.065	0.007±0.061	>0.05	NS

HS – Highly significant, NS – Not significant

Table 2: Mean ± SD of various elements according to sex of control and case subjects

Parameters	Control subjects		Malaria *falciparum* patients	
	Male	Female	Male	Female
Iron	0.990±0.119	0.977±0.178	0.785±0.106	0.750±0.057
Zinc	0.662±0.075	0.640±0.045	0.572±0.066	0.546±0.051
Mn	0.008±0.002	0.007±0.002	0.007±0.002	0.006±0.002

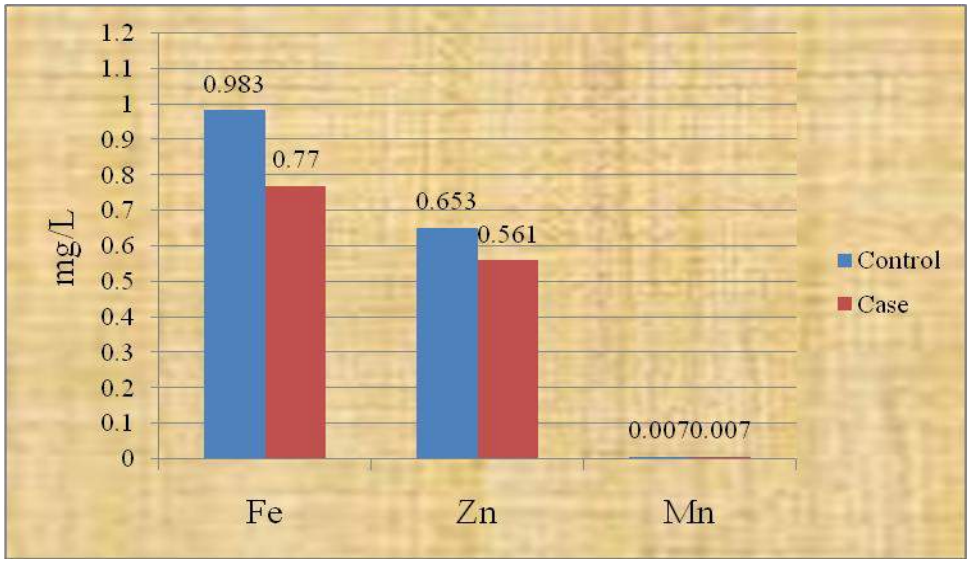

Fig 1: Mean ± SD of various trace elements of case & control group subjects

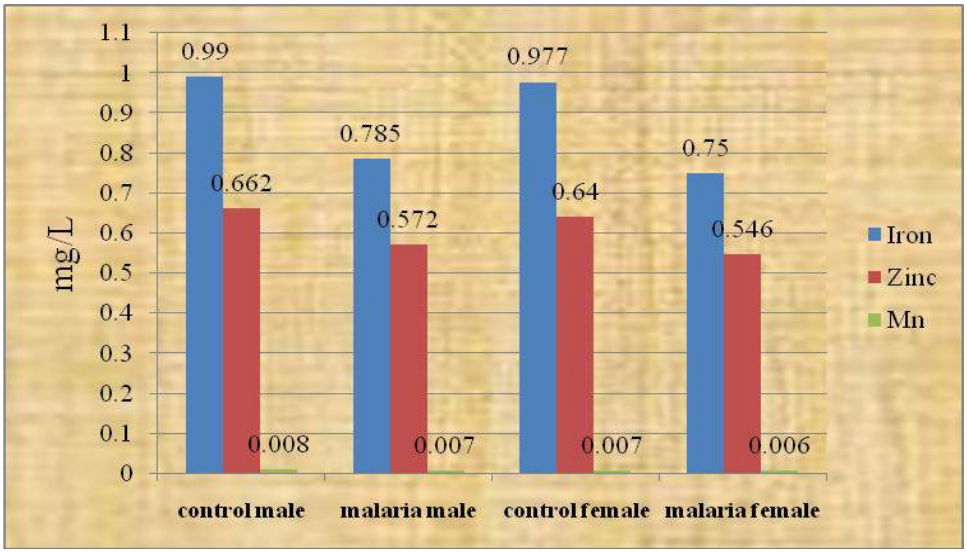

Fig 2: Mean ± SD of various elements according to sex of control and case subjects

DISCUSSION

An acute malarial episode causes anemia, mainly through decreased production of erythrocytes by the bone marrow due to suppression of erythropoiesis and increased hemolysis. Malaria also contributes to iron loss by means of its immobilization in the form of hemozoin, increased urinary excretion, and decreased intake and absorption of dietary iron. Excessive hemoglobin digestion by the parasite for its growth, or the phagocytosis of infected erythrocytes by organism for its defense, is accompanied by iron liberation. That would increase its use by the sporozoite[26] and reduce the iron status. Afterwards, the low iron observed, would result from iron utilization by the parasite and accentuation of the host defense.

When confronted with infection and inflammation the human host reallocates its iron reservoirs in an effort to deprive invading pathogens of iron. The human protein hepcidin, a rheostat of systemic iron homeostasis, signals the body to decrease absorption of iron in the proximal duodenum and orchestrates the movement of iron from serum into storage within the liver and macrophages.[27] As a result of reduced serum iron, erythropoiesis (a process exquisitely sensitive to iron levels) slows in the face of infection as well as inflammation. The human host's active reduction in bioavailable iron protects against a wide range of pathogens.[28]

Pathways of *plasmodium* require several enzyme cofactors such as iron-sulphur clusters[29] and possibly zinc, this may lead to deficiency of these nutrients in the host. Zn is required for each step of cell cycle in microorganisms.[30] Zinc is an essential component of cuprozinc superoxide dismutase, an enzyme in the erythrocytes essential for host defense as well as parasite growth.[31] In acute phase of malaria, zinc is redistributed from plasma to lymphocytes and to the liver, consequently decreasing plasma zinc levels in microbio-static environment [31](Shankar, B.2000).

P. falciparum is capable of sticking to blood vessels (a process known as cytoadherence). This sticking leads to obstruction of microcirculation which results in dysfunction of multiple organs and breakdown in the body's immune system. These resultant effects of *P. falciparum* cytoadherence could modify micronutrient levels in serum of infected patients. Malarial parasites invade and destroy red blood cells, and patients infected with the malarial parasites, also experience recurrent gastrointestinal symptoms such as nausea, vomiting and diarrhea. This may modify trace elements contents in serum of infected individuals.

Malaria provokes an overproduction of free radicals. Overproduction of activated oxygen species will have adverse effects by inducing a series of biological reactions that may impair antioxidant defenses (trace elements, vitamins) and cause of cellular damage.

CONCLUSION

It is clear that the level of Iron and of Zinc were lower in malaria *falciparum* patients as compared to controls. However, there was no significant variation in level of manganese. The present study gives us an idea that the deficiency in trace elements like iron and zinc might be one of the causes of malaria *falciparum* due to decrease immunity. However, since the size of study group was very small, it needs to be studied further with groups.

Considering all that, the correction of serum trace elements concentration would have a beneficial effect on treatment, complication and progression of the diseases, which may be a subject of further study.

The fact that serum titers of iron and zinc in both the malaria infected subjects and the control group were lower than the reference levels raises the question of bioavailability and insufficient nutritional intake of these micronutrients, which may be a subject of further study.

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