Research Article

Investigation of lipids profile with special reference to vitamin C in *vivax* malaria patients in region of Sargodha

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Abstract

Pakistan stands among 10 countries presumed to be largest population susceptible for the *vivax* malaria. *Vivax* malaria is most often benign tertian malaria. It is often mistakenly speculated to be benign however it is actually severe and acute. Body needs vitamin C for normal physiological processes. Due to the fact vitamin C act as a strong antioxidant agent its deficiency may cause weak the body immunity. By using standard procedures and kits, the parameters estimated were as follows: vitamin C, Lipid profile (Total cholesterol, Very low-density lipoprotein (VLDL), Low-density lipoprotein (LDL) cholesterol, High-density lipoprotein (HDL) and Total triglycerides, were evaluated by using automated analyzer, Micro Lab 300. At the end of the study it was concluded that by comparison of normal (n=43) and malarious (n=65) subjects of two age groups (≤ 15) and (> 15) year, there were significant ($p < 0.05$) differences observed. A significant decrease was found in vitamin C, HDL Cholesterol and LDL Cholesterol in *vivax* malaria patients. It was also positively correlates with severity of disease. No significant difference was observed in total cholesterol, total triglycerides and VLDL Cholesterol. The conclusion of this study was that alteration in these parameters suggests proper monitoring during treatment in order to reverse them to normal levels.

Keywords: *Vivax* Malaria; Vitamin C; Lipid profile; Total cholesterol; HDL; VLDL

Introduction

A person suffering from *Vivax* malaria shows the symptoms of paroxysms of chills, headache, fatigue, high fever and musculoskeletal pain. Nowadays a kind of severe disease syndromes that appears just similar to *falciparum* malaria including cerebral malaria, anemia, jaundice, renal failure, acute respiratory distress syndrome,

seizures, pulmonary edema, thrombocytopenia, hyperparasitemia, spleen rupture. Death has been reported in individuals with *vivax* malaria [1]. *Vivax* malaria is spread in 95 countries and is responsible for the bulk of the global burden of malaria in Asia, southern and Central America, south Eastern Europe, Oceania and some areas of Africa [1].

As advancing state malaria inflicts a huge public health issue in Pakistan. Both falciparum and vivax malaria are declared to be preset in Pakistan. Pakistan stands among 10 countries presumed to be largest population susceptible for the vivax malaria. Extended agricultural practices followed by widespread irrigation network, considerable population in movements, monsoon rains and complicated political situations in some border areas significantly increase the risk of malaria in Pakistan. Malaria is extensively more predominant in the rural areas [2].

According to the survey conducted in Pakistan about infectious disease, malaria is commonly known to be the second most frequent clinically problematic disease, due to poor sanitary conditions and high poverty level. The survey showed that about 4.5 million people suffering from this reoccurring disease and usually along with respiratory infection. Suspected data obtained from district health system public sector in 2008. The speculated total number of malarial patients ranging about 1.5 million patients per year is considerably less [3].

Malaria instigates the eradication of white blood cells. Antioxidants present in the body aid in the battle alongside free radicals which is enhanced after an infection especially malaria infection proliferate [4]. The oxidative stress is one of the reasons in suffering from anemia cause by malaria. All diseases like malaria stimulate the defense system of body. By the help of immune system of host, malaria parasite stimulates the production of oxygen reactive species that result in hemolysis of hemoglobin [4].

Various biochemical and physiological functions required vitamins and nutrients that are essential for the body requirement. Human body does not make them mostly, so they are supplied through the diet in the required amount. Ascorbic acid (vitamin C) a water-soluble antioxidant is an easily

oxidized, unstable acid and can be damaged by alkali, oxygen and high temperature. Studies suggested that absence of the active enzyme, l-gulonolactone oxidase, in liver is the main reason of human inability to produce ascorbic acid [5].

Body needs vitamin C for normal physiological process such as wound healing and tissue growth. Because it regulate the synthesis of carnitine and catecholamine which mainly regulate nervous system for possible action through reduction of blood cholesterol level. Due to the fact vitamin C act as a strong antioxidant agent its deficiency may cause varieties of diseases such as HIV, hepatitis, common cold flue and pylori infection [6].

L-ascorbic acid, and its congener L-dehydroascorbic acid, collectively known as vitamin C have been recognized to arbitrate a wide variety of cellular functions [7]. Connection to blood ascorbic acid and its relationship with leukocyte working, it was watched that being extraordinary presence of ascorbic acid, the parasite danger may initiate leukocytes to discharge their ascorbate stores as an additional response to parasite forced anxiety. In addition, the release of lethal compounds, including oxidant items, as a consequence of erythrocytic merogony, and the related hemolysis of red blood cells, may advance incite a need on the patient for upgraded preparation of antioxidants. Leukocytes can be instantly source of ascorbic acid [8].

It has been seen that various diseases are related by fever demonstrates diminished blood levels of ascorbic acid, showing an expanded requirement for this vitamin in infectious diseases. Vitamin C's critical part is to kill free radicals as an antioxidant. Since ascorbic acid is water soluble, it can act both inside and outside the cells to struggle free radical harms. Free radicals look for an electron pair to recover their stability. Vitamin C antioxidant behavior is

linked with its ability to enhance the defensive mechanism of immune system by improving the activity of phagocytic cell and neutrophils. It is an efficient source of electron donor during any infectious disorder it plays the same role and increase body defense against disease [9].

Vitamin C works against destruction by the free radicals released by the body in its fight against the infection [10]. Numerous studies discovered that the oxidative anxiety gives off an impression of being a typical wonder in intense infection, it might bring about a particular result in malarial pathogenesis. Hyperlipidemia, which is one of the markers of malarial disease could results in exhaustion of antioxidants and encourage the creation of receptive oxygen species which is fit to respond with every single organic particle in the body framework and apply cytotoxic consequences on cell segments [11].

Lipoproteins are significant lipid part in plasma, and absolutely the person with oxidative stress. Malarial disease produces reasonable changes in plasma lipid shape in man, with normal ascent in serum triglyceride and decrease in HDL. These adjustments in lipid parameters are more affirmed in plasmodium falciparum [12].

The intense reaction is connected with changes in lipid digestion system incorporating a moderate rise in serum triglyceride and VLDL, yet descent in HDL and LDL. In low-level malarial infection, the level of entire cholesterol, LDL, and HDL are lessened while triglyceride levels are expanded. Temporary changes in the plasma levels of lipids, cholesterol and triglycerides have been seen subsequent to quite a while by numerous researchers in various intense infections, hypertriglyceridemia, hypocholesterolemia and amazing diminishing in HDL and LDL parts were observed in complicated and uncomplicated malaria. The size of these

progressions appears to be identified with the seriousness of malaria [12]. In this study we were motivated to investigate the level of different parameters to check concentration in malaria patients.

Materials and methods

In present study 65 subjects suffering from malarial parasitic infection were selected on first come first serve bases in Sargodha division. These all were newly diagnosed and blood samples were collected from these patients who come to different local Hospitals at their OPD clinics. Similarly 43 control subjects were also selected and their blood samples were also collected. Blood of all subjects was analyzed for water soluble vitamin C level, Lipid profile (T. cholesterol, HDL cholesterol, LDL cholesterol, VLDL cholesterol and T. triglycerides).

Sample collection and storage

Collection of blood sample did with 5mL syringe. Blood is transferred from syringe into two different kinds of vials both vials have 2.5mL blood of equally sized. Vial one contains EDTA inside which is specialized to avoid blood clotting and plasma portion was used for estimation of vitamin C level, second vials was consist of simple gel containing vial. Serum was separated with the help of centrifugation at 3000 rpm which was used for lipid profile (T.cholesterol, HDL cholesterol, LDL cholesterol, VLDL cholesterol and T.triglycerides) analysis.

Lab diagnosis of malaria

The benign malarial infection plasmodium falciparum or plasmodium vivax is diagnosed with the help of lateral flow of chromatographic method which is commonly referred as strip component method. The strip is coated with two types of anti-body bands one is mouse anti-Pv-LDH antibody and other is anti-pHRP-II antibody which are used for detection of Pv and Pf based infection. One control band is also present on strip, it contain pre coated

anti-mouse IgG for the justification of result [13].

Ascorbic acid

Ascorbic acid was measured by the modified method of Roe and Keuther [14] by Nino and shah [15]. The ascorbic acid is converted to dehydro ascorbic acid by shaking with freshly prepared metaphosphoric acid. This is then coupled with 2, 4 dinitrophenylhydrazine in the presence of thiourea as a mild reducing agent. Sulphuric acid then converts the dinitrophenylhydrazine into red compound which was estimated spectrophotometrically

Estimation of lipid profile parameters

All the above mentioned parameters were measured by using a spectrophotometry comparison of the absorbance of samples with the standards solutions at different wavelengths the first (plasma ascorbic acid) was measured by using laboratory chemicals such as mentioned above in the method and the rest were measured by using internationally known kits of Analyticon Germany for total cholesterol estimation [16], while VLDL-cholesterol concentration was measured by dividing the serum TG concentration by 5.

$VLDL-c \text{ (mg/dL)} = \text{Triglyceride (TG)} / 5$

Low density lipoprotein cholesterol (LDL-c) was measured by Friedwald's formula [17]. LDL-cholesterol was obtained by subtracting HDL-cholesterol and VLDL-cholesterol from T. cholesterol as shown in given formula

$LDL-c = TC - (HDL-c + VLDL-c)$

Statistical analysis

The values acquired from the assessment of these parameters were organized in the form of data on excel and then this data was further analyzes by paired sample T-test and ANOVA. Mean and standard error (S.E.) data were also figured. Statistical software SPSS-20 was used for this resolution [18].

Results

The project was designed to analyze the blood vitamin C level with some lipid profile parameters in newly diagnosed patients of malarial infection and compared with normal healthy individuals. In order to check the effect of ascorbic acid on patients of malarial infection and to compare their levels with the normal healthy subjects in Sargodha division of Pakistan.

Subjects were selected by simple random technique from the out patients department of local and district headquarter hospital Sargodha. On the day of enrollment and sample collection, the subjects were explained fully about the protocol of the study and detailed history of subjects was taken including their locality, occupation and diet history.

Firstly most of the patients were reluctant to give blood samples and to satisfy them was the most problematic task and it had taken lot of energy and time to convince them. Majority of patients were belonging to nearby villages. In contemporary study 65 subjects suffering from malarial infection were selected. Subjects were distributed on the basis of their age and Gender. 43 normal healthy subjects were included in this protocol.

Ascorbic acid (vitamin C)

Table 1 and Figure 1 show mean $\pm$ SE results, data are divided into two groups. First was ≤ 15 years age which subdivided into two groups male and female. The comparison of vitamin C in male (≤ 15) between normal and malarious showed the significant decrease in malarious subjects ($p < 0.01$). In females (≤ 15) also show the same significant decrease in malarious subjects as compared to normal in vitamin C ($p < 0.01$).

In the other group where the age was (> 15 years) further subdivided into male and female. The comparison of vitamin C in male (> 15) and female (> 15) both depicts

statistically by applying the independent sample t-test significant decrease in

malarious subjects ($p < 0.01$).

Table 1. Comparison of vitamin C between normal and malarious subjects

Statistical Parameters	Less than or equal to 15 years (mg/dl)				Greater than 15 years (mg/dl)			
	Male		Female		Male		Female	
	Normal	Malarious	Normal	Malarious	Normal	Malarious	Normal	Malarious
Mean	0.3503	0.2006	0.3040	0.2151	0.4382	0.2844	0.3878	0.2771
S.E	0.02891	0.00678	0.01647	0.00932	0.01821	0.01212	0.01110	0.01449
p-value	0.005**		0.000**		0.000**		0.000**	

Level of significance as (**=0.01 Highly Significant and *=0.05 Significant), While non-significant is indicated by (ns).

Figure 1. Comparison of vitamin C between normal and malarious subjects

Lipid Profile

Table 2 tells about mean and standard error, results data of our study was divided into two groups. First is ≤ 15 years age which was subdivided in to two groups male and female. In male (≤ 15) the comparison of total cholesterol and HDL cholesterol between normal and malarious showed significant ($p < 0.01$) decrease in malarious and non-significant values for LDL cholesterol, VLDL cholesterol and total triglyceride. In females (≤ 15) revealed that there was non-significant variation in total cholesterol, LDL cholesterol, VLDL cholesterol and total triglyceride in

malarious subjects as compared to normal but In females (≤ 15) revealed that significant ($p < 0.01$) decrease in HDL cholesterol in malarious subjects as compared to normal.

In the second group where the age was (> 15 years) further subdivided into male and female. The comparison of HDL cholesterol in male (> 15) group revealed a significant ($p < 0.01$) decrease the values of rest of parameters in male (> 15) and female (> 15) groups both tells about non-significant in malarious subjects than normal subjects by applying the independent sample t-test.

Table 2. Comparison of lipid profile between normal and malarious subjects

Bio chemical parameters	Statistical Parameters	Less than or equal to 15 years (mg/dl)						Greater than 15 years (mg/dl)					
		Male			Female			Male			Female		
TC		Normal	Malarious	p-value	Normal	Malarious	p-value	Normal	Malarious	p-value	Normal	Malarious	p-value
	Mean	158.40	136.46	0.00**	145.67	133.71	0.10 ^{ns}	182.06	172.96	0.13 ^{ns}	176.29	174.82	0.87 ^{ns}
	S.E	6.90	2.55		6.42	2.89		3.50	4.70		6.00	6.40	
HDL-C	Mean	45.40	39.54	0.00**	43.17	38.43	0.00**	43.72	41.82	0.01**	41.42	41.29	0.84 ^{ns}
	S.E	1.12	0.48		0.60	0.30		0.56	0.41		0.43	0.43	
LDL-C	Mean	85.60	68.77	0.01**	81.00	69.57	0.05 [*]	103.47	97.16	0.39 ^{ns}	106.14	97.25	0.24 ^{ns}
	S.E	6.11	2.49		5.29	1.95		5.55	4.64		4.40	5.61	
VLDL-C	Mean	25.40	27.77	0.37 ^{ns}	21.67	25.14	0.23 ^{ns}	35.21	34.25	0.60 ^{ns}	34.43	35.45	0.67 ^{ns}
	S.E	3.03	1.13		2.12	1.78		1.25	1.19		0.70	2.22	
TG	Mean	127.00	139.85	0.34 ^{ns}	108.33	127.86	0.18 ^{ns}	163.94	172.04	0.45 ^{ns}	174.36	177.35	0.82 ^{ns}
	S.E	15.13	5.75		10.62	8.53		9.30	5.98		4.40	11.19	

T-test for independence reveals as ** when $P\text{-value} \leq 0.01$ (Highly Significant), * when $P\text{-value} \leq 0.05$ (Significant) and non-significant (ns) when $P\text{-value} > 0.05$

Discussion

Uzuegbu [19] reported in his study on extreme instances of malaria, in male youngsters plasma ascorbate was detected to be 0.28 ± 0.88 mg/dL and in female 0.41 ± 0.33 mg/dL. Amongst the control samples, plasma ascorbate level for the male kids was detected to be 1.41 ± 0.23 mg/dL and in females it was 1.66 ± 0.22 mg/dL, in male grown-ups it was observed to be 1.34 ± 0.39 mg/dL and in females it was observed to be 1.37 ± 0.51 mg/dL. From the presentation above, it was found that the mean ascorbate level for grown-ups was observed to be higher than that of youngsters. In contrast with their control, it was found that the ascorbate level of grown-up control was greater than that of the youths. The finding values of my concentrate were totally concurred with study directed by Uzuegbu [19].

How vitamin C impact on malarial infection was clearly demonstrated in present study. My results were also confirmed by another study conducted by Raza *et al.* [20], in which they assessed the serum vitamin C concentration in 150 severe falciparum-malaria patients (aged 02 to 10 years). Serum vitamin C level estimate of twenty healthy volunteers (aged 02 to 10 years) was also included as control. The mean serum ascorbic acid concentration of fit controls was 1.163 ± 0.059 mg/dL and that of unhealthy cohort was 0.685 ± 0.0145 mg/dL. The diseased cohort demonstrated significant reduction in concentrations of ascorbic acid in comparison to healthy controls ($p < 0.001$).

However work by Hassan *et al.* [21] couldn't help, contradicting my outcomes. Their outcomes were as per the following. The mean serum level in healthy guys was 1.54 ± 0.10 mg/dL and 2.02 ± 0.20 mg/dL in infected guys, ($p < 0.05$). Among the females, the mean serum ascorbate focus was observed to be 1.54 ± 0.10 mg/dL in

healthy adults and 2.03 ± 0.24 mg/dL in their infected partners, ($p < 0.05$). The mean serum grouping of ascorbate in youngsters was 2.9 ± 0.17 mg/dL in healthy kids and 1.95 ± 0.20 mg/dL in infected kids. This obviously demonstrates an inversion pattern when contrasted with the outcomes acquired in adults. The serum ascorbate grouping of 2.9 ± 0.20 mg/dL in healthy kids was fundamentally higher than the typical adults serum centralization of 1.54 ± 0.10 mg/dL ($p < 0.05$), being roughly two times value in healthy adults.

A study conducted by D'Souza V and D'Souza B [22] revealed that vitamin C level significantly decreased in Plasmodium Vivax infection 0.784 ± 0.434 mg/L. Similarly present study showed also significantly decrease level in vitamin C. A significant decrease was observed in the vitamin C, as malarial infection associated with significant destruction of erythrocytes lead to the release of metabolic and toxic products, including some oxidant compounds. Productions of oxidants cause the decrease in level of antioxidants including vitamin C level. Chikezie *et al.* [23] presented that serum lipid profile of non-malarious subjects among the age brackets of 11 to 20 and 21 to 31 years were usually inside reference intermissions. The lipid parameters were relatively upper in individuals within age brackets of 21 to 31 years ($p < 0.05$). Likewise, serum T of malarious subjects (11 to 20 years) was within reference intervals and was not significantly different ($p > 0.05$) from non-malarious counterparts. The present study showed agreed result except (≤ 15) year male group.

HDL-C = 31.10 ± 7.12 mg/dl ($p < 0.05$) of malarious subjects, which was below reference intervals. Serum concentrations of HDL-C ($p > 0.05$) of the two age brackets of malarious subjects were lower than corresponding non-malarious individuals.

The present study showed agreed result with work done by Chikezie *et al.* [23].

LDL-C = 30.90 ± 7.10 mg/dl ($p < 0.05$) of malarious subjects, which was below reference intervals. Serum concentrations of LDL-cholesterol ($p < 0.05$) of the two age brackets of Malarious subjects were lower than corresponding non-malarious individuals. The present study showed disagreed result with worked done by Chikezie but agreed with result of male (≤ 15).

Karishna *et al.* [11] had worked on comparison of lipid profiles of control group and malaria patients showed that Total cholesterol of Control group 130 ± 28.6 mg/dL and MP positive 199.4 ± 27.2 showed mg/dl Significant $P < 0.05$ variation clearly showed reversal trend. HDL cholesterol of Control group 43.25 ± 12.15 mg/dl and MP positive 36.16 ± 5.04 showed mg/dl Significant $P < 0.05$ variation. LDL cholesterol of control group 92.5 ± 8.3 mg/dl and MP positive 135.56 ± 32.57 showed mg/dL significant $P < 0.05$ variation clearly disagreed with the present study results. VLDL cholesterol of control group 76.9 ± 8.8 mg/dL and MP positive 79.6 ± 3.6 showed mg/dL non-Significant variation clearly agreed with the present study results. TG of control group 148.25 ± 16.59 mg/dl and MP positive 190.21 ± 31.47 showed mg/dl significant ($p < 0.05$) variation clearly disagreed with the present study results.

A significant decrease was observed in HDL cholesterol and LDL cholesterol, such changes are essentially enhanced mobilization of free unsaturated fats from fat tissue to react anxiety. Fat tissue lipolysis, builds de novo hepatic fatty acid synthesis and inhibition of unsaturated fat oxidation in serious disease are basic. Now and again there might be some destruction in lipoprotein lipase. Total cholesterol, T. triglycerides and VLDL cholesterol were

found not noteworthy, which likewise rely on the seriousness of malarial infection.

Conclusion

Malaria has a significant impact on biochemical and hematological parameters, mostly caused deficiency in vitamin C and deranged parameters in lipid profile. Clinical presentation of various plasmodium species was almost similar with few differences. Malaria must be considered as a leading cause in differential diagnosis in acute febrile patients with more abnormalities like deficiency in vitamin C. Peripheral smear study is the gold standard investigation for identification of different forms of parasites and determination of parasitemia level. The conclusion of this study was that alteration in these parameters suggests proper monitoring during treatment in order to reverse them to normal levels.

Authors' contributions

Conceived and designed the experiments: MZU Hassan & T Mehmood, Performed the Experiments: R Mushtaq, Analyzed the Data: R Mushtaq & T Mehmood, Contributed reagents/ materials/ analysis tools: T Mehmood, Wrote the paper: R Mushtaq & T Mehmood.

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