OXIDATIVE STRESS AMONG PATIENTS WITH SOME DIFFERENT PARASITIC INFECTIONS

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ABSTRACT

Objective: To estimate the oxidative stress status in patients with different parasitic infections.

Methods: A total of 230 blood samples obtained from individuals aged 1-65 years during the first 9 months of 2007 in Basrah, Iraq were used. One hundred samples collected from patients with parasitic infections (35 patients with toxoplasmosis, 32 patients with leishmaniasis, 24 patients with amoebiasis and 9 patients with giardiasis) while the rest 130 were apparently healthy subjects and considered as a control group. The oxidative stress status was determined by measuring serum malondialdehyde (MDA) level as indicator of lipid peroxidation, erythrocytes superoxide dismutase (SOD) as indicator of endogenous antioxidant enzymes level and zinc level as cofactor for antioxidants enzymes.

Results: Serum MDA level for patients group was (1.02+0.26 µmol/L) which was significantly higher than that in the control group (0.83±0.12 µmol/L) ((P<0.001). Serum MDA levels were increased significantly with increasing age (P<0.001). The levels of erythrocytes SOD were significantly lower (P<0.001) in patients than in control subjects and its level is decreasing with increasing age. There were significant negative correlation between serum MDA and erythrocytes SOD level among studied groups. Serum zinc level was decreased significantly in patients group (84.95±13.31µg/dl) when compared to healthy subjects (94.48±6.6µg/dl) (P<0.001).

Conclusion: The parasitic infections are associated with significant degree of free radicals formation as indicated by significantly higher MDA and lower SOD levels among those patients.

INTRODUCTION

Reactive oxygen species (ROS) formed during multiple normal processes in tissues and cells as well as their excessive formation in state of oxidative stress play an important role in the pathogenesis of various diseases.[1] ROS-generating systems kill murine malaria parasites *in vitro*[2] and *in vivo*[3,4] and kill *Plasmodium falciparum* in vitro. [5] The possibility that phagocyte-derived ROS are involved in the host antimalarial immune response which has been investigated only indirectly in past studies. Trophozoites of *E. histolytica* have been well known to induce apoptosis of host cells including neutrophils, T-lymphocytes, and macrophages *in vitro*.[6] Higher concentrations of H_2O_2 increased the loss of motility as well as cell death of *Leishmania donovani*, suggesting that the degree of oxidative stress that the cells are exposed to, is very important for the ability of the cell to survive. Similar phenomenon with a superoxide generation system has been observed in *Trypanosoma brucei brucei*.[7] Myeloperoxidase is believed to be involved in augmenting the cytotoxic activity of H_2O_2. Low nicotinamide adenine dinucleotide phosphate (NADH)-oxidase and myeloperoxidase activity were observed in monocytes of patients with active visceral leishmaniasis as compared with healthy controls. These results suggest that low NADPH-oxidase, may account for the persistence of Leishmania parasites in visceral leishmaniasis.[8] The main objectives were to estimate the oxidative stress status in patients with different parasitic infections by measuring serum malondialdehyde (MDA) level as indicator of lipid peroxidation, erythrocytes superoxide dismutase (SOD) as indicator of endogenous antioxidant status and zinc level as a cofactor for antioxidants enzyme (SOD).

MATERIALS AND METHODS

This study was conducted during the first 9 months of 2007 in Basrah, Iraq. A total of 230 subjects aged (1-65) years were included in the study. There were 35 patients with toxoplasmosis, 32 patients with leishmaniasis (13 of them were with cutaneous leismaniasis and the rest, 19 were with visceral leishmaniasis), 24 patients with amoebiasis, 9 patients with giardiasis, while the rest 130 subjects were apparently healthy individuals and considered as control group. Patients were diagnosed and followed up by specialist physicians at Al-Sader Teaching Hospital and Basrah Hospital for Maternity and Children. The work has been approved by the ethical committee of Basrah Medical College. About

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5ml of fresh venous blood was collected from each patient and control subjects. About 2ml of it was added to EDTA anticoagulant tubes to be used immediately for antioxidant enzymes (SOD).[9] While the rest was allowed to clot in a clean plain tube, centrifuged at 3000 rpm for 10-15 min and sera were separated to be used for measuring MDA [10] and zinc level. [11]

The results were expressed as mean ± SD. The data were analyzed statistically by one-way analysis (ANOVA) while correlation between the data was tested statistically by simple linear regression test using SPSS program.

P-value of less than 0.05 was considered as statistically significant, P-value less than 0.01 as highly significant and P-value of less than 0.001 as extremely significant.

RESULTS

The general characteristics of all subjects participated in this study were presented in table (1).

Table 1. Basic clinical characteristics of all studied groups.

Variables		Control	Patients				
			Toxoplasmosis	Leishmaniasis		Giardiasis	Amoebiasis
				Cutaneouse leishmaniasis	*Visceral leishmaniasis*		
Total (No.)		130	35	13	19	9	24
Age (years)		(1-62)	(16-43)	(2-65)	(1-9)	(1-8)	(1-58)
Sex	***Male***	53		4	10	8	14
	female	77	35	9	9	1	10

Serum level of MDA in patients was (1.02± 0.26 µmol/L) which was significantly higher than that in the control group (0.83±0.12 µmol/L) (P<0.001) (Table-2). Serum level of SOD was significantly lower in patients compared to the control (1045.37 ± 138.55 U/g Hb vs 1496.85±156.52 U/g Hb, P<0.0001). Serum level of zinc was also significantly lower in patients compared to the control (84.95 ±13.31 µg/dl vs 94.48± 6.6 µg/dl, (P<0.001).

Table 2. Basic biochemical characteristic in all studied groups

Parameters	Patients N=100	Control N=130	P-value
SOD U/g Hb	1045.37±138.55	1496.85 ±156.52	0.000
MDA µmol/L	1.02±0.26	0.83±0.12	0.001
S. Zn µg/dl	84.95±13.31	94.48±6.60	0.001

Value were expressed as mean ±SD

Table (3) shows all biochemical characters of different groups of patients. Serum level of MDA was increased in all patients sub-groups as toxoplasmosis, cutaneous and visceral leishmaniasis, amoebiasis and giardiasis, and this increase was statistically significance when compared with control subjects. Patients with cutaneouse leishmaniasis shows the highest level of serum MDA (1.93±0.35µmol/L) as compared with other subgroups of patients, while patients with toxoplasmosis shows the lowest levels of serum MDA (1.09±0.28 µmol/L) among patients subgroups. However, this increase was statistically not significant among subgroups of patients. The erythrocyte SOD activity was decreased in all patients with toxoplasmosis, cutaneous or visceral leishmaniasis, amoebiasis or giardiasis, which was statistically significant when compared with healthy subjects. The lowest level was in amoebiasis (1012.7±143.66 U/g Hb) and the highest level was in giardiasis (1082.11±135.08 U/g Hb). However there were no significant differences between patient's subgroups.

Table 3. Basic biochemical character according to the type of parasites in patients and control groups.

Parameters	Toxoplasmosis N=35	C.leishmaniasis N=13	V.leishmaniasis N=19	Giardiasis N=9	Amoebiasis N=24	Control N=130
S. MDA µmol/L	1.09***[a] ±0.28	1.93***[b] ±0.35	1.17***[c] ±0.24	1.16***[d] ±0.24	1.30***[e] ±0.32	0.83 ±0.12
Erythrocyte SOD U/gHb	1068.57***[a] ±131.14	1053.15***[b] ±131.69	1021.15***[c] ±152.46	1082.11***[d] ±135.08	1012.70***[e] ±143.66	1496.85 ±156.52
S. Zn µg/dl	81.11***[a] ±11.92	80.11***[b] ±13.94	84.12***[c] ±12.21	85.86***[d] ±12.25	83.13***[e] ±10.45	94.48 ±6.6

Values expressed as mean ± SD

***significance difference as compared with healthy subject (P<0.001).

a. significance difference as compared between patients with toxoplasmosis and healthy subjects .

b. significance difference as compared between patients with cutaneouse leishmaniasis and healthy subjects .

c. significance difference as compared between patients with visceral leishmaniasis and healthy subjects .

d .significance difference as compared between patients with giardiasis and healthy subjects.

e. significance difference as compared between patients with amoebiasis and healthy subjects.

Serum zinc was decreased for all patient groups. These results were statistically significant as compared with control group. Marked reduction in serum zinc level was seen in patients with cutaneouse leishmaniasis wheras serum zinc level was slightly reduced in patients infected with giardiasis. There was no statistical significance as compared between patient groups. In general, all patient groups show the same results and there were no statistical differences in between them. In general there was increasing level of serum MDA in both sexes (male and female) in patients, which was significantly higher as compared with control in both sexes (P<0.001) (Table-4). Patient group showed a significant decrease in erythrocytes SOD in both sexes when compared with control group (Table-4).

Table 4. Serum level of MDA (µmol/L) and erythrocytes SOD (U/g Hb) in relation to sex in patient and control groups.

Parameters	Sex	Control	Patients	P- value
MDA	Male	0.81 ± 0.11 (53)	1.04 ± 0.28 (36)	0.001
	Female	0.84 ± 0.12 (77)	1.01 ± 0.25 (64)	0.001
Erythrocyte SOD	Male	1057.66 ± 154.04 (53)	1015.91 ± 137.42 (36)	0.001
	Female	1489.41 ± 158.77 (77)	1061.93 ± 137.47 (64)	0.001

In patients group, a statistically negative correlation was found between MDA with SOD and zinc level (Table-5). Positive correlation was determined between MDA and age. In control, SOD and serum zinc shows negative correlation with serum MDA, but it was not significant. In patients, a statistically positive correlation (P<0.01) was found between SOD with serum zinc level (Table-5). Negative correlation was found between SOD and age. In control, age with SOD showed significant negative correlation, while SOD and serum zinc shows positive correlation with serum MDA, but it was not significant.

Table 5. Correlation coefficient (r) between either MDA or erythrocyte SOD and other biochemical parameters in patient and control groups.

	Parameters	Patients	Control
MDA	Age	0.150	0.341**
	SOD	- 0.548**	-0.084
	S. Zn	-0. 266*	-0.111
SOD	Age	-0.026	-0.183*
	S. Zn	0.254*	0.062

Values expressed as correlation coefficient (r).
***,**correlation is significant at the 0.05 and 0.01 levels respectively**

DISCUSSION

Highly reactive oxygen species (ROS) have been indicated in the pathogenesis of various parasitic infections including *Leishmania,*[12-14] *Toxoplasma gondii*[15] *Giardia lamblia*[16] *Entameba histolytica*.[17] Macrophages, neutrophils and other phagocytic cells are key components of the antiparasitic, antimicrobial and tumoricidal immune responses. These cells are capable of generating large amounts of ROS and reactive nitrogen species (RNS). ROS and RNS have a possible role in the pathogenesis of parasitic diseases.[18] Increased levels of lipid peroxidation products have been associated with a variety of chronic diseases in both humans and model systems. Oxidative stress as a mediator of tissue damage concurrent with the result of the present study strongly suggest that one of the main reasons for high MDA levels in patients with parasitic infection. To the best of our knowledge, this type of work has been done for the first time in Iraq. Free radical theory of aging is one of the most widely accepted theories. In aerobic organisms, ROS are by product of their normal metabolisms. ROS are highly reactive, they attach the important molecule in cell, damage the cell components. To survive under such threats, organisms have developed defense mechanisms against free radicals. However the antioxidant mechanisms are not strong enough to remove all ROS produced during physiological or pathological processes. Then oxidative damage occurs, if the damage cannot be repaired, its effects will accumulate with age.[19] In the present study serum MDA level was increased with increasing age for all studied groups. This finding can support age theory. Two studies have suggested that oxidative damage is accumulated during aging. [19,20] Elderly patients are therefore more susceptible to free radical-induced damage and lipid peroxidation than can be accounted for by normal aging. In the present study the activity of antioxidant enzyme (SOD) in erythrocytes of patients suffering from parasitic infection was significantly lower than those of control group, this finding is in agreement with some studies[12-16,20-23] but it is against others.[24,25] Hydrogen peroxide formation as result of parasitic infection will inactivate SOD [26] which is may be one of the explanation for the decreased level of SOD in these patients; other reason also may be due to a decrease in zinc which can cause depletion in Cu-Zn- SOD activity.[27] This study showed that activity of SOD decrease with increasing age. This finding goes with the finding of other studies.[28,29] In the present study it has been found a positive correlation between serum zinc level and SOD activity which suggest that antioxidative enzymes activity depends on their cofactor concentrations. Significant negative correlation between SOD activity and MDA generation suggest an increase utilization by ROS as an important contributing factor to the lower concentrations of anti-oxidants, and this support the link between oxidative stress and infection.[14,16] Zinc is required as a cofactor for the function of intracellular enzymes that may be involved in protein, lipid and glucose metabolism.[30] The present study showed a significant decrease in serum zinc level in patient group in comparison with control group. This was in agreement with others. [16,22] This may be due to dietary factors (intake of food low in antioxidant). So, body will use endogenous SOD to scavenge the over production of ROS as a result of parasitic infection which leads to decrease serum zinc level.[31] Also it could be due to the antioxidant effect of zinc which play a structural role in the maintenance of Cu-Zn-SOD structural integrity.[31] Serum zinc level decreases with

increasing age. This finding goes with aging theory, as SOD decreases with increasing age and serum zinc level correlate positively with erythrocyte SOD and also it correlate negatively with serum MDA which will increase with increasing age. So this finding is accepted according to the theory of age.

In conclusion, the parasitic infections are associated with significant degree of free radicals formation as indicated by significantly higher MDA and lower SOD levels among those patients.

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