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Lipid Peroxidation and some Haematological Changes in *Entamoeba histolytica* Patients

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ABSTRACT

Hospital based study was conducted on 57 patients of Al-Zahrawy hospital returners in Mosul city, to investigate the role of intestinal protozoan parasite *Entamoeba histolytica* infection in lipid peroxidation and some haematological parameters in human.

The results revealed that plasma malondialdehyde (MDA) level (µmole/l.)was significantly higher in patients both females and males (5.322 ±0.478, 4.157 ± 0.722) respectively, as compared with its level in healthy female (2.266 ±0.215) and male (2.037 ±0.288). While the glutathione (GSH) level (µmole/l.) in plasma was significantly decreased in patients both in females and males (5.599 ± 0.561, 7.942 ± 0.495) respectively, as compared with its level in healthy females (12.526 ±1.445) and males (14.363 ±1.321). Hemoglobin content (g/dl.) of patients was significantly decreased both in females and males (9.329 ±1.173, 11.955±1.214) respectively, as compared with healthy females (10.664 ±2.66 and males (15.096 ± 1.836), also the packed cell volume was decreased in patients both in females and males (27.926 ±2.73 , 30.233 ±2.88) respectively, as compared with healthy females (39.05 ±2.075) and males (46.55 ±3.179).

In conclusion, *Entamoeba histolytica* infection resulted in high plasma MDA level and low GSH level beside anemia.

بيروكسدة الدهن وبعض التغيرات في مكونات الدم في المصابين بالطفيلي المعوي *Entamoeba histolytica*

الملخص

اجريت الدراسة الحالية على 57 مريضا من مراجعي مستشفى الزهراوي في مدينة الموصل، لبحث دور الاصابة بالطفيلي المعوي (*Entamoeba histolytica*) في بيروكسدة الدهن وبعض المقاييس الدموية.

اظهرت النتائج زيادة مستوى المالونداليديهايد (مايكرومول/لتر) في بلازما الدم للمصابين بمعدل بلغ في الاناث والذكور(5.322 ±0.478، 4.157 ± 0.722) على التوالي، مقارنة بمستواه في الاصحاء الاناث (2.266 ± 0.215)والذكور(2.037 ±0.288)، بينما اظهرت النتائج خفضا معنويا في مستوى

الكلوتاثايون(مايكرومول/لتر) في المصابين من الاناث والذكور(5.599 ± 0.561، 7.942 ± 0.495) على التوالي ، مقارنة بمستواه في الاصحاء االاناث(12.526 ± 1.445) والذكور (14.363 ± 1.321).

انخفض تركيز الهيموكلوبين(غم/دسيلتر) في المصابين الاناث والذكور (9.329 ± 1.173 ، 11.955 ± 1.214) على التوالي ،مقارنة بتركيزها في الاصحاء الاناث(10.664 ± 2.66) والذكور (15.096 ± 1.836). وكذلك انخفض حجم الخلايا المرصوصة في المصابين الاناث والذكور (27.926 ± 2.73 ، 30.233 ± 2.88) مقارنة بحجمها في الاصحاء الاناث (39.05 ± 2.753) والذكور(46.55 ± 3.179).

يستنتج من الدراسة الحالية ان الاصابة بأميبا الزحار تؤدي الى زيادة في تركيز المالوندايالديهايد وخفض في مستوى الكلوتاثايون الى جانب حصول فقر الدم.

INTRODUCTION

The intestinal protozoan parasite *Entamoeba histolytica* which causes two major clinical syndromes, amebic colitis and liver abscess, remains a significant cause of morbidity and mortality worldwide (Stanley and reed, 2001). Amoeba can both lyse host cell and induce tits suicide through programmed cell death, the neutrophils may play a key role in contributing the tissue damage in amebiasis and in controlling amebic infection (Stanley and Reed, 2001). Lysis of neutrophils by *Entamoeba histolytica* trophozoites may release mediators that lead to hepatocyte death and extend damage to hepatocytes (Tsutsumi et al., 1984). Nitric oxide has been linked to macrophage killing of *Entamoeba histolytica* trophozoites (Stanley and Reed, 2001)

There are important changes in the cell biochemistry of the hosts suffering from parasitic invasion depending on the species of parasite and the site of invasion (Dede et al., 2002). Also the intestinal parasite caused many hematological changes. In goats, the intestinal parasite caused anemia by decreasing the amount of hemoglobin and numbers of erythrocyte causing thrombocytopenia, and as a result of hemoglobin metabolism, free oxygen radicals and hydrogen peroxide increased leading to excess lipid peroxidation (Dede et al., 2002). Also red blood cells as most cells exposed to oxidative stress causing physiochemical changes in the membrane of these cells leading to lipid peroxidation and hemolysis producing anemia (Egwunyenga et al., 2004).

The polyunsaturated fatty acids and other lipids in the cells membrane through chain of reactions with intermediate free radicals form lipid peroxidation end products as malondialdehyde (Facundo et al., 2004). Therefor measurement of malondialdehyde is widely used as an indicator of lipid peroxidation. Increased level of lipid peroxidation product have been associated with a variety of diseases in both human and animal model systems(Suleyman et al., 2003). Reactive oxygen species (ROS) are involved in intracellular killing as a mechanism of cell defense, (ROS) and hydrogen peroxide have been shown to play important role in host defense and killing protozoa in the cells (Kocyigit et al., 1999).

Also these parasites lead to changes in antioxidant levels of the host due to oxidative stress and disturbance in free radical production. It had been shown that parasitic infections cause decrease in antioxidant capacity of the host, increased formation of

reactive oxygen species is secondary to the primary disease process (Kocyigit et al., 1999). In mice the antioxidant capacity is reduced and lipid peroxidation end product (MDA) level increased about 10% as a result of liver damaged by schistosomiasis (Facundo et al., 2004). Also increased level of (MDA) was seen in patients infected with *Ascaris lumbricoides* (Eser et al., 2003). In cutaneous leishmaniasis, some antioxidant as glutathione decreased as compared with control persons (Kocyigit et al., 1999). Also increased lipid peroxidation and decreased antioxidant level were seen in malaria patients (Kulkarni et al., 2003)

This study aimed to investigate the effect of *Entamoeba histolytica* infection on levels of plasma malondialdehyde and glutathione, beside some hematological parameters.

MATERIALS AND METHODS

Subjects

The study comprised 57 infected patients with *Entamoeba histolytica* (30 male and 27 female) chosen among 684 patients suffering abdominal pain of Al-Zahrawy hospital returners from october, 2001 to march, 2002 in Mosul city. The disease diagnosed on the bases of clinical finding and microscopically positive stool examination for *Entamoeba histolytica.* All the subjects were at age of (5-15) years and none of the patients had any chronic disease. Healthy subjects were chosen as control groups from primary and secondary schools students in Mosul city.

Blood collection

5 ml samples of venous blood was collected in an EDTA tube before antiparasite therapy. The hemoglobin and packed cell volume (PCV) were determined directly after blood collection. Plasma was separated by centrifugation for 15 min. at 3000 rpm, and stored at (- 4 °c.) till assay.

Hematological parameters

Blood hemoglobin concentration of each blood sample was determined by diluting 0.02 ml of blood with 5 ml of Drabkin solution and the absorbence was recorded at wave length of 540 nm using spectrophotometer. Packed cell volume of each sample was determined by capillary tubes containing sodium and potassium oxalate, then centrifuged at 3000 rpm for 3 min. using microhaematocrit centrifuge and the PCV was determined using microhaematocrit reader (Said and Al-Habbib, 1990).

Assay of lipid peroxidation and antioxidant
Malondialdehyde(MDA)

The plasma of venous blood of subjects was assayed for lipid peroxidation by determining the malondialdehyde (MDA) level using thiobarbituric acid (TBA) reaction (Wysocka et al., 1995), which depend on reaction of TBA with lipid peroxides. 0.2 ml of plasma was mixed with 1 ml of TBA solution in presence of 4 ml of trichloroactic acid solution. After incubation for 15 min. the mixture was centrifuged and the absorbence of the supernatant was determined at wave length 532 nm using (4050 Uv/Visible spectrophotometer LKB). Calibration curve of standard absorbence against different concentrations was prepared. The MDA concentration was expressed as µmole / liter.

Glutathione (GSH)

Plasma glutathione level was determined using modified Ellman's method. 150 microliter of plasma was incubated with equal volume of sulfosalicylic acid (4%). After 5 min. the supernatant was separated by centrifugation at 2000 rpm for 5 min., 150 microliter of supernatant was mixed with 4.5 ml of Ellman's reagent. The absorbence was determined at 412 nm using spectrophotometer. The glutathione concentration was measured according to the following formula:

$$\text{GSH (Micromole/liter)} = \frac{\text{O. D. of sample}}{E_0 \times L}$$

$E_0 = 13600\ M^{-1}\ CM^{-1}$
L = Light path (cm).

RESULTS

Among(684) patients with diarrhea and abdominal pain, only 57 had infected by *Entamoeba histolytica.* The results showed alterations in some haematological parameters. A significant decrease ($P< 0.05$) in hemoglobin concentration(g/dl) was noted in *Entamoeba histolytica* patients in both sexes, in females(9.329 ±1.173)and in males(11.955 ±1.214) as compared with its concentration in control subjects of related sex, in females (10.664 ±2.66) and in males (15.096 ±1.836) (Fig. 1).

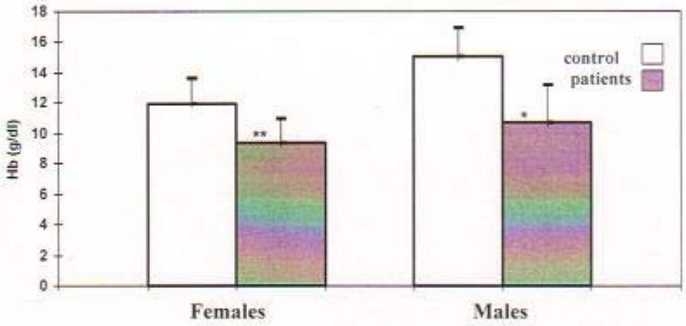

Fig.1: Hemoglobin(g/dl) content of *Entamoeba histolytica* Infected patients and healthy subjects
The values are expressed as mean ± S.E. / * ($P< 0.05$) / ** ($P<0.01$)

Figure (2) showed percentage of the packed cell volume (PCV) which was also significantly ($p<0.05$) decreased in patients with *Entamoeba histolytica* intestinal parasite both in females (27.926 ±2.73%) and males (30.233 ±2.88%) as compared to healthy subjects (39.05 ±2.075%) in females and (46.55 ±3.179%) in males.

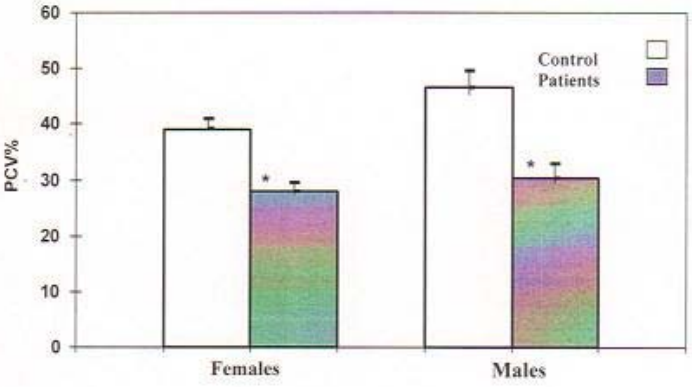

Fig. 2.:Percentage of packed cell volume of *Entamoeba histolytica* infected patients and healthy subjects
The values are expressed as mean ± S.E. / * (P< 0.05)

The results of the present study revealed a significant (P< 0.05) increase in the level of plasma MDA (μmole/l.) in females and males (5.322± 0.478, 4.157±0.722) respectively in *Entamoeba histolytica* infected patients in both sexes as compared with its level in control, female and male subjects (2.266±0.215 , 2.037±0.288) respectively. (Fig. 3).

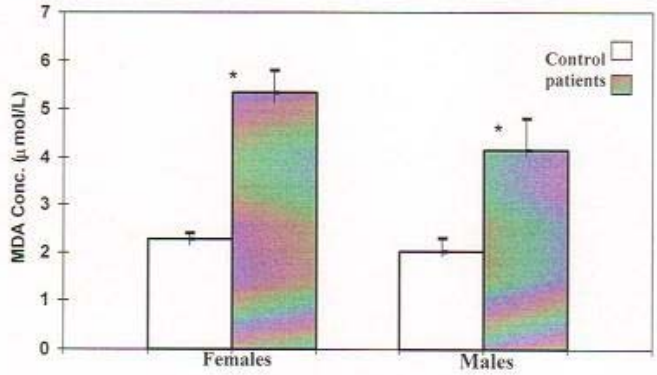

Fig. 3: Malondialdehyde(MDA) concentration of *Entamoeba histolytica* infected patients and healthy subjects
The values are expressed as mean ± S.E. / * (P< 0.05)

Figure (4) showed depression of antioxidant capacity in *Entamoeba histolytica* infected patients, the present analysis specified concurrent reduction in antioxidant in concert with the momentous increase in oxidative stress in *Entamoeba histolytica* infected patients. The glutathione level (μmol /l.) was decreased significantly (P< 0.05)

in patients (5.599 ±0.561and 7.942 ±0.495) in both females and males respectively, as compared with its level in control females and males (12.526 ±1.445 and 14.363 ±1.321) respectively.

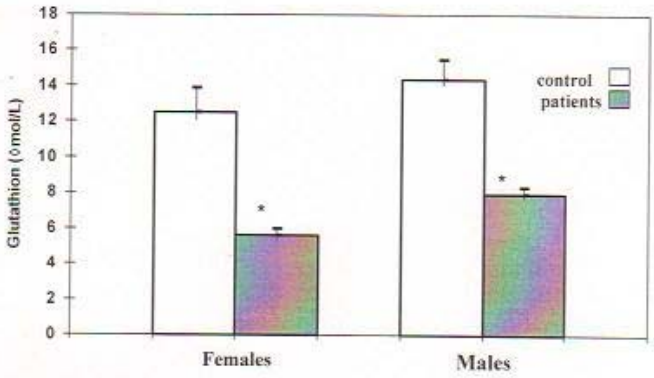

Fig. 4: Glutathione(GSH) concentration of *Entamoeba histolytica* infected patients and healthy subjects
The values are expressed as mean ± S.E. / * ($P< 0.05$)

Discussion

The results of this study demonstrated a significant decrease in the hemoglobin content and percentage of packed cell volume and a significant increase in the level of the plasma malondialdehyde while the level of the glutathione decreased significantly.

Related investigations in other protozoan parasite infected patients showed a decrease in hemoglobin content and red blood cells breakdown in malaria infected patients (Golenser and Chevion, 1989), which was contributed to that free iron which can stimulate free radical reactions to produce hydrogen peroxide that cause lipid peroxidation in membrane lipids (Egwunyenga et al., 2004). Another study demonstrated a significant increase in the level of MDA in *Toxoplasma* seropositive patients, due to a decreased activity of the defense system that protect tissue from free radical damage (Suleyman et.al., 2003). *Schistosoma mansoni* can induce the formation of certain reactive oxygen species as supperoxide anion (O^-) and hydroxyl radical (OH^-) that promotes lipid peroxidation (Facundo et al., 2004).

As it is known that lipid peroxidation is a free radical related process that may occur in biologic systems under enzymatic control or nonenzymatic for defense mechanism against free radical cellular damage as a result of oxidative stress, therefor MDA level was increased (Eser et al., 2003; Suleyman et.al., 2003). In case of parasitic infections protective enzymes as catalase, glutathione peroxidase ,and supperoxide dismutase are depleted in red blood cells of the host, increased production of hydrogen peroxide and free oxygen radicals, at the same time lead to decrease in antioxidant enzymes and other antioxidants (Egwunyenga et al., 2004). The increased level of lipid peroxidation was an ultimate toxic effect of sudden increase of reactive oxygen species production by immune

system as well as synchronized release of singlet oxygen during hemoglobin degradation, other than the production of hydrogen peroxide and hydroxyl radical during parasite infection that accelerate lipid membrane peroxidation (Kulkarni et al., 2003).

Entamoeba histolytica may induce chemical damage to erythrocyte membrane through an oxidative stress pathway. Superoxide anion, singlet oxygen and hydrogen peroxide can all contribute to erythrocyte lipid oxidation with the formation of intermediate lipid peroxides promoting lipid peroxidation related to the disease process, causing erythrocyte membrane denaturation, inducing hemoglobin breakdown, and consequently hemolysis, also the disease process leads to reduction in antioxidant defense, including consumption of glutathione during free radical scavenging.

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