

Short Communication

Serum Malondialdehyde, glutathione and nitric oxide levels in patients infected with *Entamoeba coli*

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While *Entamoeba histolytica* is a major human pathogen, other species like *Entamoeba coli* are not known to be pathogenic. Thus, there have been studies investigating the possible pathogenic nature of *E. coli*. The aim of this study is to evaluate the serum levels of malondialdehyde (MDA), glutathione (GSH) and nitric oxide (NO) levels in *E. coli*-infected patients. The study was conducted between January 2005 and December 2007. In this study the sera of 35 patients with *E. coli* infection and 40 healthy people in the control group were analyzed for MDA, NO and GSH levels. The mean values for the cases were $1.37 \pm 0.03 \mu\text{mol/l}$ for GSH, $42.38 \pm 2.96 \text{ nmol/l}$ for MDA and $44.71 \pm 1.12 \mu\text{mol/dl}$ for NO. For the control group, the mean values were 2.49 ± 0.10 for GSH, $19.47 \pm 2.25 \text{ nmol/l}$ for MDA, and $17.83 \pm 0.50 \mu\text{mol/dl}$ for NO. A statistically significant difference existed between the cases control groups. In the present study, significant increases were detected in the serum levels of MDA and NO while there was a significant decrease in the serum levels of, GSH. It is concluded that the *E. coli* infection was associated with significant oxidative stress.

Key words: *Entamoeba coli*, oxidative stress, malondiadehyde, glutathione, nitric oxide.

INTRODUCTION

Entamoeba histolytica, one of the ameobas known to parasite human, is an etiology in dysenteric illnesses. Though other species like *Entamoeba coli* are previously not known to be pathogenic, there have been studies inquiring the possible pathogenic nature of the parasite. The global incidence of the *E. coli* infection is reported as 30%. It has been also determined that the prevalence of the parasite in tropical and subtropical regions where general hygiene requirements are not met adequately may increase up to 100 % (Kaya et al., 2005; Kuman and Altintas, 1996). As reported, *E. coli* was also detected in patients with digestive system complaints, but they did not revealed any established pathogen bacteria in their examination (Kaya et al., 2005; Kuman and Altintas,

1996). The defense by the host immune system against the pa-rasites (adult and larval form) is provided through cells. Various cytotoxic agents, reactive oxygen and nitrogen by-products produced in activated phagocyte cells play role in this mechanism. These products made up of oxi-dant molecules with a nature of free radicals have an ad-verse impact on the viability of the parasite. Glutathione (GSH), also an endogen-derived tripeptid which can be synthesized in liver without any need for genetic informa-tion, consists of glutamic acid, cystein and glycine amino acids and is an important antioxidant. It protects cells against oxidative damage by reacting with free radicals and peroxides (Akkus, 1995; Amanvermez and Celik, 2004; Kurt et al., 2002).

Malondialdehyde (MDA) produced as a result of membrane lipid peroxidation, however, gives permanent damage to the cells (Akkus, 1995). A colorless gas nitric oxide (NO), on the other hand, is a simple molecule with the features of a free radical. Inducible form of Nitric

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Table 1. Comparison of the mean serum levels of GSH, MDA and NO in patients infected with *E. coli* group and the control group

	Groups	N	Mean	Standard Error	P value
GSH ($\mu\text{mol/l}$)	Patient	35	1.37	0.03	0.001
	Control	40	2.49	0.10	
MDA (nmol/l)	Patient	35	42.38	2.96	0.001
	Control	40	19.47	2.25	
NO ($\mu\text{mol/l}$)	Patient	35	44.71	1.123	0.001
	Control	40	17.83	0.50	

oxide syntheses (iNOS) exists in various cells, cytokines and bacterial toxins induce particularly phagocytic leucocytes and its synthesis. Since the activity of the iNOS enzyme is free from the calcium and cannot be controlled, it is active as long as there is arginine involved and it can catalyze NO synthesis at long-termed and high concentration (Kilinc and Kilinc, 2002).

The aim of this study was to confirm whether infection with *E. coli* provokes oxidative stress in the host by measuring changes in serum levels of anti-oxidants and oxidative agents such as MDA, NO, and GSH in patients diagnosed *E. coli*.

MATERIALS AND METHODS

Prior to the study Ethical clearance was obtained from the ethical council of the institution. Only those patients who volunteered to participate in the study were recruited. The stool and serum samples were examined in the Parasitology and Biochemistry Laboratory in Faculty of Medicine at Inonu University, Malatya, Turkey between January 2005 and December 2007.

Given the fact that an increase in the MDA levels can be observed in other parasitic diseases, the participants in both patient and control groups were examined for the intestinal parasites and cyst hydatid using manual IHA (indirect hemagglutination technique) and IFAT (indirect immunofluorescent technique) methods. Then the cases were recruited from those patients with *E. coli* alone. Exclusion criteria included other forms of parasitosis, on-going hormonal therapy, smoking and alcoholism since these conditions can also provoke alterations in MDA, NO or GSH level.

Controls were patients in whom hydatid cyst was not detected using manual IHA and IFAT methods. A total of 35 *E. coli*-infected patients were consecutively selected and included in scope of the study. These cases checked for serum levels of MDA, NO and GSH, and compared to a 40 cases of control.

The MDA levels in the serums of both groups were examined using Uchiyama and Mihara methods (1978). The method is based on the production of the pink compound producing maximum absorbance at 535 nm as a result of tiyobarbutiric acid's reaction with MDA. The GSH

level was examined using the Ellman method (Fairbanks and Klee, 1986). The level of NO was measured by reading the maximum absorbance at 545nm after cadmium-reduction of nitrate to nitrite (Kortas and Waked, 1990). The data are presented in mean values and standard errors. Normality test was done with Shapiro-Wilk method. Independent samples t-test was used for the statistical analysis. $P < 0.05$ was considered significant and the statistical analysis was done with SPSS 11.5 software.

RESULTS

The feces examination of the patients in the experiment group revealed *E. coli* alone, and the serum specimen obtained from patients showed negative cyst hydatid in manual IHA and IFA test. The mean age of the cases was 37.97 ± 8.3 years. They comprised 31 males and 4 females. On the other hand, all the 40 control subjects were males and their mean age was 40.23 ± 7.2 years.

The comparison of the serum MDA level, and serum NO and GSH activities are shown in Table 1. The serum GSH level in the cases was significantly lower than that of controls, while serum MDA and NO levels were significantly higher than those in control group ($p < 0.05$).

DISCUSSION

Some previous studies have reported an increase at MDA level in parasitic diseases (Atambay et al., 2006; Kilic et al., 2003; Yazar et al., 2004). MDA, an important product of lipid peroxidation, is produced as a result of the peroxidation of fatty acids containing three or more double bonds. The MDA product can cause the cross linkage of membrane elements by affecting the ion exchange from cell membranes, which results in a change in ion permeability and enzyme activity. It was reported that due to this feature, MDA can react with DNA's nitrogen bases and can thus be mutagenic, and genotoxic and carcinogenic for cell culture (Cortas and Wakid, 1990; Mercan, 2004). Atambay et al. (2006) reported that increased MDA activity in lymphocytes and erythrocytes in the dust-mite positive/skin test positive group shows the presence of the oxidative stress in patients with dust-mite infestation. In this study, the MDA level was found

significantly higher than that in the control group suggesting that the presence of *Entamoeba coli* provoked oxidative stress in the hosts.

Peroxynitrite (ONOO⁻), which is formed by oxidation of nitric oxide, nitrogen dioxide (NO₂), dinitrogen trioxide (NO₂O₃), and nitroxyl ions can cause oxidation. Nitrogen oxide species can initiate continuous lipid peroxidation reactions (Kilinc and Kilinc, 2002). Reactive species derived from NO are able to perform enzymatic inhibition, DNA fragmentation, base modification, oxidative destruction of membrane lipids and consumption of cellular antioxidants (Kilinc and Kilinc, 2002). It was reported that NO production could increase as a free radical following infections (Yarim et al., 2006). This claim is supported by the findings in this study wherein the serum NO level in patients infected with *E. coli* was significantly higher than that in control group.

Glutathione was also reported to protect the cells against oxidative damage. It plays a role in preventing the transformation of hemoglobin into methemoglobin due to oxidation. Moreover, it maintains the sulfhydryl (-SH) groups in proteins in a reduced state and protects these groups against oxidation, thus preventing the inactivation of the functional proteins and enzymes (Akkus, 1995; Amanvermez and Celik, 2004; Kurt et al., 2002). A decrease in the GSH activation was observed in patients with confirmed *E. coli* infection. This suggests that the presence of this parasite may be associated with remarkable oxidative stress resulting in the consumption of GSH. Thus it is suggested that antioxidant vitamins such as E and C, which increase the GSH activity (one of the cell-protective factors), should be used additionally in *E. coli* infections. This circumstance is due to the observation of a probable decrease in GSH level, which occurred as a result of consumption of whole body antioxidants due to an increase in the level of oxygen radicals, such as MDA and NO.

To our knowledge, there has been no previous research studying the serum MDA, NO levels and GSH activities in *E. coli* infected patients.

The increase in the MDA level in patients with *E. coli* as a result of lipid peroxidation is an evidence of an adverse effect on the host. The increase in the NO level can be associated with the stimulation of the cell-mediated immune system. The decrease in the GSH activity, however, indicates a decline in the antioxidant activity. These values led us to consider the possibility of the parasite to be pathogen.

All the findings obtained from the current study suggest *E. coli* as the probable pathogen. Nevertheless, molecular techniques such as Polymerase Chain Reaction, to strengthen the diagnosis were not studied, since they

were not currently available at our laboratory. We hope the findings obtained from the present study will encourage larger experimental and clinical studies about the oxidative stress that *E. coli* causes. Moreover, controlled experiments on the likely role of vitamins with antioxidant properties, such as Vitamin A and C in suppressing the pathogenicity of *E. coli* could be carried out.

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