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Original Article

Comparison of malondialdehyde, nitric oxide, adenosine deaminase and glutathione levels in patients with *Entamoeba coli, Enterobius vermicularis, Giardia intestinalis, Demodex* spp. positive, hydatid cyst and *Toxoplasma gondii* serum positive

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Abstract

Aim: In this study we aimed to compare glutathione (GSH), adenosine deaminase(ADA), nitric oxide (NO) and malondialdehyde (MDA) values between the patients with and without different parasitic infections.

Material and Methods: MDA, NO, ADA and GSH levels were studied in the serums of the patients group with *T. gondii* and cyst hydatid seropositivity and *E. vermicularis* and *E. coli* positive and the control group, while ADA levels alone were studied in *G. intestinalis* and *Demodex* spp.

Results: There was a statistically significant difference between the groups in terms of the amounts of ADA, GSH, MDA, and NO according to results of the variance analysis ($p<0.001$).

Conclusion: Given the change in the levels of GSH and ADA activities and MDA and NO levels observed in patients with parasitic infection, over production of active neutrophils, macrophages, reactive oxygen radicals and reactive nitrogen species may be an indicator of accelerated oxidative stress and lipid peroxidation in these patients. Increased serum ADA activity in the group infected by *E. coli* might be resulted from fight of the immune system with parasites. Low serum ADA activity in the other parasitic infection groups may be explained by suppression of lymphocyte proliferation by macrophages that were activated by sporozoite antigens in the late period of the infection. Accordingly, determination of the levels of MDA, NO, ADA and GSH may be important in treatment follow up and control of parasitic infections.

Keywords: Parasites; malondialdehyde; nitric oxide; adenosine deaminase; glutathione.

INTRODUCTION

Parasitic infections are an important health problem which negatively affects life quality in billions of people worldwide. Therefore research on the reaction given by immunity system of the host against possible parasitic infection is important for the treatment and protection.

The prevalence of *Entamoeba coli* (*E. coli*) infection has been reported as 30%. It has been found that the prevalence may raise up to 100% in the populations in tropical and subtropical areas, which this regard general hygienic measures. It has been reported that the parasite which is known as nonpathogenic may cause digestive system complaints (1,2).

Toxoplasma gondii (*T. gondii*) is also an obligate intracellular protozoan parasite (3). The disease is asymptomatic in many people infected by this parasite. However, the parasite may lead to serious pathological diseases in some cases including toxoplasmosis hepatitis, pneumonia, blindness and severe neurological disorders. Such diseases are seen especially in persons

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with impaired immune system. In addition it has been reported that the central nervous system is sensitive against free radical because of its high lipid content and oxygen consumption (4,5).

Echinococcos granulosus (*E. granulosus*) which larvae cause hydatid cyst in humans is seen in many countries worldwide. Echinococcus spp. is a cestode that can not synthesis of lipids which play an important role in the regulation of enzymes which are the major part of cell membranes, cell surface recognition, cellular interaction, glycoprotein synthesis and secretion of surface antigenic determinants in membrane transport and provides the necessary lipid from the host (6).

Enterobius vermicularis (*E. vermicularis*), is a nematode which is the most common cause of parasite diseases seen in children. The most commonly observed symptoms of the disease include gastrointestinal symptoms such as abdominal pain, diarrhea, nausea, vomiting, constipation and loss of appetite, and skin rashes (7).

Giardiasis is a disease caused by *Giardia intestinalis* (*G. intestinalis*) in the small intestine. The infection may be asymptomatic as well as it may cause symptoms such as periodic diarrhea, nausea, vomiting, loss of appetite, epigastric pain, weakness and weight loss. Furthermore absorbing discs of the parasite cause irritation in the mucosa, excessive mucus secretion and various absorption disorders (2).

Demodex spp has been reported to exacerbate clinical picture of some diseases including rosacea, blepharitis, phthririasis folliculorum, perioral dermatitis, Grovers' disease, eosinophilic folliculitis and pustular folliculitis (8,9). Prevalence of the parasite varies between 23.5% and 100%. The disease is more common in adults compared to children (10). Whereas Demodex folliculorum is found in the infundibular part of the hair follicle, D. brevis is located in depth of the sebaceous gland. It has been reported that the mite is passively located in the damaged follicles and the giant cell reaction develops against the parasite (8,10).

Tissue injuries occur with the effect of the toxins secreted by parasites. In addition it has been reported that these can produce oxygen radicals such as superoxide and hydrogen peroxide, and contain enzymes producing these radicals (11,12).

The defence of host immune system against parasites (adult and larval forms) is carried out through cells. Various cytotoxic agents, reactive oxygen and nitrogen intermediate products that are produced by the activated phagocytic cells play a role in this mechanism. These products are oxidant molecules of free radical nature and may negatively affect viability of the parasite (13,14).

Cytokines play a role in the regulation of immune response in organisms. Whereas Th1 cells strengthen defence mechanism of the host by releasing IL-2, IFN-ɣ and lymphotoxin, Th2 cells make the host susceptible to infections by synthesizing IL-4, IL-5, IL-6 and IL-10 (14, 15,16).

Lipid peroxidation is the oxidative degradation of polyunsaturated fatty acids (PUFA) to various products such as peroxide, peroxynitrite, alcohols, aldehydes and hydroxy fatty acids by free oxygen radicals. Malondialdehyde (MDA), which is the degradation product of three or more double-bond fatty acids, has been associated with many diseases as a marker of oxidative stress, because it shows a well correlation with the degree of lipid peroxidation (17).

Nitric oxide (NO) is a free radical which mediates physiological and pathological events. NO is endogenously synthesized from L-arginine and oxygen during the formation of citrulline and catalyzed by nitric oxide synthase (NOS). NO, which is highly secreted by active macrophages and neutrophils, is critical in defence against tumor cells, parasitic fungi, protozoa, helminthes, and microbacteria. NO has antimicrobial, antitumoral, neurotransmitter, and cytotoxic functions. Nitric oxide (NO) is considered as an integrative component of host arming against invading parasites (18,19).

Adenosine deaminase (ADA) is an amidohydrolase, which plays a role in the catabolism of purine nucleotides, and irreversibly deanimases adenosine and deoxyadenosine to inosine and deoxyinosine. ADA activities of T lymphocytes is higher than B lymphocytes, and in addition ADA activity significantly increases during differentiation of T cells and especially in immature and undifferentiated stages. For all these reasons, ADA serum levels were detected in different diseases, because ADA is considered as a marker of cellular immunity (20,21).

Glutathione (γ-Glutamyl Cysteinyl Glycine) is an important low-molecular weight tripeptide, which can be synthesized in the liver without a need for genetic information. Glutathione (GSH) is an intracellular antioxidant, found in low concentration at extracellular distance. GSH is an important intracellular antioxidant because of cysteine-bound thiol group and its high concentration. GSH protects the cell against detrimental effects of endogenous and exogenous oxidants by conjugation of reactive species and detoxification of lipid peroxidation products (22).

In this study, we aimed to compare serum levels of GSH, ADA, NO, and MDA in patients with and without different parasitic infections.

MATERIAL and METHODS

Considering possible increases in MDA level in parasitic diseases, *E. vermicularis, G. intestinalis, and E. coli* were studied in patient and control groups with Native-Lugol, perianal cellophane-tape examination and sedimentation methods. Whereas, *T. gondii* was studied in the serum samples collected from the patients, with cyst hydatid manual IHA and IFAT methods. Among the patients with *T. gondii*, cyst hydatid seropositivity, *E. vermicularis, G. intestinalis,* and *E. coli* detected, patients with other parasites, those receiving any hormone drug, smokers, and alcohol abusers were excluded from the study considering that these factors may cause differences in the levels of

MDA, NO, ADA, and GSH *T. gondii* IgM was seronegative in all samples. Patients with only one parasite detected (one from *T. gondii*, cyst hydatid, *E. vermicularis, G. intestinalis* and *E. coli*) were included in the experimental group. Again patients with *Demodex* spp. detected with standard superficial skin biopsy method who had no any other parasite in stool and serum constituted the experimental group.

Volunteers participating to this study, the control group consisted of the persons who had no any parasitic infection, were non-smokers, not receiving any hormonal drug and not using alcohol. The patients were informed about the study, 5mL blood samples were collected from the persons who consented giving sample. The serums were separated and kept at -20oC until the analysis.

The levels of MDA, NO, ADA, and GSH were studied in the serum samples of patients with *T. gondii* and cyst hydatid seropositivity, and *E. vermicularis*, and *E. coli* positivity and the control group, while ADA alone was examined in *G. intestinalis* and *Demodex* spp.

Biochemical Measurements

The collected blood samples were put into serum tubes, centrifuged at 4000 rpm for 7 minutes, and the serums were portioned in Eppendorf tubes and kept at -80oC in deep freezer until the analysis.

The measurement of MDA, which is a lipid peroxidation product, was made with Uchiyama and Mihara method. The method is based on the measurement of the absorbance of pink-red color, which was formed as a result of the reaction of MDA in the serum with TBA at 95oC, at 532 nm wavelength (23).

In this study, determination of glutathione was made according to the method developed by Fairbanks and Klee. The method is based on the spectrophotometric measurement of the absorbance of yellow product, which was formed as a result of the reaction ofsulfhydryl group with Ellman's reagent, at 410 nm wavelength (24).

The colored compound, which was formed by reaction of NO, which was produced by the activity of NOS in the medium with Griess reactive was measured at 545 nm with the spectrophotometer and studied with the method described by Cortas and Wakid (25).

Serum ADA levels were studied with the method developed by Ellis and Goldberg. The green-blue indophenol complex, which was formed as a result of the Berthelot reaction of ammonium ion, which was released by the effect of adenosine deaminase enzyme was read at 632 nm with the spectrophotometer (26).

Statistical Analysis

The data were tested for normality using the Anderson-Darling Test and for homogeneity of variance using the Levene's Test prior to the analyses. Descriptive statistics are presented as mean and standard deviation (SD). One-way ANOVA was used to evaluate the differences among the groups and multiple comparisons were performed with Tukey's range test. Data of the groups were illustrated using interval plots with confidence interval. Statistical analysis was done using SPSS software SPSS v25 (IBM Inc., Chicago, IL, USA) statistical software.

RESULTS

Descriptive statistics and comparison results for ADA (µmol/l), GSH (µmol/l), MDA (nmol/l) and N0 (µmol/dl) are given in Table 1. Presence of a statistically significant difference between the groups (Control, *Entamoeba coli* etc.) for the studied variables was examined with One-way ANOVA analysis. The difference between the groups was statistically significant, and the different groups were determined with Tukey's post hoc test. The results of Tukey's post hoc test are presented as letters along with the mean values.

There was a significant difference between the groups in terms of ADA levels (µmol/l) ($p<0.001$). The mean ADA value (µmol/l) was significantly higher in the patients with *E. coli* parasite both than the control and other parasite groups ($p<0.05$). Whereas the amount of ADA was significantly lower in the other parasite groups compared to the control group ($p<0.05$). No statistically significant difference was found between the groups with *Demodex* spp., *E. vermicularis*, *G. intestinalis*, cyst hydatid, and *T. gondii* parasites ($p>0.05$).

There was statistically significant difference between the groups in terms of the amount of GSH (µmol/l) ($p<0.001$). The highest amount of GSH was found in the control group with statistically significantly higher mean value compared to the other groups ($p<0.05$). The patients in the cyst hydatid group which had 50% lower GSH level than the control group, had significantly higher mean value compared to the other groups ($p<0.05$). There was no significant difference between *E. coli* and *E. vermicularis* groups that have the lowest GSH values ($p>0.05$), while the amount of GSH (µmol/l) was significantly lower in these two groups than in *T. gondii* group ($p<0.05$).

The difference between the groups in the amount of MDA (nmol/l) was statistically significant ($p<0.001$). The amount of MDA (nmol/l) was significantly lower in the control group compared to the other groups ($p<0.05$). The mean MDA value was higher in *E. coli* compared to *E. vermicularis* group ($p<0.05$), while no statistically significant difference was found between cyst hydatid and *T. gondii* groups ($p>0.05$).

There was a statistically significant difference between the groups in the amount of NO (µmol/dl) ($p<0.001$). No significant difference was found between *E. coli* and *T. gondii* groups ($p>0.05$), but both groups have significantly higher mean NO (µmol/dl) value compared to the other groups ($p<0.05$). The control group which had the lowest NO (µmol/dl) level was found to have statistically significantly lower levels than all the other groups ($p<0.05$). All values of the groups are shown in Figure 1.

Table 1. Descriptive statistics and comparison results for the ADA (µmol/l), GSH (µmol/l), MDA(nmol/l) and NO (µmol/dl)

	ADA (µmol/l)			GSH (µmol/l)			MDA(nmol/l)			NO (µmol/dl)		
	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD
Control	40	21.11b	14.08	40	24.95a	6.55	40	26.52c	19.42	40	0.28c	0.05
E. coli	35	29.32a	11.63	35	1.38d	0.22	35	57.71a	23.87	35	44.71a	6.68
Demodex spp.	30	11.03c	4.96									
E. vermicularis	40	11.96c	16.33	40	1.17d	0.17	40	39.30b	18.87	40	20.75b	3.82
G. intestinalis	50	10.87c	7.91									
Cyst hydatid	46	10.99c	6.53	42	11.23b	3.39	42	47.01ab	20.21			
T. gondii	32	9.26c	5.09	37	3.97c	0.65	37	56.27a	17.01	37	47.48a	6.13
P-Value	.000*** (F=18.42)			0.000*** (F=345.02)			0.000*** (F=16.03)			0.000*** (F=16.03)		

SD, Standard deviation; F, One-way ANOVA; ***, Statistically significant (p<0.001)According to Tukey test, means that do not share a letter are significantly different (p<0.05)

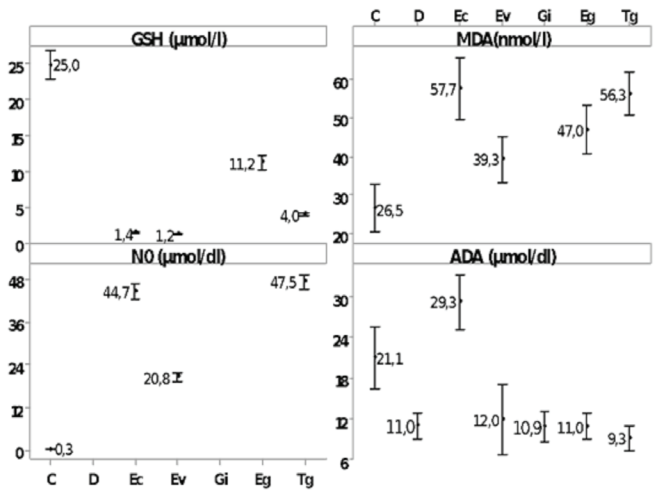

Figure 1. The interval plot for the level of GSH (µmol/l), MDA (nmol/l), NO (µmol/dl) and ADA (µmol/l) (95% C, Control; D, *Demodex* spp; Ec, *E. coli*; Ev, *E. vermicularis*; Gi, *G. intestinalis*; Eg, cyst hydatid; Tg, *T. gondii*

DISCUSSION

Parasitic infections are important public health problems, affecting lives of billions people worldwide. Therefore, determination of the reaction given by the host immunity system is critical for diagnosis and appropriate treatment.

In the present study, serum NO levels were statistically significantly higher in the patients infected by E. coli, *E. vermicularis*, and *T. gondii* (p<0.05). There was no significant difference between *E. coli* and *T. gondii* groups (p>0.05), but both groups have significantly higher mean NO value than the other groups (p<0.05). Engin et al. (27) found significantly higher serum levels of NO and MDA in brain, liver, and spleen of mice infected by *T. gondii* compared to the control group. In parallel with the results of the current study, Marchioro et al. (28) found significantly higher serum NO levels in pregnant patients infected with *T. gondii*. Given that different immune responses would be caused by differences between the parasites, common finding of increased NO production could be thought to be induced as a metabolic reaction because of the exposure to the secretion of proinflammatory cytokines and specific parasite antigens during the infections. Higher NO levels in the groups infected by *E. coli* and *T. gondii* could be explained by higher metabolic reaction in these species. It was thought that, NO is important in follow up of these two parasites. One of the remarkable results of this study was that, NO level was increased, despite *E. coli* is known as non-pathologic. It was concluded that, further studies are needed about pathogenicity of E. coli.

Serum MDA levels were statistically higher in patients with positive *E. coli*, *E. vermicularis*, *T. gondii*, and cyst hydatid (p<0.005). Whereas MDA level was higher in *E. coli* group compared to *E. vermicularis* group (p<0.05), no significant difference was found between cyst hydatid and *Toxoplasma gondii* groups (p>0.05). Increased reactive oxygen species (ROS) and MDA, and decreased glutathione (GSH) levels were found in placental tissue of mice infected by *T. gondii* (29). Cinar et al. (30) found significantly increased erythrocyte MDA levels and significantly decreased erythrocyte superoxide dismutase (SOD) and catalase (CAT) activity compared to the control group in sheep infected with *Dicrocoelium dendriticum* and cyst hydatid. In another study, urinary MDA levels were found to be significantly higher in parasite-infected groups created as helminth, protozoa, and helminth+protozoa compared to the healthy control subjects (31).

Among the products of lipid peroxidation, malondialdehyde is known as the best indicator determination of the level of reactive oxygen species (ROS) that lead to systemic biological damage (32). The mean MDA level was found as significantly higher in *E. coli*, which is known as non-pathogenic compared to *E. vermicularis*, and this could be explained by that this parasite induced lipid peroxidation in a higher rate. In addition, free radicals generated during parasitic infections have found to induce lipid peroxidation in organs, tissues, and cells.

GSH levels were statistically significantly lower in serums of the patients infected with *E. coli*, *E. vermicularis*, *T. gondii*, and cyst hydatid compared to the control groups (p<0.05). The patients in the cyst hydatid group which

had 50% lower GSH level than the control group, had significantly higher mean value compared to the other groups (p<0.05). Jafari et al. (33) found no significant difference between *T. gondii* positive male patient serums and the control groups in terms of glutathione activities and malondialdehyde levels, while glutathione activity was lower and MDA level was significantly higher in female patient serums. Al-Hadraawi et al. (34) found that GSH levels showed a significant decrease in male patients serums infected by *G. intestinalis* compared to the control group. Reduction in GSH activity in the patients groups might be resulted from lipid peroxidation-induced oxidative stress and depletion of endogenous antioxidant GSH concentration. Significantly higher mean value in patients with cyst hydatid group compared to the other groups, might be resulted from higher rate of this infection than the others. This may be interpreted as that pathogenicity might be higher in cyst hydatid disease. It was concluded that, further controlled studies should be conducted on this issue.

ADA activity was significantly higher in the serums of the patients with *E. coli* parasite both than the control and *D. folliculorum*, *E. vermicularis*, *G. intestinalis*, *T. gondii* and cyst hydatid (p<0.05). Whereas ADA levels were significantly lower in *D. folliculorum, E. vermicularis*, *G. intestinalis*, *T. gondii* patient groups compared to the control group (p<0.05). E-ADA levels were significantly decreased in hepatic lymphocytes of mice infected by *T. gondii*, again in another study infected cells were decreased in human fibroblast cells compared to the control group (35,36).

ADA activity was found to be statistically significantly higher in spleen, and serums of mice infected by *T.gondii,* and in another study in camel serums infected with *T.gondii* compared to the control group (37,38). Vakili et al. (39) found significantly higher ADA levels in cattle blood samples that were naturally infected with liver parasite (Cystic echinococcosis) compared to the control group. In the study, ADA levels were found to be lower in parasite groups except for *E. coli* compared to the control group.

The most important biological activity of adenosine deaminase is protecting lymphocytes against the toxic effects of 2-deoxyadenosine and deoxyadenosine triphosphate that suppress immune system. Different ADA levels in different parasites compared to the control group suggest that this was associated with increased cellular immune stimulation by the host in order to be protected against host parasite. It was concluded that further controlled studies are needed to evaluate ADA levels according to parasite types.

Since polymorphonuclear neutrophils and monocyte/macrophage cells play an important role in host defence, these cells can produce highly toxic molecules such as reactive oxygen radicals (ROS) and reactive nitrogen species such as nitric oxide (RNS). As is known, parasites, bacteria and tumor cells activate macrophages for NO synthesis. ROS and RNS that are overproduced in the picture of oxidative stress, which emerges by impaired antioxidant and oxidant balance contribute to the degradation of many biomolecules such as DNA, carbohydrates and proteins. The free radicals formed can attack polyunsaturated fatty acids, initiating lipid peroxidation chain reaction, which causes disruption of the cellular structure and function. It is obvious that lipid peroxidation lead to the formation of many degradation products including malondialdehyde (MDA).

CONCLUSION

In conclusion the levels of GSH, ADA, MDA and NO observed in patients with parasitary infection could be a marker of active neutrophils and macrophages, and accelerated oxidative stress and lipid peroxidation caused by the over production of ROS and RNS. Increased serum ADA activity in the group infected with *E. Coli* may be resulted from fight of the immune system with parasites. The lower ADA activity in the other parasitary infections may be explained by the suppression of lymphocyte proliferation by macrophages that are activated by antigens in late periof of the parasite. Accordingly, determination of MDA, NO, ADA and GSH levels in parasitary infections could be important for follow up and control of treatment.

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