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Lipid Peroxidation Level in Patients with Blastocystosis

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Aim: To investigate the oxidative stress hypothesis in patients infected with *Blastocystis hominis*.
Method: Serum malondialdehyde concentration activity was measured in 52 patients who were positive for intestinal parasite of *Blastocystis hominis*. Scores were obtained for the positives and their age-and sex-matched 60 *Blastocystis hominis* negative healthy controls. For comparison of two groups of continuous variables, independent samples t-test was used.
Results: There were no significant difference between malondialdehyde levels of patients with *Blastocystis* and control group both for females ($p>0.05$) and males ($p>0.05$). In addition, in the patient and control group, no correlation was found between age and malondialdehyde levels ($p>0.05$) both in females and males.
Conclusion: No change was observed in malondialdehyde levels in the patients with *Blastocystis* compared to controls.

Key Words: *Blastocystis Hominis*, Malondialdehyde

Blastocystosisli Hastalarda Lipid Peroksidaz Seviyesi

Amaç: *Blastocystis hominis*'le enfekte hastalarda oksidatif stres hipotezinin incelenmesi.
Metod: Serum malondialdehid konsantrasyon aktiviteleri 52 *Blastocystis hominis* pozitif hastada ölçüldü. Elde edilen sonuçlar yaş ve cinsiyete göre 60 kontrol ile karşılaştırıldı. Grupların karşılaştırılmasında bağımsız t-testi kullanıldı.
Bulgular: Kontrol grubu ile *Blastocystis hominis* ile enfekte hastalarda malondialdehid seviyelerinde istatistiksel olarak hem erkeklerde hem de bayanlarda bir fark bulunmadı. Aynı zamanda yaş ve malondialdehid seviyeleri arasında hem erkeklerde hem de bayanlarda kontrol grubu ile *Blastocystis hominis* ile enfekte hastalarda bir fark gözlenmedi.
Sonuç: *Blastocystis hominis*'le enfekte hastalarda malondialdehid seviyelerinde kontrol grubuna göre bir değişiklik gözlenmedi.

Anahtar Kelimeler: *Blastocystis Hominis*, Malondialdehid

Blastocystis hominis (*B. hominis*) is increasingly recognized to be a cause of human enteric disease, with symptoms often like those in giardiosis.[1] *B. hominis* is thought to be nonpathogenic.[2] Consequently, the true role of this organism in terms of colonization or disease is still somewhat controversial. However, the incidence of this organism appears to be higher than suspected in stools submitted for parasite examination, in symptomatic patients in whom no other etiologic agent has been identified, *B. hominis*, should certainly be considered the possible pathogen. Several reports have appeared that support the importance of the protozoan *B. hominis* as an intestinal pathogen in humans and consequently, the pathogenicity of *B. hominis* is extensively debated in the medical literature. *B. hominis* may be the cause of diarrhea, fever, vomiting, cramps, nausea and abdominal pain and may require therapy, the improvement probably represents elimination of some other undetected pathogenic organism.[3] It has been previously the prevalence and clinical significance of *B. hominis* in a large group of patients infected with human deficiency virus (HIV) was investigated, and data from this study indicated that the isolation of *B. hominis* does not justify treatment, even in symptomatic, severely immunocomprimised patients.[4]

Lipid peroxidation is a well-established mechanism of cellular injury in human, and is used as an indicator of oxidative stress in cells and tissues. Lipid peroxides, derived from polyunsaturated fatty acids, are unstable and decompose to form a complex series of compounds. These include reactive carbonyl compounds, which is the most abundant malondialdehyde (MDA). Therefore, measurement of malondialdehyde is widely used as an indicator of

lipid peroxidation. Increased levels of lipid peroxidation products have been associated with a variety of diseases in both humans and model systems.[5-7] For example; HIV infection is associated with oxidative stress, as it has been demonstrated in adult *Blastocystis* positive individuals. It has been shown in one study that serum MDA concentration of HIV-infected children was significantly higher than in control children.[8,9]

The aim of the study was to investigate and to test the hypothesis of decreased activity of defense system protecting tissues from free radical damage in patients with B. hominis by measuring the level of MDA (an end-product of lipid peroxidation), in serum samples.

MATERIALS AND METHODS

We assayed MDA activities of 112 subjects in human serum (blood was obtained from the antecubital vein) aged between 11-69 years (38 males and 74 females). None of them were smokers, had any known pathologies and taking steroids or medications such as iron for anemia at the time of sampling. Serum samples for control group were obtained from healthy people who have come to the different departments of Erciyes University, Medical Faculty for regular check-up and students or employees of the University. All subjects fasted after midnight before blood collection the next morning. 52 patients and 60 controls were examined in this study. The mean age of the patient group, which consisted of 28 men (aged 36±17 years) and 24 women (aged 31±14 years). The mean age of the control group, which included 10 men (aged 38±14 years) and 50 women (aged 27±5 years). Wet mount preparations in 0,9 % NaCl, diluted Lugol's iodine and flotation technique in saturated saline solution were used.[10] 52 *B. hominis* positive patients and 60 negative healthy subjects were selected as control group.

Assay
All venous blood samples taken between 8 and 9 a.m. after 12 h of fasting were collected in polystyrene tubes and vacutainers containing heparin. The tubes were centrifuged at 500xg for 15 min. Sera were then removed and stored at -20ºC until analysis.

Serum MDA levels were measured by the double heating method.[11] The principle of the method was based on the spectrophotometric (Shimadzu 1601 UV-Vis spectrophotometer) measurement of the color occurred during the reaction to thiobarbituric acid with MDA. Concentration of thiobarbituric acid reactive substances (TBARS) was calculated by the absorbance coefficient of malondialdehyde-thiobarbituric acid complex and expressed in nmol/ml. As a standard MDA bis (dimethyl acethal)-TBA (thiobarbituric acid) complex was used.

Statistical Analysis
Statistical analysis was performed with SPSS software package (Version 9.0 for Windows). Data were expressed as mean±standard deviation (SD). For comparison of two groups of continuous variables, independent samples t-test was used. A probability value of $p<0.05$ indicated a statistically significant difference.

RESULTS

MDA, scores are given in Table 1.

Table 1. MDA levels of patients with *B. hominis* and control group.

	Patients	
	Age	**MDA levels (nmol/ml)**
Female (24)	31±14	0,35±0,12
Male (28)	36±17	0,37±0,14
	Controls	
	Age	**MDA levels (nmol/ml)**
Female (50)	27±5	0,27±0,19
Male (10)	38±14	0,18±0,15

No statistically difference between MDA levels of patients and control group was found both for females ($p>0.05$) and males ($p>0.05$) (Table 1). In addition, in the patient and control group, no correlation was found between age and MDA levels ($p>0.05$) both in females and males. Moreover no significant correlation could be found between MDA levels of both females and males for patients and control groups ($p>0.05$).

DISCUSSION

Our work was first aimed to evaluate and characterize the relationship between intestinal parasite of *B. hominis* infection, which can cause serious pathology and oxidative stress mechanism as a mediator of tissue damage concurrent with *B. hominis* infection.

This is the first study to characterize the relationship between *B. hominis* and MDA (lipid peroxidation), which is a well-established mechanism of cellular injury in human, and is used as an indicator of oxidative stress in cells and tissues.[6]

Levels of MDA were seemed to be numerically but statistically increased in patients with *B. hominis*. The

results of our study possibly suggest that one of the main reasons for this numerically (but as indicated above not statistically) high MDA levels in patients with *B. hominis* could be decreased activity of defense system protecting tissues from free radical damage. However, in the patients and control group, no correlation was found between age and MDA levels both in females and males. In addition, no significant correlation could be found between MDA levels of both females and males for *B. hominis* infected and control groups.

As it is known that lipid peroxidation is a free radical-related process that in biologic systems may occur under enzymatic control, e.g., for the generation of lipid-derived inflammatory mediators, or non-enzymatically. This latter form is associated mostly with cellular damage as a result of oxidative stress, which also involves cellular antioxidants in this process[7]. *B.hominis* is strictly anaerobic, normally requires bacteria for growth, and is capable of ingesting bacteria and other debris. It is usually seen in the human stool specimen and diarrheal fluid.[2,3] Thus, infection/control ratio of MDA concentration and the insignificant but numerically increased correlation weakly but possibly indicate the occurrence of oxidative stress and lipid peroxidation somehow as a mechanism of tissue damage in cases of *B. hominis*.

In conclusion, the results of our study possibly suggest that one of the main reasons for this numerically high MDA levels in patients infected with B. hominis could be decreased activity of defense system protecting tissues from free radical damage.

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