Advanced Journal of Microbiology Research Volume 2014
Available online at http://advancedresearchjournals.org/AJMR

Full Length Research Paper

Adenosine deaminase level in the serum of the patients *Toxoplasma gondii* seropositive and *Giardia intestinalis*

Ülkü Karaman[1], Leyla Beytur[2], Tu ba Raika Kıran[3] and Cemil Çolak[4]*

[1]Department of Parasitology Beyda ı State Hospital, Malatya, Turkey.
[2]Department of Gynecology and Obstetrics, Gözde Hospital, Malatya, Turkey.
[3]Inonu University Medical Faculty Department of Biochemistry, Malatya, Turkey.
[4]Department of Statistics, Firat University, Elazig, Turkey.

Accepted 27 August, 2013

Adenosine deaminase (ADA) is an aminohydrolase making adenosine, deoksiadenozini inozin, and deocsiniozine deaminise irreversibly and plays role in the catabolism of purine nucleotids. *Toxoplasma gondii* is a zoonoses intracellular parasite that causes infection in animals and humans. This parasite encompasses enzymes that produce free radicals such as superoxide and hydrogen peroxide. In addition, *Giardia intestinalis* is another parasite that causes irritations in mucosa, over mucus discharge, aggravating former inflammations, and various absorption defects. In the present study, it has been aimed to compare ADA levels between *T. gondii* seropositive (IgG seropositive but symptomless patients), *G. intestinalis* positive patients, and control group. Thus, ADA levels between 32 patients being *T. gondii* seropositive and 29 controls and between 50 patients' *G. intestinalis* positive and 40 controls have been evaluated. The results were analyzed using independent samples *t* -test at the level of $p < 0.05$. According to this, in the statistical comparison between the parameters of patient and control groups, a meaningful decrease could be determined in ADA levels. This situation can be commented in the way that toxoplasmosis infection being inactive does not necessarily cause an increase in T lymphocytes. In addition, this decrease can be due to increasing oxidative stress in parasitic infections.

Key words: *Toxoplasma gondii, Giardia intestinalis,* adenosine deaminase.

INTRODUCTION

Toxoplasmosis is an infection developing asymptomatic and when it is passed in pregnancy period, it can cause abortion, dead birth and births with congenital anomalies (Kuman, 1987; Roman et al., 2006). It is stated that incidence of the disease increases with age and it is predicted that 40% of the world population is infected (Yazar, 2000).

Giardiasis is a disease caused by *G. intestinalis* in small intestine. The infection can be asymptomatic and also, causes periodic diarrhea, nausea-vomiting, inapetence, pain in epigastrium, malaise and loss of weight. In addition, sucking disks of parasite can cause irritations in irritations in mucosa, over mucus discharge and various absorption defects (Kuman and Altintas, 1996; Unat et al., 1995).

The defense of host immune system against parasites (mature and larval form) is made by means cells. Sitotoxic agents, reactive oxygen and nitrogen intermediary products in the activated cells play important role. These products are oxidant molecules and affect parasite viabilities in negative way (Akkus, 1995; Amanvermez and Celik, 2004; Kurt et al., 2002). Adenosine deaminase (ADA) is an aminohydrolase playing role in the catabolism of nucleotids, catalyzes adenosine, deoksiadenosine, and known ribozids in mammals (Ates et al., 2005; Ozvaran et al., 2004). Its structural gene is located in 20th chromosome and exists in multiple molecular forms having wide distribution (Ates et al., 2005; Alatas et al., 2003). On the other hand, ADA activity can be found in lymphocytic cells 10 fold more compared to erythrocytes and also, it is higher in T lymphocytes than to B

*Corresponding author. E-mail: cemilcolak@yahoo.com. Tel.: +90 505 887 04 98. Fax: + 90 312 416 65 93.

Table 1. Serum ADA level of *T. gondii* and *G. intestinalis* value.

Parasite	Groups	n	Mean ± SD	P value
T. gondii	Patient	32	9.25± 5.09	0.002
	Control	29	15.75± 10.23	
G. intestinalis	Patient	50	10.87 ± 7.91	<0.001
	Control	40	21.11± 14.08	

lymphocytes (Ates et al., 2005; Ormen et al., 2005). Considering ADA as a cellular immunity indicator, in this direction researchers have determined ADA serum levels for different diseases. According to this, it has been demonstrated that ADA activity increases in the activation period of autoimmune diseases such as; typhoid, infectious mononucleus, brucella, acute pneumonia, tuberculosis, sarcoidosis, liver diseases, acute leukemia, various malignites and rheumatoid arthritis, systemic lupus erythematous (SLE), and Behcet's syndrome (Hatipoglu et al., 2003; Ozgen et al., 2007) As both cellular and humoral immunity have disrupted in ADA deficiency, it has been concluded that ADA activity is significant for normal lymphocyte function (Ozgen et al., 2007; Cenesiz et al., 2007).

In the present study, it has been aimed to compare ADA levels between *T. gondii* seropositive (IgG seropositive but symptomless patients), *G. intestinalis* positive patients, and control group.

MATERIALS AND METHODS

Ethical council report has been taken for the research and only patients desiring to give sample have been assessed. 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. In the patient group, only persons seropositive to *T. gondii* infection that were determined using ELISA and IFAT tests and the ones positive to *G. intestinalis* using fecal inspection have been comprised in test group. In addition, the patients who had different parasites in their fecal samples, taking some illness medicine such as typhoid, infectious mononucleus, brucella, acute pneumonia, tuberculosis, sarcoidosis, liver diseases, acute leukemia and egg. (Hatipoglu et al., 2003; Ozgen et al., 2007), smoking cigarette and using alcohol had not been concluded in the research, considering these situations could cause variable differences at ADA levels.

In order to determine antitoxoplasma antibodies, ELISA and IFAT tests were used. Meddens and Zeus brands were used for ELISA and IFAT tests, respectively. The volunteer persons without the history of any parasitic infection, smoking cigarette, taking some illness medicine such as typhoid, infectious mononucleus, brucella, acute pneumonia, tuberculosis, sarcoidosis, liver diseases, acute leukemia and egg. (Hatipoglu et al., 2003; Ozgen et al., 2007), and using alcohols were taken to assessment in control group.

The patients that were positive using mentioned tests were called again and after making necessary explanations, 5 ml whole blood samples were taken from the volunteer persons. Its serum has been separated and stored -20 degree until studying.

Serum ADA levels have been examined by Ellis and Goldberg (Ellis and Galdberg, 1970). According to this method, ammonium ion that was released from adenosine by the effect of adenosine deaminase enzyme, forms indophenol complex in green and blue color as a result of boertholet reaction, also, the intensity of color increased proportional to enzyme concentration. This complex was evaluated at 632 nm using spectrophotometer.

Data was described with mean ± standard deviation and normality test was made by Shapiro-Wilk test. For statistical analysis, independent samples *t*-test was used through SPSS 13.0 package program and $p < 0.05$ value was accepted as a significant result.

RESULTS

In the present study, all the sera samples were negative to IgM titers. Descriptive statistic for ADA level has been shown in Table 1. Toxoplasmosis ELISA IgG results of patient group were in the range of 1012.97 ± 543.81 (mean ± SD) and values of control group were also negative. In the toxoplasmosis IFAT results of patient group, 1/16 and higher values were assessed as positive Table 1. Average age of the group showing *T. gondii* seropositive results was 29.57 ± 5.02; including 3 men and 29 women. Average age of control group was 35.22 ± 6.03; including 7 men and 22 women.

Average age of the group with *G. intestinalis* positive results was 30.5 ± 8.07; including 33 men and 17 women. Average age of control group was 40.22 ± 7.01; including 41 men and 9 women.

Significant differences between group of patients showing toxoplasmosis and their control group and between group of patients infected with *G. intestinalis* and their control group were determined ($p = 0.002$) and decreasing in ADA levels of both patient groups could be observed compared to control groups.

DISCUSSION

It has been stated that parasites have enzymes that produce free oxygen radicals such as super oxide and hydrogen peroxide and the tissue damages that occurs in parasitic infections take place by the effect of toxin discharged by these organisms (Kurt et al., 2002).

The growth of *T. gondii* as an intracellular parasite and forming its cysts in human body is controlled by T cells of cellular immune system. In addition, macrophages, fibroblast cells, astrocytes, and microglia cells found in brain are cells that can restrict reproduction of toxoplas -mosis (Daubener et al., 1999). Conversely, it has been determined that some *T. gondii* strains show high virulence in persons with T lymphocyte deficiency and T lymphocytes

play significant role in stimulation of immune reply, and the resistance occur against tachizoites or intermediary parasite (Denkers et al., 1998).

G. intestinalis reproduce and place in gastrointestinal mucosa of humans and animals. In general, 6 - 15 days after contagion, the special symptoms of parasitic infection start to appear. Immune system in intestines is very complex and this system can distinguish commensal bacteria from pathogens. Nitric oxide, reactive oxygen products, lactoferrin, defensin, phagocyte, mast, and dentric cells play significant role in natural immune response. Regarding to acquire immune response, IgA antibodies and T cells have undertaken significant roles (Roxström-Lindquist et al., 2006). The role of T cells in giardiosis infection in humans could not be determined. Also, it has been confirmed that chronic giardiosis develops in rats with CD4+ T cell deficiency (Singer and Nash, 2006).

The cells that proliferate in tissues have higher ADA activity and their ADA percentage is higher in cytoplasm than nucleus. Also, the highest concentration of ADA has been in lymphoid tissues (Carson and Seegmiller, 1976). Furthermore, the activity of lymphocytes increase along with mitogenic and antigenic response, meanwhile ADA activity in T lymphocytes becomes higher than B lymphocytes (Tripathi and al., 2008).

The lack of ADA in erythrocytes and lymphocytes cause extreme immune deficiency. The inhibition of this enzyme weakens the maturing and the function of lymphocytes and other immune cells; in addition, when monocytes and macrophages are infected with intracellular parasites, they show an increasing in ADA activity. During the lack of ADA in all cells, immune system damage occurs and repetitive vital, fungal, protozal, bacterial infections and lymphopenia can be seen (Cenesiz et al., 2007). Until now, a study directed to the determination of ADA level in giardiosis infection has not been encountered. However According to Tripathi et al. (2008), high ADA levels could be determined in patients showing visceral leishmaniasis. Again it is stated that ADA levels increase in malaria (Daddona et al., 1984) and Gakis (1996) acute toxoplasmosis for rats infected with Hitoglou et al. (2004) *Trichinella spiralis.* In the present study, a meaningful difference between *T. gondii* seropositive and control group could be determined and a decrease in ADA level has been observed according to controls, either too. Also, as mentioned before, only ADA levels of patients with *T. gondii* IgG seropositive were examined. The low levels in ADA can be commented in the way that toxoplasmosis infection being inactive does not cause an increase in T lympdocytes number or the oxidative stress emerging in parasitic infectios.

In our study, a significant difference between *G. intestinalis* and control group could be determined and a decrease in ADA levels could be observed according to controls. The duty of T lymphocytes in *G. intestinalis* infec-tions has not been determined completely yet. It can be thought that ADA levels have been decreased due to oxidative stress increasing resulted from parasitic infec-tion. Since the lymphocytes of patients infected by *T. gondii* and *G. intestinalis* and control group have not been examined, data concerning lymphocyte levels does not exist. It is considered that this research will bring light to studies which will be made in the future in regard to controlled test animals on this subject.

ACKNOWLEDGEMENTS

The authors would like to thank to Prof. Nilgün Daldal and Assist. Prof. Metin Atambay and Aysun Bay Karabulut for their contribution in the study.

REFERENCES

Akkus I (1995). Effect of free radicals and pathophsiological. Mimoza Pup. Konya pp: 35, 76.

Alatas F, Uslu S, Moral H, Alatas O, Metintas M, Erginel S, Ucgun I (2003). Serum adenosine deaminase activity in pulmonary tuberculosis. Tub. Thorax J. 51(3): 277-281.

Amanvermez R, Celik C (2004). Superoxide dismutasis, glutation, vitamin C, total tiyol levels in hydatic cysts. T. Klin. J. Med. Sci. 24: 213-18.

Ates Y, Ergun H, Tuzun A, Bagcı S, Kurt I, Inal A, Polat Z, Karaeren N, Dagalp K (2005). Serum adenosine deaminase levels and lymphocyte subgroups in familial mediterranean fever. Ankara Aca. J. Gastroent. 4(2): 112-116.

Carson DA, Seegmiller JE (1976). Effect of adenosine deaminase inhibition upon lymphocyte blastogenesis. J. Clin. Invest. 54: 274.

Cenesiz S, Nisbet C, Yarim GF, Arslan HH, Ciftci A (2007). Serum adenosine diaminase activity and nitric oxide level in cows with trichophytosis. Vet. J. Ank. Univ. 54(3): 155-158.

Daddona PE, Wiesmann WP, Lambros C, Kelley WN, Webster HK (1984). Human malaria parasite adenosine deaminase. Characterization in host enzyme-deficient erythrocyte culture. J. Biol. Chem. 259(3): 1472-1475.

Daubener W, Posdziech V, Hadding U, Mackenzie CR (1999). Inducible anti-parasitic effector mechanisms in human uropethelial cells: tryptophan degradation vs. NO production. Med. Microbiol. Immunol. 187: 143-147.

Denkers EY, Gazzinelli RT (1998). Regulation and Function of T-Cell-Mediated Immunity during *Toxoplasma gondii* Infection. Clin. Microbiol. Rev. 11(4): 569–588.

Ellis G, Goldberg DM (1970). A reduced nicotinamide adenine dinucleotide-linked kinetic assay for adenosine deaminase activity. J. Lab. Clin. Med. 76*:* 507-517.

Gakis C (1996). Adenosine deaminase (ADA) isoenzymes ADA1 and ADA2: diagnostic and biological role. Eur. Respir. J. 9(4): 632-633.

Hatipoglu K, Yuksekol I, Ozkan M, Balkan A, Koparan EZ, Bedirhan I, Bilgic H, Demirci N (2003). The Importance of measurind serum adenosine deaminase in tuberculosis. Gulhane J. Med. 45(2): 165–168.

Hitoglou S, Frydas S, Hatzistilianou M, Pappa S, Gougoustamou D, Kotsis A (2004). Response of ADA and its isoenzymes in mice infected by *Trichinella spiralis* and treated with mimosine. Int. J. Immunopathol. Pharmacol. 17(2): 191-200.

Kuman A, Altintas N (1996). Protozoon Diseases Ege Universty Publishing House, First Edition. Izmir pp. 60 – 68.

Kuman HA, Ak M, Altintas N, Uner A (1987). In the last ten years, the aegean region of cases toxoplasmosis. Acta Parasitol.Tur. 11: 54-62.

Kurt O, Ok UZ, Ertan P, Yuksel H (2002). Antioxidant substances and malaria. Acta Parasitol. Tur. 26(1): 108-112.

Ormen B, Ormen M, Turker N, Coskun NA, Onvural B, Kaptan F, El S, Ural S, Vardar I (2005). Adenosine deaminase activity in cerebrospinal fluid in tuberculous meningitis. Turk. J. Infect. 19(4): 415-418.

Ozgen G, Yasa H, Bektas A, Beyler AR, Buyukkocak S, Canpolat O

(1998). Adenosine deaminase activity in patients with carcinoma of rectum. T. Klin. J. Med. Sci. 18: 369-371.
Ozvaran MK, Okur H, Ozgel M, Ertugrul M, Baran R (2004). Diagnostic value of adenosine deaminase and its isoenzym in tuberculous pleural effusions. Thorax J. 5(3): 166-170.
Rorman E, Zamir CS, Rilkis I, David HB (2006). Conjenital toxoplasmosis-prenatal aspects of *Toxoplasma gondii* infection. Reprod. Toxicol. 21: 458-72.
Roxström-Lindquist K, Palm D, Reiner D, Ringqvist E, Svärd SG (2006). Giardia immunity-an update. Trends. Parasitol. 22(1): 26-31.
Singer SM, Nash TE (2006). T-Cell -Dependent Control of Acute *Giardia lamblia* Infections in Mice. Infect. Immun. 68(1): 170-175.
Tripathi K, Kumar R, Bharti K, Kumar P, Shrivastav R, Sundar S, Pai K (2008). Adenosine deaminase activity in sera of patients with visceral leishmaniasis in India. Clin. Chim. Acta 388(1-2): 135-8.
Tung A, Silber B, Quagliata C (1976). Adenosine deaminase activity in chronic lymhocytic leukemia: relationship to B cell and T cell subpopulation. J. Clin. Invest. 57: 756-61.
Unat EK, Yucel A, Altas K, Samastı M (1995). Unat's Medical Pathology. 5th Edition. Cer. Med. Fac. J., Istanbul p. 565.
Yazar S, Karagoz S, Altunoluk B, Kılıc H (2000). Investigation of anti-*Toxoplasma gondii* antibodies in patients suspected of having toxoplasmosis. Acta Parasitol. Tur. 24: 14-16.