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Full Length Research Paper

Assessment of the levels of nitric oxide (NO) in chronic diarrhea patients infected with *Giardia intestinalis*

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Giardia intestinalis **is an enteric pathogen causing certain digestive disorders by attaching itself on the human intestine and, especially on the duodenum. It is reported that there is a strong relationship between the nitric oxide (NO) level and the clinical prognosis of the infection in infections of *G. intestinalis.* The aim of the study was to investigate serum NO levels in samples taken from patients with *G. intestinalis* detected in the microscopic stool examination and from the control group consisting of healthy people and the presence of any relationship with Giardiosis. The study consisted of 60 patients diagnosed with *G. intestinalis* in microscopic stool examination, [31 (51.7%) male, 29 (48.3%) female] and 60 healthy people [28 (46.7% male, 32 (53.5%) female] as the control group. All patients in the patient group with gastrointestinal symptoms had chronic diarrhea and cysts and trophozoites were observed in microscopic examination of the feces in all of them. Measurement of serum NO level was performed using Cortas method. When the data were evaluated by T-test in the Independent Groups, NO levels in the patient group was found low at a statistically significant level. In Giardiosis, the decline in the NO level suggested that the disease causes a more severe clinical presentation. In addition to this, we consider it necessary that extensive experimental or clinical studies be conducted on the subject.**

Key words: *Giardia intestinalis,* nitric oxide, diarrhea, prognosis.

INTRODUCTION

Giardia sp is a parasitic intestinal pathogen very commonly seen throughout the world. It is known as *Giardia intestinalis*, *Giardia lamblia* or *Giardia duodenalis*, a species that infects humans. When the global distribution of *G. intestinalis* was examined, it was seen that this flagellate intestinal protozoa is very commonly seen throughout the world, in particular, in tropical and sub-tropical regions (Kucik et al., 2004). *G. intestinalis*, is a very common protozoa throughout the world presenting such findings in infected people as diarrhea and malabsorption. Infection starts with the oral intake of cysts. *G. intestinalis* does not need intermediate hosts for its evolution (Kucik et al., 2004; Pavanelli et al., 2010). Pathogenic effect of *G. intestinalis* is contingent upon factors such as the virulance of the parasite, the number of parasites received, the age and the immune response of the infected person. Pathologic changes such as ulcerations in the small intestine and partial constriction in the lumen, acute inflamation developed at the ulcer site

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were reported to have been observed in giardiosis (Pavanelli et al., 2010). It also hampers food intake by physically blocking the absorptive function of a high number of parasites attached onto the mucous surface (Kucik et al., 2004; Buret, 1994). Although, most infections can be controlled by an effective immune system, some people may develop chronic diseases. Furthermore, it is not clear which immune mechanisms are associated with controlling the infection effectively. Defense against parasites (adult or in larval form) in the immune system of the host is made by variety of cells and molecules capable of specific recognition and elimination of pathogens. Various cytotoxic agents produced by activated phagocytic cells, reactive oxygen and nitrogen intermediates take part in this defense mechanism. These intermediates are oxidant molecules in the nature of free radicals, and they negatively affect parasite viability (James, 1995; Nathan and Shiloh, 2000).

While NO has been said to be a simple atmospheric waste as late as 15-20 years ago, nitric oxide synthase (NOS) was discovered during the isolation of the structure known as endothelium-derived relaxing factor (EDRF) from the venous endothelium in 1987 and EDRF was reported as NO in the following years (Nathan and Shiloh, 2000). Nitric oxide is a nitrogen-centered radical produced purposefully and to perform the essential biological functions in higher living organisms. Nitric oxide (NO) is known to play a significant role in controlling the infections caused by various factors such as *Leishmania* spp*, Toxoplasma gondii*, *Salmonella typhimurium* and *Mycobacterium tuberculosis* (James, 1995; Nathan and Shiloh, 2000). NO plays an active role in many physiological and pathological processes in the human body. In the gastrointestinal system, NO plays a significant role in the relaxation of the muscularis externa, the regulation of mucosal blood flow and the protection of the mucosa. Also, NO plays a significant role in protecting the mucose by providing blood flow to the intestinal mucosa. Having the properties of a free radical, NO is formed by oxidation of L-arginine by NOS enzyme in the tissues (Palmer et al., 1987; Eckman et al., 2000). And the reactive nitrogen oxide species produced as a result of nitric oxide oxidation may cause nitrosylation and nitration of cellular molecules, DNA damage, destruction of membrane lipids and the inactivation of proteins/ enzymes (Palmer et al., 1987). Oxygen tolerance of *G. intestinalis* is low and they mostly colonize on the upper parts of the small intestine (duodenum and jejunum) and they do not have respiratory oxidase enzymes such as catalase or superoxide dismutase. NO is known to modulate the immune system directly and indirectly through certain microbicidal free radicals predominantly in macrophages and leukocytes. It has been demonstrated that especially NOS-1 is effective in the clearance of *G. intestinalis* infection and inhibits the *in-vitro* proliferation, excitation and incitation of *G. intestinalis* trophozoites (Cortas and Wakid, 1990). It has been observed that arginine is consumed at a higher level in the period when reproduction is rapid in *G. intestinalis* medium and as the arginine in the medium runs out, the reproduction slows down (Palmer et al., 1987). Eckmann et al. (2000) reported that *G. intestinalis* inhibits NO production in intestinal epithelial cells by reducing the availability of arginine.

The present study aims to investigate and compare serum NO levels in samples obtained from patients diagnosed with Giardiosis after microscopic stool examination and control group with no complaints and no parasite detected by gaita microscopy.

MATERIALS AND METHODS

The study consisted of 60 patients detected with *G. intestinalis* in microscopic stool examination, [31 (51.7%) male, 29 (48.3%) female] and 60 healthy people [28 (46.7% male, 32 (53.5%) female] as the control group. Patients detected with intestinal parasites apart from *G. intestinalis* in the patient group, patients receiving hormone/steroid medication, smoking and taking alcohol, patients with positive fecal occult blood and a history of chronic systemic disease in the patient and the control group were excluded from the study. Ten milliliters of blood was taken from the control group and their serums were taken out on the same day at 3000 rpm +4℃ and kept in the deep freeze at -70℃ until the analys is was performed. Serums were brought to room temperature before analysis.

NO levels were measured by the enzymatic Griess method in the form of total nitrite (as described by Cortas and Wakid), as total nitrite levels are considered to be the index of endogenous NO production (Cortas and Wakid, 1990). Using the Cortas method (kinetic cadmium-reduction method), the sensitivity limit was reported to be 0.1-1 µM. In the serum samples taken from the study group, NO levels were analyzed by measuring the NOS activity based on the amount of nitrite, that is by reducing nitrate to nitrite using cadmium. Statistical analysis was carried out using the SPSS 11.5 software package with the independent sample T-test and the level $P<0.05$ was taken as significant.

RESULTS

The average age of the patients group of 60 patients detected with *G. intestinalis* in the stool samples were 36.4±4.3 years, and the gender distribution were 31 (51.7%) male and 29 (48.3%) female. The average age of the control group of sixty people were 40.2±2.1 years and, the gender distribution were 28 (46.7%) male and 32 (53.3%) female. All individuals from both the patient and control groups were negative for other intestinal parasites and hydatid cyst, and none had any history of hormonal/steroid medication use, smoking or drinking.

All patients in the study group with gastrointestinal symptoms had chronic diarrhea and cysts and trophozoites were observed in microscopic examination of the feces in all of them. It was determined that most patients had low socioeconomic standing and were living in regions with infrastructure problems.

Mean values of NO levels for both the patient and the

Table 1. NO levels in the study and the control group.

Diagnosis	N	NO (µmol/L)	p
Study group	60	70.84 ± 11.60	0.0001*
Control group	60	165.83 ± 10.10	

*T-test in independent groups.

control group and their standard deviations are given in Table 1. When the results of the study were evaluated by T-test in the Independent Groups, NO levels in the patient group was found low at a statistically significant level (*P<0.0001*).

DISCUSSION

G. intestinalis is a parasite causing diarrhea cases throughout the world by infecting the human intestine. *G. intestinalis* inhibits epithelial NO production by using arginine which is an important substrate to produce NO by NOS. NO is an antimicrobial agent released against most bacteria and parasitic pathogens and has many functions including the regulation of intestinal mucosa structure and vascular tonus and the regulation of neuro-transmission (Eckmann et al., 2000). NO is synthesized enzymatically from arginine with the help of NOS enzyme with three isoforms which are neuronal NOS (nNOS), inducible NOS (iNOS) and endothelial NOS (eNOS). NO, prevents *G. intestinalis* from forming cysts or trophozoites. *G. intestinalis* is known to use arginine as energy source. Arginine has been reported as an important substrate which NOS enzyme needs to pro-duce NO (Assreuy, 1997). By inhibiting the NO synthesis, *G. intestinalis* creates a more convenient environment for reproduction. Also, the disruption of the NO synthesis speeding up the recovery period is thought to be causing a slowdown in the clinical picture of the patient. Ringqvist et al. (2008) reported that recombinant arginine deiminase (rADI) secreted by *G. intestinalis* in contact with intestinal epithelium disrupts the immune mechanism by affecting NO production and facilitates intestinal colonization. In a study conducted on a mouse with NO deficiency, it was demonstrated that neuronal NOS-1 which is a NOS isoform contributed to the elimination of *G. intestinalis*. In the same study, it was reported that NO removed the parasite by increasing intestinal motility rather than its toxicity towards the parasite (Li et al., 2006). Other researchers studied the role of NO and superoxide in *G. intestinalis* killing and verified that from the vast array of cytotoxic molecules potentially produced by defense cells, NO seems to account for the majority, if not all, of macrophage giardicidal effects (Fernandes and Assreuy, 1997). Furthermore, it was observed that NO also inhibited *G. intestinalis* excitation and encystations *in vitro* (Eckmann et al., 2000). Growth inhibition may be important for the infected host, because local trophozoite growth is probably crucial for the ability of *G. intestinalis* to establish and maintain infection of the proximal small intestine. In contrast, inhibition of encystation by NO could reduce the formation and passing of infectious cysts and, thereby, transmission to other potential hosts.

In our study, NO levels were found significantly lower in comparison with the control group (*P<0.0001*). This may be due to the failure to increase NO levels as a response because of chronic infection in our patients. Interestingly, Karna et al. (2011) found IFN-γ and IL-13 levels high to a statistically significant level in addition to NO levels in patients infected with *G. intestinalis* and reported that the levels did not drop with antiparasitic treatment. Capacity to increase NO levels in healthy individuals is significant as a defense mechanism in the removal of the parasite. Eckmann (2003) established that NO has cytotoxic and cytostatic effects on *G. intestinalis* trophozoites. The increase of NO level causes the number of parasites to decline and the balance between NO and anti-inflammatory cytokines determines the activity of immune response and morbidity (Brunet, 2001). As demonstrated in our study, the disruption of the balance in favour of *G. intestinalis* may cause chronic diarrhea and malabsorption.

As the disease is more severe in children, it was found convenient to compare the relationship between *G. intestinalis* and NO in adults and in children. In giardiasis, the decline in the NO level suggests that the disease causes a more severe clinical picture.

Based on the results of our study, reduced levels of NO, which is a significant mediator of the host immune system and the immune defense in infections of *G. intestinalis* detected in chronic diarrhea cases due to the parasite may explain why the infection and the symptoms progress more severely in some people. Still, as the immune mechanisms effectively controlling the infection can not be fully explained, more studies are needed to be conducted on the pathophysiology of *G. intestinalis*. Shedding light on these mechanisms will not only allow us to understand the mucosal immune response to the parasite, but will also provide an insight on the develop-ment of rational treatment strategies to activate the most effective host defense against *G. intestinalis*.

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