

## Serum malondialdehyde level in patients infected with *Ascaris lumbricoides*

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### Abstract

**AIM:** The aim of the study was to investigate the changes of serum malondialdehyde level, i.e; the oxidative stress hypothesis in patients infected with *Ascaris lumbricoides*.

**METHODS:** Serum malondialdehyde activity was measured in 43 patients who were positive for intestinal parasite of *Ascaris lumbricoides*. Scores were obtained for the positives and their age-and sex-matched 60 *Ascaris lumbricoides* negative healthy controls.

**RESULTS:** The difference between malondialdehyde levels of patients infected with *Ascaris lumbricoides* and control group was statistically significant both for females ( $P<0.05$ ) and for males ( $P<0.05$ ). In the patient and control groups, no correlation was found between age and malondialdehyde levels ( $P>0.05$ ) both in females and in males. In addition, no significant correlation could be found between malondialdehyde levels of both females and males for patients and control groups ( $P>0.05$ ).

**CONCLUSION:** Malondialdehyde levels clearly increase in patients infected with *Ascaris lumbricoides*.

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### INTRODUCTION

*Ascaris lumbricoides* (*A. lumbricoides*) is one of the largest nematode (roundworm) parasitizing the human intestine<sup>[1-5]</sup>. It is estimated that 25 % of the world's population was infected with this nematode<sup>[4]</sup>. The adult worms live in the small intestine and eggs are passed in the feces. A single female can produce up to 200 000 eggs each day. About two weeks after passage in the feces the eggs contain an infective larval or juvenile stage, and humans are infected when they ingest such infective eggs. The eggs hatch in the small intestine, the juvenile penetrates the small intestine and enters the circulatory system, and eventually the juvenile worm enters the lungs. In the lungs the juvenile worm leaves the circulatory system and enters the air passages of the lungs. The juvenile worm then migrates up the air passages

into the pharynx where it is swallowed, and once in the small intestine the juvenile grows into an adult worm. Why *Ascaris lumbricoides* undergoes such a migration through the body to only end up where it started is unknown. Such a migration is not unique to *Ascaris lumbricoides*, as its close relatives undergo a similar migration in the bodies of their hosts<sup>[1-3,5]</sup>.

*Ascaris lumbricoides* infections in humans can cause significant pathology. Infection with ascarids is called ascariasis. The migration of the larvae through the lungs causes the blood vessels of the lungs to hemorrhage, and there is an inflammatory response accompanied by edema. The resulting accumulation of fluids in the lungs results in "ascaris pneumonia", and this can be fatal<sup>[1,3,5,6]</sup>. The large size of the adult worms also presents problems, especially if the worms physically block the gastrointestinal tract. *Ascaris lumbricoides* is notorious for its reputation to migrate within the small intestine, and when a large worm begins to migrate there is not much that can stop it. Instances have been reported in which *Ascaris lumbricoides* has migrated into and blocked the bile or pancreatic duct or in which the worms have penetrated the small intestine resulting in acute (and fatal) peritonitis. *Ascaris lumbricoides* seems to be especially sensitive to anesthetics, and numerous cases have been documented where patients in surgical recovery rooms have had worms migrating from the small intestine, through the stomach, and out the patient's nose or mouth<sup>[1-3,5]</sup>.

Infections are diagnosed by finding the typical eggs in the patient's feces, on occasion the larval or adult worms are found in the feces, or especially for *Ascaris lumbricoides*, in the throat, mouth, or nose<sup>[1]</sup>. This infection may self-cure after the larvae have matured into adults or may require anthelmintic treatment. In severe cases, surgical removal may be necessary. Allergic symptoms (especially but not exclusively of the asthmatic sort) are common in long-lasting infections or upon reinfection in ascariasis<sup>[1,3,7]</sup>. Eggs of *Ascaris lumbricoides* have been detected on fresh vegetables<sup>[2,3,5]</sup>. This infection is cosmopolitan, but ascariasis is more common in North America and in Europe. Relative infection rates on other continents are not available<sup>[3,4]</sup>.

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 can decompose to form a complex series of compounds. These include reactive carbonyl compound, 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 chronic diseases in both humans and model systems<sup>[8-10]</sup>. The aim of this study was to test the hypothesis of decreased activity of defense system protecting tissues from free radical damage in patients with *A. lumbricoides* by measuring the level of MDA (an end-product of lipid peroxidation) in serum samples.

### MATERIALS AND METHODS

#### Patients

We assayed MDA activities of 103 subjects aged between 12-44 years (48 males and 55 females). None of them was smoker,

and 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 came to the different departments of Medical Faculty Erciyes University, for regular check-up and students or employees of the University. All subjects were fasted after midnight before blood collection the next morning. 43 patients and 60 controls were examined in this study. The mean age of the patient group, which consisted of 21 men and 22 women were  $25 \pm 13$  years and  $27 \pm 13$  years, respectively. The mean age of the control group, which included 27 men and 33 women were  $30 \pm 14$  years and  $29 \pm 12$  years respectively. Wet mount preparations in 0.9 % NaCl, diluted Lugol's iodine and flotation technique in saturated saline solution were used for the detection of intestinal parasites.

### Assay

All venous blood samples taken between 8 and 9 a.m. after 8 h of fasting were collected in polystyrene tubes and vacutainers containing heparin. The tubes were centrifuged at  $500 \times g$  for 15 min. Sera were then removed and stored at  $-20^\circ \text{C}$  until analysis. Serum MDA levels were measured by the double heating method<sup>[11,12]</sup>. The principle of the method was based on the spectrophotometric 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.

### Statistical analysis

Statistical analysis was performed with SPSS software package (Version 11.0 for Windows). The data were expressed as mean  $\pm$  standard deviation (SD). For comparison of two groups of continuous variables, independent sample *t*-test was used. A probability value of  $P < 0.05$  indicated a statistically significant difference.

## RESULTS

Malondialdehyde scores are given in Table 1.

**Table 1** MDA levels of patients infected with *A. lumbricoides* and control group

Patients	Age(year)	MDA levels (nmol/ml)
Female (22)	$27 \pm 13$	$0.67 \pm 0.16$
Male (21)	$25 \pm 13$	$0.62 \pm 0.11$
Controls	Age(year)	MDA levels (nmol/ml)
Female (33)	$29 \pm 12$	$0.21 \pm 0.15$
Male (27)	$30 \pm 14$	$0.22 \pm 0.14$

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

## DISCUSSION

This present study was aimed to evaluate and characterize the relationship between intestinal parasite infection of *ascariasis*, which can cause pathology and oxidative stress mechanism as a mediator of tissue damage concurrent with ascariasis infection.

Ascariasis is the most common human worm infection. Human can become infected after touching mouth with hands

contaminated with eggs from soil or other contaminated surfaces. Infection has occurred worldwide and has been most common in tropical and subtropical areas where sanitation and hygiene were poor<sup>[1-3,5]</sup>. Children are infected more often than adults. Estimates suggest that 1 in 4 of the world's population, or more than 1 billion people, are infected with the intestinal roundworm *A. lumbricoides*. In Europe, infection was common, but the most common in rural areas of the southeast<sup>[3-5]</sup>.

Although *Ascaris lumbricoides* has only a single host and it is found in the small intestine, its life cycle is far from simple. It has been suggested that from an evolutionary perspective that *Ascaris lumbricoides* originally had two hosts and has secondarily lost its intermediate host<sup>[1-3,5-7]</sup>. Although most people have no symptoms, symptoms can be broken down into 2 categories: early (larval migration, 4-16 days after egg ingestion) fever, cough and wheezing and late (mechanical effects, 6-8 weeks after ingestion). All symptoms resulted from mechanical irritation include; vague abdominal complaints (i.e. cramping, nausea, vomiting), small bowel obstruction (mostly in children), pancreatitis (secondary to worm migration), cholecystitis (secondary to worm migration), appendicitis (less common, secondary to worm migration). Secondary complications could arise with *Ascaris lumbricoides* infections because sometimes when the worms were undergoing this migration they appeared to get lost and started wandering through other organs such as the brain, bile duct, pancreas or appendix<sup>[1-3,5,7]</sup>.

*Ascaris lumbricoides* proteins are very immunogenic and people can become very sensitive to the worm and have strong allergic reactions. The parasite could be treated very easily with drugs such as mebendazole or pyrantel pamoate<sup>[1,3,5,13,14]</sup>. However, reinfections frequently occur if other control measures are not taken. This is a particular problem where night soil is used as a fertilizer.

Oxidative stress as a mediator of tissue damage concurrent with *A. lumbricoides* infection was investigated. This was the first study to characterize the relationship between *A. lumbricoides*, (may cause no symptoms however, some complaints of cramping, nausea, vomiting, small bowel obstruction, pancreatitis, cholecystitis, appendicitis can be seen) 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.

Levels of MDA were significantly increased in patients infected with *A. lumbricoides*. The results of our study strongly suggested that one of the main reasons for high MDA levels in patients infected with *A. lumbricoides* could be decreased activity of defense system protecting tissues from free radical damage. However, in the patients and control groups, no correlation was found between age and MDA levels both in females and in males. In addition, no significant correlation could be found between MDA levels of both females and males for *A. lumbricoides* infected and control groups. These results for patients infected with *A. lumbricoides* could possibly be explained as that with high MDA activity in all ages.

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 nonenzymatically. This latter form was associated mostly with cellular damage as a result of oxidative stress, which also involved cellular antioxidants in this process<sup>[10]</sup>. The high infection/control ratio of MDA concentration and the significant correlation strongly indicate the occurrence of oxidative stress and lipid peroxidation as a mechanism of tissue damage in cases of *A. lumbricoides* infection.

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