ANTIOXIDANT ENZYME ACTIVITY AMONG ORPHANS INFECTED WITH INTESTINAL PARASITES IN PATHUM THANI PROVINCE, THAILAND

Aongart Mahittikorn, Rapeepan Prasertbun, Hirotake Mori and Supaluk Popruk

Department of Protozoology, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand

Abstract. Intestinal parasitic infections can negatively impact growth and nutrition in children. The infections can induce oxidative stress, resulting in a variety of illnesses. We measured antioxidant enzyme levels in orphan children infected with intestinal parasites to investigate the influence of nutritional status on antioxidant enzymes. This cross sectional study was conducted at an orphanage in Thailand. Stool samples were obtained from each subject and examined for intestinal parasites. Anthropometric measurements, complete blood count and biochemical parameters, including serum superoxide dismutase (SOD) and glutathione peroxidase (GPx) levels, were obtained from studied subjects. One hundred twenty-eight children were included in the study. Intestinal parasites were found on microscopic examination of the stools in 36.7% (47/128); 18% (23/128) had a mixed parasite infection. Intestinal protozoa were found in 34.4% of subjects and intestinal helminthes were found in 2.3%. The median GPx level in children infected with intestinal parasites (2.3 ng/ml) was significantly lower than in non-infected children (7.7 ng/ml) ($p<0.05$). However, there was no significant difference in SOD levels between the two groups. When comparing GPx levels in children with 1) pathogenic parasites, 2) non-pathogenic parasites and 3) no intestinal parasite infection, GPx levels differed significantly among three groups (2.2 ng/ml, 2.4 ng/ml and 7.7 ng/ml, respectively) ($p<0.05$). When separating children by BMI and type of infection, the median SOD level in underweight children infected with pathogenic parasites (107.2 ng/ml) was significantly higher than in underweight children infected with non-pathogenic parasites (68.6 ng/ml) and without intestinal parasite infections (72.2 ng/ml). The present study identified two key findings: low GPx levels in children with intestinal parasitic infections, and the potential impact of malnutrition on some antioxidants.

Keywords: orphanage, antioxidant, intestinal parasites, nutritional status, Thailand

INTRODUCTION

Intestinal parasitic infections are common worldwide, especially in developing countries (Mehraj *et al*, 2008; Silva *et al*, 2009; Yami *et al*, 2011). They cause gastrointestinal (GI) and extra-GI symptoms, and have a negative impact

Correspondence: Dr Supaluk Popruk, Department of Protozoology, Faculty of Tropical Medicine, Mahidol University, 420/6 Ratchawithi Road, Ratchathewi, Bangkok 10400, Thailand. Tel: +66 (0) 2354 9100-4; Fax: +66 (0) 2643 5601 E-mail: supaluk.pop@mahidol.ac.th

on both nutrition and growth in children (Crompton, 1986; Ertug *et al*, 2007; Buret, 2008; Mondal *et al*, 2009; Lander *et al*, 2012). Intestinal parasitic infections may be asymptomatic (Checkley *et al*, 1997; Leder *et al*, 2005; Prasertbun *et al*, 2012) and as a result go untreated. Intestinal parasitic infections can disturb absorption of nutrients resulting in weight loss, failure to thrive and other physical and mental health problems (Sivakumar and Reddy, 1975; Brown *et al*, 1980; Evans and Stephenson, 1995; Checkley *et al*, 1997; Mondal *et al*, 2009; Bhandari *et al*, 2011). The long-term effects of asymptomatic intestinal parasitic infections in humans are not fully understood.

The study of oxidative stress and antioxidants among humans with parasitic infections may help to clarify the effect of asymptomatic intestinal parasitic infections. Oxidative stress can be of benefit to host inflammatory cells, stimulating the production of reactive oxygen species (ROS) to suppress invading parasites (Saran *et al*, 1999; Gookin *et al*, 2005; El-Taweel *et al*, 2007). However, they can also damage the body, leading to diseases like cancer, diabetes and ischemic heart disease (Valko *et al*, 2007). Oxidative enzymes are elevated in individuals with intestinal parasitic infections, even in those with less pathogenic protozoa such as *Blastocystis* (Demirci *et al*, 2003; Chandramathi *et al*, 2009a,b, 2010). Antioxidants, which play a role in suppressing oxidation, have been studied in tissue and blood parasites (Deger *et al*, 2008; Esmaeilnejad *et al*, 2012a; Heidarpour *et al*, 2013). However, few studies have focused on antioxidant enzyme activity among humans infected with intestinal parasites.

Most antioxidant studies have focused on individuals who are overweight or who have metabolic syndrome; few have been conducted among underweight children in developing countries. Inadequate protein intake can influence antioxidant enzyme levels (Fang *et al*, 2002). The impact of undernutrition on antioxidant enzymes may be significant. A dietary deficiency of protein can impact the synthesis of antioxidants and reduces tissue concentrations of antioxidants resulting in a compromised antioxidant status (Machlin and Bendich, 1987; Sies, 1999).

The present study had two objectives: determine antioxidant enzyme levels among children infected with intestinal parasites and investigate the influence of nutritional status on antioxidant enzyme levels.

MATERIALS AND METHODS

Study area, subjects and sample collection

We conducted this cross sectional study at the Maharaj Foundation, an orphanage in Pathum Thani Province, Thailand. Stool and blood samples were collected from 128 Thais, aged 13-20 years. Fifteen to 30 grams of stool were collected from each participant. Stool samples were kept cool and shipped within 2-3 hours to the Department of Protozoology, Faculty of Tropical Medicine, Mahidol University. The stool samples were examined for intestinal parasites using the formalin-ethyl acetate technique. Six milliliters of blood was collected from each participant before 6.00 AM on the same day of stool collection.

Anthropometric measurements

The nutritional status of the subjects was assessed using anthropometric measurements. The body weight of each individual dressed in light clothing was obtained using a beam balance scale (Detecto®, Webb City, MO). The height of each individual was measured using a vertical

measuring rod. Body mass index (BMI) was calculated as weight in kilograms/(height in meters)^2. The BMI was compared to a CDC growth chart. Participants were grouped according to their BMI percentile: underweight was a BMI less than the 5th percentile; at risk of becoming underweight was a BMI between the 5th and 15th percentiles; normal weight was a BMI between the 15th and 85th percentiles; at risk of becoming overweight was a BMI between the 85th and 95th percentiles; overweight was a BMI greater than the 95th percentile.

A complete blood count (CBC) was obtained from each subject. Glucose, total cholesterol, high-density lipoprotein cholesterol (HDL-C) and triglyceride levels were evaluated for each subject using an enzymatic method. The low-density lipoprotein cholesterol (LDL-C) was calculated as: LDL-C = total cholesterol – HDL-C – (triglycerides/5). Total protein and albumin levels were determined using a colorimetric method. Globulin was calculated as globulin = total protein – albumin.

Superoxide dismutase and glutathione peroxidase analysis

Superoxide dismutase (SOD) and glutathione peroxidase (GPx) levels were measured as follows: the clotted tube blood was centrifuged at 2,000*g* for 10 minutes. Cu/Zn-SOD levels were measured by sandwich enzyme-linked immunosorbent assay (ELISA), according to the manufacturer's instructions (IBL International, Hamburg, Germany). The principle of this assay was based on a general ELISA test. The studied samples each added to a specific well of a microtiter plate which had been pre-coated with a monoclonal antibody specific to human SOD. Unbound SOD and other components of the sample were removed by washing, then biotin-conjugated monoclonal antibody specific to SOD was added. Avidin conjugated to horseradish peroxidase (HRP) was added to each microplate well, followed by a 3, 3', 5, 5'-tetramethylbenzidine (TMB) substrate solution. Finally, a sulfuric acid solution was added, and the resulting yellow-colored product was evaluated at 450 nm. GPx levels were measured with a GPx ELISA Kit (Abnova, Taipei, Taiwan) according to the manufacturer's instructions. A sandwich ELISA technique similar to that used to measure SOD levels was used, but the microtiter plate was pre-coated with a monoclonal antibody specific for human GPx.

Statistical analysis

The data was tested for normal distribution by a Kolmogorov-Smirnov test. The results showed a non-normal distribution, therefore nonparametric statistics were used for analysis. Statistical analyses were carried out using SPSS for Windows, version 18.0 (IBM, Armonk, NY). Medians, ranges and the 95% confidence intervals for the medians (CI for median) were used to determine the middle range for each variable and the distribution of the variables. Using the non-parametric method, possible differences between two independent groups was tested using the Mann-Whitney *U* test. The ANOVA Kruskal-Wallis test was used to calculate statistical differences among three groups. Statistical significance was set at $p < 0.05$.

Ethical consideration

This study was approved by the Department of Public Welfare, the Maharaj Foundation, and the Ethics Committee for Research, Faculty of Tropical Medicine, Mahidol University, Thailand (MUTM 2012-006-01). Written informed consent was obtained from each participating guardian and for children participant

under their care. In addition, each participating child (under age 18) gave written assent. Thus, informed consent/assent was obtained for all children involved in the study. The guardians have the right to decline or allow their children to participate or to withdraw at any point in this study without penalty or loss of benefits. The children have the same rights to decline to participate or withdraw from the study at any time.

RESULTS

A total of 128 subjects were included in the study. Blood and stool specimens were obtained from each subject, but anthropometric information was not obtained from 7 subjects because they were adopted or moved out of the orphanage during the study.

The prevalence of intestinal parasitic infections detected by microscopic examination of the stool samples was 36.7% (Table 1). *Giardia duodenalis*, *Blastocystis*, *Entamoeba coli*, *Endolimax nana*, *Entamoeba histolytica*-like parasites, hookworm and *Opisthorchis viverrini* were identified. *Blastocystis* was found in 17.4% of samples, *E. coli* was found in 14.1%, *G. duodenalis* in 12.5%, and *E. nana* in 12.5%. We classified *G. duodenalis, E. histolytica*-like parasites, hookworm and *O. viverrini* as pathogenic parasites; and *Blastocystis*, *E. nana* and *E. coli* as non-pathogenic.

Of the 121 subjects for whom anthropomorphic measurements were available, 29.7% were underweight, 28% were at risk of becoming underweight, 40.5% had a normal weight, and 1.6% were at risk of becoming overweight. The median biochemical lab results, CBC results and antioxidant enzyme levels were not significantly different between normal weight and underweight subjects (data not shown).

No significant differences were found in the median anthropometric measurements, biochemical lab results, CBC results and SOD levels between infected and non-infected children, but the median GPx level in the infected group (2.3 ng/ml) was significantly lower than in the non-infected group (7.7 ng/ml) (Table 2). However, when children were grouped by type of infection (pathogenic parasite infection, non-pathogenic parasite infection and no intestinal parasite infection), the median SOD levels in the pathogenic parasite-infected children were slightly higher (107.2 ng/ml) than in the non-pathogenic parasite and non-infected children (68.6 ng/ml and 77.2 ng/ml) but this was not statistically significant. In contrast, the GPx levels differed significantly among the non-pathogenic parasite, pathogenic parasite and non-infected children (2.4 ng/ml, 2.2 ng/ml, 7.7 ng/ml; $p<0.05$) (Table 3).

When separating children according to BMI and type of infection (pathogenic parasites, non-pathogenic parasites and no intestinal parasite infection), the SOD levels differed significantly among the non-pathogenic parasites, pathogenic parasites and non-infected children in the underweight group ($p<0.05$) (Table 4).

There was no correlation between biochemical parameters and SOD or GPx levels in non-infected children. However, SOD levels were significantly negatively correlated with the median glucose level in infected children. A significant positive correlation was found between the median GPx and LDL-C levels (data not shown).

DISCUSSION

Our objectives were to determine antioxidant enzyme levels in children infected with intestinal parasites and to

Table 1
Intestinal parasite infection rates among Thai orphans.

Types of parasites	Total examined *N*=128 Number (%)
Single infection	
Protozoa	
G. duodenalis	6 (4.7)
Blastocystis	5 (3.9)
E. coli	6 (4.7)
E. nana	6 (4.7)
Total	23 (18.0)
Helminths	
Hookworm	1 (0.8)
Total	1 (0.8)
Mixed infections	
Protozoa	
G. duodenalis + *Blastocystis*	3 (2.3)
G. duodenalis + *E. coli*	3 (2.3)
G. duodenalis + *Blastocystis* + *E. coli*	3 (2.3)
G. duodenalis + *Blastocystis* + *E. coli* + *E. nana*	1 (0.8)
Blastocystis + *E. nana*	5 (3.9)
Blastocystis + *E. nana* + *E. coli*	1 (0.8)
Blastocystis + *E. nana* + *E. histolytica* like	1 (0.8)
Blastocystis + *E. coli*	1 (0.8)
Blastocystis + *E. histolytica like* + *E. coli* + *E. nana*	1 (0.8)
E. histolytica like + *E. coli*	1 (0.8)
E. nana + *E. histolytica* like	1 (0.8)
Protozoa + Helminths	
Hookworm + *E. coli*	1 (0.8)
O. viverrini + *Blastocystis* + *E. coli*	1 (0.8)
Total	23 (18.0)

investigate the influence of nutritional status on antioxidant enzymes. To the best of our knowledge, this is the first study to identify significantly low antioxidant levels (GPx) in humans infected with intestinal parasites.

A high overall prevalence of intestinal protozoa (34.4%) and a low infection rate with intestinal helminths (2.3%) were found in this study. *G. duodenalis*, *Blastocystis*, *E. nana*, and *E. coli* were commonly found; consistent with a previous study in Thai orphanages (Saksirisampant *et al*, 2003). The transmission route might be the reason for this discrepancy in prevalence. The water supply was clean and toilet use at the study site was common; soil-transmitted helminths (STHs) require soil to progress to their infective stage, so transmission is blocked through good toilet hygiene. Most intestinal protozoa are infective immediately after excretion, thus can be transmitted by direct fecal-oral routes (Pipatsatitpong *et al*, 2012). Another

Table 2
Medians, ranges and 95% CI for BMI, CBC, biochemical parameters and antioxidant enzymes (SOD, GPx) among infected and non-infected subjects.

Parameters	Non-infected group (n=81)		Infected group (n=47)		p-value
	Median (range)	95%CI	Median (range)	95%CI	
Body mass index (kg/m^2)	16.6 (11.2-25.7)	15.9-17.4	16.8 (12.9-27.7)	15.5-17.8	0.54
White blood cell count (×10^3/mm^3)	7 (4.4-12.5)	6.6-7.6	6.9 (5.0-10.8)	6.5-7.9	0.57
Neutrophil (%)	53.4 (27.8-70.5)	50.1-55.9	52.4 (26.0-69.0)	49.6-56.0	0.38
Lymphocyte (%)	37.1 (20.5-60.7)	34.0-39.2	34.2 (20.8-59.1)	31.8-40.4	0.59
Monocyte (%)	4.0 (0.6-12.8)	3.5-4.7	4.1 (0.0-8.1)	3.7-4.5	0.71
Eosinophil (%)	3.9 (0.9-29.3)	3.2-4.8	4.2 (1.4-25.0)	3.3-6.1	0.67
Basophil (%)	0.4 (0.0-0.9)	0.4-0.5	0.5 (0.0-0.8)	0.4-0.5	0.06
Red blood cell count (×10^6/mm^3)	5.0 (4.0-6.8)	4.9-5.2	5.0 (4.3-6.4)	4.9-5.2	0.55
Hemoglobin (g/dl)	13.3 (7.9-17.2)	12.9-13.6	12.9 (9.9-16.2)	12.6-13.2	0.39
Hematocrit (%)	39.1 (28.6-50.5)	37.9-40.7	38.1 (13.6-47.0)	37.2-39.2	0.53
Platelet count (×10^3/mm^3)	264.0 (115.0-449.0)	250.0-294.6	271.0 (35.0-402.0)	262.1-290.0	0.39
Total cholesterol (mg/dl)	159.0 (37.0-252.0)	154.2-165.8	166.0 (106.0-233.0)	156.0-172.6	0.48
Triglyceride (mg/dl)	57.0 (16.0-190.0)	49.4-62.7	67.0 (23.0-181.0)	58.3-81.0	0.48
High-density lipoprotein cholesterol (mg/dl)	49.0 (32.0-90.0)	47.0-52.0	48.0 (30.0-75.0)	44.3-52.6	0.53
Low-density lipoprotein cholesterol (mg/dl)	102.0 (56.0-195.0)	97.2-111.0	106.0 (56.0-182.0)	97.8-116.6	0.42
Glucose (mg/dl)	69.0 (22.0-117.0)	67.7-71.2	68.5 (35.0-97.0)	63.9-70.0	0.26
Protein (mg/dl)	7.6 (6.7-9.2)	7.4-7.6	7.5 (6.5-8.8)	7.3-7.7	0.54
Albumin (mg/dl)	4.1 (3.2-4.6)	4.0-4.2	4.0 (3.5-4.8)	3.9-4.1	0.30
Globulin (mg/dl)	3.5 (2.7-5.6)	3.3-3.6	3.4 (2.7-4.9)	3.3-3.5	0.50
Superoxide dismutase (ng/ml)	77.2 (11.1-331.5)	68.9-82.1	84.1 (24.1-479.8)	65.3-108.1	0.46
Glutathione peroxides (ng/ml)	7.7 (0.7-32.0)	6.8-10.0	2.3 (0.0-15.6)	1.2-3.6	0.01

p<0.05 was considered statistically significant.

Table 3
Medians, ranges and 95% CI for antioxidant enzymes levels among children infected with non-pathogenic and pathogenic parasites, and with no parasites.

Parameters	Non-pathogenic parasites (*n*=22)		Pathogenic parasites (*n*=25)		No parasites (*n*=81)		*p*-value
	Median (range)	95% CI	Median (range)	95% CI	Median (range)	95% CI	
Superoxide dismutase (ng/ml)	68.6 (24.1-372.8)	64.5-90.1	107.2 (29.3-479.8)	58.7-162.0	77.2 (11.1-331.5)	68.9-82.1	0.19
Glutathione peroxidase (mg/ml)	2.4 (0.0-26.2)	0.0-3.9	2.2 (0.0-3.6)	1.4-7.4	7.7 (0.7-32.0)	6.8-10.0	0.00

$p<0.05$ was considered statistically significant; CI, confidence interval.

Table 4
Medians, ranges and 95% CI for superoxide dismutase enzyme among normal weight and underweight children infected with non-pathogenic and pathogenic parasites and with no parasites.

Parameters	Non-pathogenic parasites (*n*=22)		Pathogenic parasites (*n*=25)		No parasites (*n*=81)		*p*-value
	Median (range)	95% CI	Median (range)	95% CI	Median (range)	95% CI	
SOD (ng/ml) Normal weight	76.2 (38.1-166.0)	43.5-116.5	87.9 (31.1-166.9)	39.4-125.7	76.9 (33.0-331.5)	68.2-93.3	0.64
SOD (ng/ml) Underweight	67.3 (37.6-93.9)	47.3-87.3	162.7 (45.1-297.2)	76.0-247.2	81.8 (11.1-220.0)	71.1-98.1	0.03

$p<0.05$ was considered statistically significant; CI, confidence interval.

explanation for the lower prevalence rates of STHs in our study could be the ongoing helminth control programs underway during the study period; deworming is conducted regularly in schools and orphanages in the study area.

Nutritional status indicators, including BMI and biochemical parameters, were not significantly different between infected and non-infected children. There are two possible reasons for this. First, malnutrition in the underweight group may not severe in the present study. This was seen with the biochemical parameters, total protein and albumin, which were not significantly different between the underweight and normal weight children; only the BMI was lower in underweight individuals.

Secondly, contradictory data exists regarding intestinal parasitic infections and child growth. Several studies have demonstrated links between helminth infection and undernutrition or stunted growth (Moore *et al*, 2001; Muniz *et al*, 2002), while other studies have found none (Pegelow *et al*, 1997). The same is true for intestinal protozoa; some studies reported a significant association between undernutrition and intestinal protozoa, such as *Cryptosporidium*, *G. duodenalis* and *Blastocystis* (Checkley *et al*, 1997; Simsek *et al*, 2004; Ertug *et al*, 2007), while others found no connection (Hollm-Delgado *et al*, 2008). *G. duodenalis* has been reported to be linked to symptoms (Ignatius *et al*, 2012). Further genosubtyping is required to properly elucidate the pathogenicity of intestinal protozoa and the relationship with undernutrition.

The median GPx levels in children infected with both pathogenic and non-pathogenic intestinal parasites were lower than in the non-infected group; even non- or less-pathogenic parasites may induce oxidative stress and subsequently lower antioxidant levels, which is consistent with a previous report (Chandramathi *et al*, 2010a). GPx and SOD activities differ by type of parasitic infection. GPx and SOD levels have been found to be depressed in sheep and goats infected with *Babesia ovis* (Esmaeilnejad *et al*, 2012a,b). Echinococcosis and distomatosis tissue parasitic infections have been associated with a variety of GPx and SOD levels in different studies; some have reported increased GPx and decreased SOD levels in cattle, hamsters, and sheep (Sanchez-Campos *et al*, 1999; Deger *et al*, 2008; Heidarpour *et al*, 2013), while others have reported decreased GPx levels in camels and humans (Lilic *et al*, 2007; Heidarpour *et al*, 2012). During intestinal parasitic infections, serum ferric-reducing antioxidant power (FRAP) levels have been reported to be elevated in humans (Chandramathi *et al*, 2009b, 2010). An animal model found FRAP was elevated during the initial stage of infection, but later decreased significantly during follow-up testing (Chandramathi *et al*, 2010). Erythrocytic SOD was found to be decreased in humans infected with *G. duodenalis* (Demirci *et al*, 2003).

GPx decomposes intracellular lipid peroxidase. Elevated lipid peroxidation found in patients with parasitic infections may be related to decreased antioxidant enzymes (Grewal *et al*, 2005; Heidarpour *et al*, 2012). Another possibility is the stage of infection influences GPx levels. In the early stages, antioxidant enzymes may increase, then decrease later on; the antioxidant status of the host may also be overwhelmed by free radical-induced oxidative damage (Chandramathi *et al*, 2010). In this study, SOD levels were higher among children with pathogenic intestinal parasitic infections and GPx

levels were significantly lower than among the non-infected group (Table 3). The reason for these different results is unclear. Further large-scale longitudinal studies will be required to monitor antioxidant enzyme levels among patients with intestinal parasitic infections over a longer time period. The long term effect of oxidative stress due to chronic intestinal parasitic infections has also not yet been determined. Reactive oxygen species induced by oxidative stress can damage lipids, proteins and nucleic acids, leading to DNA mutations, apoptosis and more rapid aging (Clark *et al*, 1986; Valko *et al*, 2007). Such a study could help increase understanding of the factors that resulted in these differences between children with and without intestinal parasitic infections.

Nutritional status appears to influence antioxidant levels. SOD levels were higher among underweight individuals, but were not elevated in otherwise healthy subjects of normal weight infected with pathogenic intestinal parasites (Table 4). One possible explanation is that the SOD enzyme in underweight children infected with pathogenic parasites may not function properly due inadequate trace elements which are important components of the enzyme. SOD may be produced in higher levels as enzyme activity decreases. The influence of undernutrition on antioxidant enzyme activity has not yet been determined; further studies measuring trace elements are required to evaluate the association between them.

A positive correlation was identified among children infected with intestinal parasites between GPx and total and LDL cholesterol levels and a negative correlation was seen between SOD and glucose levels. Abnormalities in the antioxidant defense system and increased oxidative stress due to parasite infection may result in higher susceptibility to lipid peroxidation. Free radicals can react with biomolecules, such as lipids, protein and DNA, and break down lipids to aldehydes and ketones (Rahman, 2007). However, the influence of reactive oxygen species and antioxidant enzymes on carbohydrate metabolism has not yet been clarified. Contradictory results for antioxidant enzymes have been reported for diabetic patients (Hartnett *et al*, 2000; Menon *et al*, 2004).

In summary, in this study, we found low GPx levels among children with intestinal parasitic infections and an association between malnutrition and antioxidant activities. GPx levels were lower in children with pathogenic and non-pathogenic intestinal parasitic infections. Longitudinal large-scale investigations are needed to further evaluate the relationship between antioxidant enzyme activity and malnutrition. The long-term risks associated with asymptomatic intestinal parasitic infections need to be explored. Interventional studies to assess the results of treating asymptomatic *G. duodenalis* and *Blastocystis* infections on oxidative stress and antioxidant enzymes are also needed.

ACKNOWLEDGEMENTS

We would like to thank all the guardians and the volunteers at the study orphanage. We would also like to thank the staff of the Department of Protozoology, Faculty of Tropical Medicine, Mahidol University for their help with data collection and for their valuable advice. Thanks also to Tim Cole for editing the manuscript. This study was supported by a Research Grant from the Faculty of Tropical Medicine, Mahidol University for 2011-2012.

REFERENCES

Bhandari N, Kausaph V, Neupane GP. Intestinal parasitic infection among school age children. *J Nepal Health Res Counc* 2011; 9: 30-2.

Brown KH, Gilman RH, Khatun M, Ahmed G. Absorption of macronutrients from a rice-vegetable diet before and after treatment of ascariasis in children. *Am J Clin Nutr* 1980; 33: 1975-82.

Buret AG. Pathophysiology of enteric infections with *Giardia duodenalis*. *Parasite* 2008; 15: 261-5.

Chandramathi S, Suresh K, Anita ZB, Kuppusamy UR. Elevated levels of urinary hydrogen peroxide, advanced oxidative protein product (AOPP) and malondialdehyde in humans infected with intestinal parasites. *Parasitology* 2009a; 136: 359-63.

Chandramathi S, Suresh KG, Anita ZB, Kuppusamy UR. Attenuation of hydrogen peroxide and ferric reducing/antioxidant power serum levels in colorectal cancer patients with intestinal parasitic infection. *Malays J Med Sci* 2009b; 16: 15-20.

Chandramathi S, Suresh K, Shuba S, Mahmood A, Kuppusamy UR. High levels of oxidative stress in rats infected with *Blastocystis hominis*. *Parasitology* 2010; 137: 605-11.

Checkley W, Gilman RH, Epstein LD, *et al*. Asymptomatic and symptomatic cryptosporidiosis: their acute effect on weight gain in Peruvian children. *Am J Epidemiol* 1997; 145: 156-63.

Clark IA, Hunt NH, Cowden WB. Oxygen-derived free radicals in the pathogenesis of parasitic disease. *Adv Parasitol* 1986; 25: 1-44.

Crompton DW. Nutritional aspects of infection. *Trans R Soc Trop Med Hyg* 1986; 80: 697-705.

Deger Y, Ertekin A, Deger S, Mert H. Lipid peroxidation and antioxidant potential of sheep liver infected naturally with distomatosis. *Turkiye Parazitol Derg* 2008; 32: 23-6.

Demirci M, Delibas N, Altuntas I, Oktem F, Yonden Z. Serum iron, zinc and copper levels and lipid peroxidation in children with chronic giardiasis. *J Health Popul Nutr* 2003; 21: 72-5.

El-Taweel H., El-Zawawy LA, Said DE, Sharara GM. Influence of the antioxidant drug (Antox) on experimental giardiasis and microsporidiosis. *J Egypt Soc Parasitol* 2007; 37: 189-204.

Ertug S, Karakas S, Okyay P, Ergin F, Oncu S. The effect of *Blastocystis hominis* on the growth status of children. *Med Sci Monit* 2007 Jan; 13 (1): CR40-3. Epub 2006 Dec 18.

Esmaeilnejad B, Tavassoli M, Asri-Rezaei S, Dalir-Naghadeh B. Evaluation of antioxidant status and oxidative stress in sheep naturally infected with *Babesia ovis*. *Vet Parasitol* 2012a; 185: 124-30.

Esmaeilnejad B, Tavassoli M, Asri-Rezaei S, Dalir-Naghadeh B, Malekinejad H. Status of lipid peroxidation and antioxidant enzymes in goats naturally infected with *Babesia ovis*. *Acta Parasitol* 2012b; 57: 228-34.

Evans AC, Stephenson LS. Not by drugs alone: the fight against parasitic helminths. *World Health Forum* 1995; 16: 258-61.

Fang YZ, Yang S, Wu G. Free radicals, antioxidants, and nutrition. *Nutrition* 2002; 18: 872-9.

Gookin JL, Allen J, Chiang S, Duckett L, Armstrong MU. Local peroxynitrite formation contributes to early control of *Cryptosporidium parvum* infection. *Infect Immun* 2005; 73: 3929-36.

Grewal A, Ahuja CS, Singha SP, Chaudhary KC. Status of lipid peroxidation, some antioxidant enzymes and erythrocytic fragility of crossbred cattle naturally infected with *Theileria annulata*. *Vet Res Commun* 2005; 29: 387-94.

Hartnett ME, Stratton RD, Browne RW, Rosner BA, Lanham RJ, Armstrong, D. Serum markers of oxidative stress and severity of diabetic retinopathy. *Diabetes Care* 2000; 23: 234-40.

Heidarpour M, Mohri M, Borji H, Moghdass E. Oxidative stress and trace elements in camel (*Camelus dromedarius*) with liver

cystic echinococcosis. *Vet Parasitol* 2012; 187: 459-63.

Heidarpour M, Mohri M, Borji H, Moghdass E. Oxidant/antioxidant status in cattle with liver cystic echinococcosis. *Vet Parasitol* 2013; 195: 131-5.

Hollm-Delgado MG, Gilman RH, Bern C, *et al*. Lack of an adverse effect of *Giardia intestinalis* infection on the health of Peruvian children. *Am J Epidemiol* 2008; 168: 647-55.

Ignatius R, Gahutu JB, Klotz C, *et al*. High prevalence of *Giardia duodenalis* Assemblage B infection and association with underweight in Rwandan children. *PLoS Negl Trop Dis* 2012; 6: e1677.

Lander RL, Lander AG, Houghton L, *et al*. Factors influencing growth and intestinal parasitic infections in preschoolers attending philanthropic daycare centers in Salvador, Northeast Region of Brazil. *Cad Saude Publica* 2012; 28: 2177-88.

Leder K, Hellard ME, Sinclair MI, Fairley CK, Wolfe R. No correlation between clinical symptoms and *Blastocystis hominis* in immunocompetent individuals. *J Gastroenterol Hepatol* 2005; 20: 1390-4.

Lilic A, Dencic S, Pavlovic SZ, *et al*. [Activity of antioxidative defense enzymes in the blood of patients with liver echinococcosis]. *Vojnosanit Pregl* 2007; 64: 235-40.

Machlin LJ, Bendich A. Free radical tissue damage: protective role of antioxidant nutrients. *FASEB J* 1987; 1: 441-5.

Mehraj V, Hatcher J, Akhtar S, Rafique G, Beg MA. Prevalence and factors associated with intestinal parasitic infection among children in an urban slum of Karachi. *PLoS One* 2008; 3: e3680.

Menon V, Ram M, Dorn J, *et al*. Oxidative stress and glucose levels in a population-based sample. *Diabet Med* 2004; 21: 1346-52.

Mondal D, Haque R, Sack RB, Kirkpatrick BD, Petri WA Jr. Attribution of malnutrition to cause-specific diarrheal illness: evidence from a prospective study of preschool children in Mirpur, Dhaka, Bangladesh. *Am J Trop Med Hyg* 2009; 80: 824-6.

Moore SR, Lima AA, Conaway MR, Schorling JB, Soares AM, Guerrant RL. Early childhood diarrhoea and helminthiases associate with long-term linear growth faltering. *Int J Epidemiol* 2001; 30: 1457-64.

Muniz PT, Ferreira MU, Ferreira CS, Conde WL, Monteiro CA. Intestinal parasitic infections in young children in Sao Paulo, Brazil: prevalences, temporal trends and associations with physical growth. *Ann Trop Med Parasitol* 2002; 96: 503-12.

Pegelow K, Gross R, Pietrzik K, Lukito W, Richards AL, Fryauff DJ. Parasitological and nutritional situation of school children in the Sukaraja district, West Java, Indonesia. *Southeast Asian J Trop Med Public Health* 1997; 28: 173-90.

Pipatsatitpong D, Rangsin R, Leelayoova S, Naaglor T, Mungthin M. Incidence and risk factors of *Blastocystis* infection in an orphanage in Bangkok, Thailand. *Parasit Vectors* 2012; 5: 37. doi: 10.1186/1756-3305-5-37.

Prasertbun R, Sukthana Y, Popruk S. Real-time PCR: benefits for detection of mild and asymptomatic *Giardia* infections. *Trop Med Health* 2012; 40: 31-5.

Rahman K. Studies on free radicals, antioxidants, and co-factors. *Clin Interv Aging* 2007; 2: 219-36.

Saksirisampant W, Nuchprayoon S, Wiwanitkit V, Yenthakam S, Ampavasiri A. Intestinal parasitic infestations among children in an orphanage in Pathum Thani province. *J Med Assoc Thai* 2003; 86: S263-70.

Sanchez-Campos S, Tunon MJ, Gonzalez P, Gonzalez-Gallego J. Oxidative stress and changes in liver antioxidant enzymes induced by experimental dicroceliosis in hamsters. *Parasitol Res* 1999; 85: 468-74.

Saran M, Beck-Speier I, Fellerhoff B, Bauer G. Phagocytic killing of microorganisms by radical processes: consequences of the reaction of hydroxyl radicals with chloride yielding chlorine atoms. *Free Radic Biol*

Med 1999; 26: 482-90.

Sies H. Glutathione and its role in cellular functions. *Free Radic Biol Med* 1999; 27: 916-21.

Silva RR, da Silva CA, de Jesus Pereira CA, *et al*. Association between nutritional status, environmental and socio-economic factors and *Giardia lamblia* infections among children aged 6-71 months in Brazil. *Trans R Soc Trop Med Hyg* 2009; 103: 512-9.

Simsek Z, Zeyrek FY, Kurcer MA. Effect of *Giardia* infection on growth and psychomotor development of children aged 0-5 years. *J Trop Pediatr* 2004; 50: 90-3.

Sivakumar B, Reddy V. Absorption of vitamin A in children with ascariasis. *J Trop Med Hyg* 1975; 78: 114-5.

Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. *Int J Biochem Cell Biol* 2007; 39: 44-84.

Yami A, Mamo Y, Kebede S. Prevalence and predictors of intestinal helminthiasis among school children in Jimma zone; a cross-sectional study. *Ethiop J Health Sci* 2011; 21: 167-74.