

Relationship Between Interleukin-10, Cholesterol and Blood Glucose Levels in Geohelminth Positive Adolescents and Adults

by Eryati Darwin2

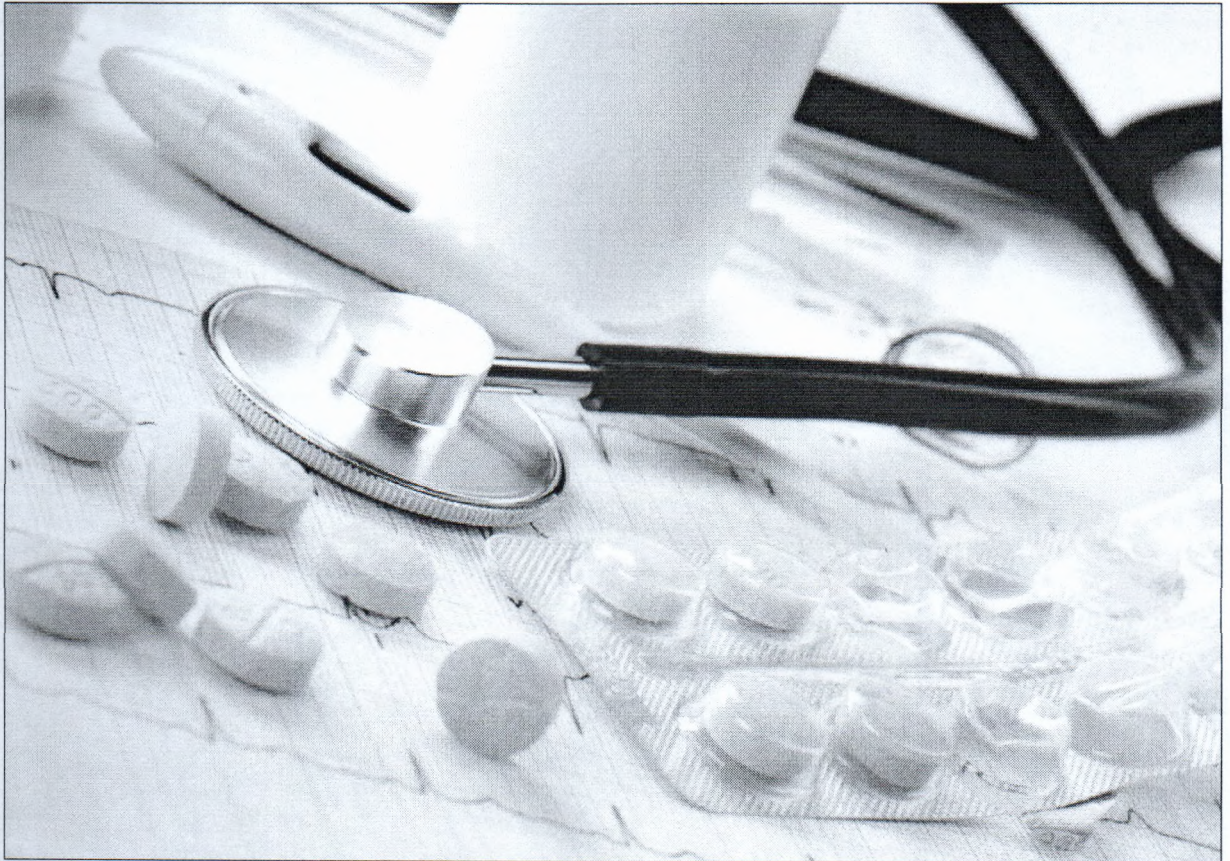
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Research Article

Relationship Between Interleukin-10, Cholesterol and Blood Glucose Levels in Geohelminth Positive Adolescents and Adults

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Abstract

Background and Objective: Geohelminth is a common cause of chronic infections in humans. Worm infections have been shown to have a protective effect against some diseases. The effect is related to the ability of the worm to modulate the host immune response through Th2. The aim of study was to investigate the correlation between IL-10 levels and blood cholesterol and glucose in geohelminth positive human subjects with mature immune systems. **Materials and Methods:** Stool examination for geohelminth was done by direct method using iodine and by Kato Katz method. IL-10 levels were measured by indirect enzyme-linked immunosorbent assays (ELISA) method. Total plasma cholesterol levels were examined by CHOD-PAP method and fasting blood glucose was measured using hexokinase method. **Results:** All subjects infected with geohelminth classified as a mild infection. There was no correlation between IL-10 levels and EC/GC ($p > 0.05$) but there was a negative correlation between IL-10 levels with TC levels ($p < 0.01$) and between IL-10 levels and FBG levels ($p < 0.01$). **Conclusion:** A negative correlation between IL-10, TC and FBG further strengthens support for the role of Th2 especially IL-10 to low cholesterol and blood glucose levels in geohelminth infections.

Key words: Protective effect, geohelminth, infection, interleukin-10, total cholesterol, blood glucose

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Intestinal parasitic worms, also called helminths are a common cause of chronic infections in humans. Recent global estimates indicate that more than one-third of the world's population is infected by one or more types, most commonly soil-transmitted worms or *intestinal* geohelminth such as *Ascaris lumbricoides*, *Necator americanus*, *Ancylostoma duodenale* and *Trichuris trichiura*¹.

Geohelminth infections are most frequent and persistent in children and individuals living in endemic areas since they are continually exposed from immediately after birth to adulthood. The highest prevalence is found in elementary school-age children since they frequently play on the ground and their cellular immunity is not fully developed. Geohelminth can also affect adults who are susceptible due to their environment or study conditions².

Worm infections cause malnutrition and anemia³, especially in children whose food intake is marginal. Nutrition disorders are more evident in children as they have a higher need for nutrition for growth. But despite these negative impacts, worm infections have been shown to have a protective effect against some disorders such as allergies^{4,7}, autoimmune disorders like inflammatory bowel disease⁸, rheumatoid arthritis⁹ and multiple sclerosis¹⁰.

Chen *et al.*¹¹ found that the prevalence of diabetes and metabolic syndrome was low in Chinese over 60 who had a history of *Schistosoma mansoni* infection. These people tended to have low blood cholesterol, blood glucose and all other components of metabolic syndrome. Previous study in Nigeria also found that children infected with intestinal worms had lower total cholesterol levels than uninfected children in the same age group¹². This seems to indicate that worm infections reduce metabolic syndrome factors such as high blood cholesterol and glucose levels and hence a protective effect against related diseases, like diabetes and cardiovascular disease.

Wiria *et al.*¹³ in his study in Flores, Indonesia, also found that geohelminth infected individuals had lower total cholesterol levels than non-infected individuals. He also found that geohelminth infections may increase insulin sensitivity¹⁴. These results suggested that the relationship between geohelminth and the diseases related to metabolic syndrome like diabetes mellitus, which is becoming an increasing problem worldwide, is worthy of further research.

Low blood cholesterol and blood glucose levels in geohelminth infections could be explained because the worms absorb nutrients from the intestine of the host but there is some indication that low cholesterol and blood

glucose levels associated with geohelminth infections are related to the ability of the worms to modulate the host immune response¹⁵⁻¹⁷ but this needs to be explored further.

In chronic infections, the worms trigger macrophage differentiation from classically activated macrophages (CAMs) into alternatively activated macrophages (AAMs) which are characterized by the production of anti-inflammatory cytokines such as interleukin-10 (IL-10) and/or Transforming Growth Factor - β (TGF- β)^{18,19}. IL-10 is an anti-inflammatory cytokine and considered to be one of the key cytokines neutralizing the effects of the inflammatory responses that arise in some diseases.

The anti-inflammatory effects of IL-10 triggered by geohelminth infections are assumed to increase insulin sensitivity so lowering glucose and cholesterol levels in the blood. This was demonstrated by Lumeng²⁰ in mice where AAMs were able to maintain insulin sensitivity in fat tissue through the production of IL-10 which inhibited the TNF α effect that triggers insulin resistance. Hussaarts *et al.*²¹ also found that in obese mice chronic worm infections and worm derivative antigens decreased systemic insulin resistance by 23%, increased peripheral glucose uptake by 25% and insulin sensitivity. There is, therefore, good reason to discover to what extent these effects are evident in human subjects. While previous research has indicated that similar processes do occur in humans, the relationship between IL-10 levels and blood cholesterol and glucose in geohelminth positive human subjects with mature immune systems has yet to be explored fully. The aim of this study is to increase understanding of this area of study.

MATERIALS AND METHODS

Location and population of study: This research was conducted in West Sumatra, Indonesia. Subjects were geohelminth positive children above the age of 11 years from 3 elementary schools in Padang Pariaman and geohelminth positive farmers from the outskirts of Padang. None of the subjects were using cholesterol, diabetes, allergy or steroid medication. Cancer and tuberculosis sufferers were also eliminated from the study. The total sample size was 41 individuals along with an age, gender and BMI matched control sample of uninfected individuals. This study was conducted in Parasitology Laboratory and Biomedical Laboratory in June, 2017 to March, 2018.

Research ethic: This study was already passed the ethics clearance and has been approved by the Ethics Committee of

the Faculty of Medicine andalus University, Padang. All subjects were briefed on the study including the objectives, risks and benefits of the study and gave consent to be involved. For those younger than 18, consent forms were signed by their parents.

Stool sample collection and examination: On the day before the stool examination, the subjects were given stool collection containers and asked to return stool samples the next morning. Stool examinations were conducted at the Parasitology Laboratory, Faculty of Medicine andalus University, using a direct method with iodine²² and by the Kato Katz method²³ to calculate the number of geohelminth eggs.

Collection of blood samples: Subjects were requested to fast for 8-10 h before the taking of blood samples. Blood was taken using an aseptic procedure from the *mediana cubiti* vein by trained personnel, using 3 cc syringe. 2 cc was collected in an EDTA tube for total plasma cholesterol and fasting blood glucose and 1 cc collected into tube marked by a yellow cap for ELISA examination. The samples were taken to biomedical laboratory Faculty of Medicine andalus University, Padang for further analysis.

Measurement of levels of IL-10: The IL-10 levels were measured according to the manufacturer's instructions using a Human IL-10 ELISA Kit (Sensitivity 2.59 pg mL⁻¹, assay range 5pg mL⁻¹-1500 pg mL⁻¹, Catalog Number E0102Hu, Bioassay Technology Laboratory, Shanghai) and read with an ELISA reader at 450 nm using an automatic microplate reader (xMark, BioRad, USA).

Estimation of total plasma cholesterol: Total plasma cholesterol levels were examined using an enzymatic method according to Allain *et al.*²⁴. Cholesterol levels were measured using a spectrometer at 520 nm following Ochei and Kolhatkar²⁵.

Fasting blood glucose estimation: Fasting blood glucose levels were examined using hexokinase according to Passey *et al.*²⁶.

Statistical analysis: As neither IL10 levels, the number of eggs/gram of stool (EC/GS), total cholesterol (TC) nor fasting blood glucose (FBG) were normally distributed. The mean differences of IL-10, TC and FBG levels among geohelminth-infected individuals and controls were analyzed using T-student test but the result was not normally and homogenly distributed, the data were analyzed by a

Table 1: Correlation between EG/GS, IL-10, TC and FBG level in infected geohelminth subjects

Parameters	EC/GS	IL-10	TC	FBG
EC/GS	1.000	0.081	-0.364*	-0.217
IL-10	0.081	1.000	-0.367*	0.357*
	p = 0.213			
TC	-0.364*	-0.367*	1.000	0.623**
	p = 0.019	p = 0.006		
FBG	-0.217	-0.357*	0.623*	1.000
	p = 0.156	p = 0.004	p = 0.001	

*,**Significant correlation

Mann-Whitney U test. Correlations between continuous variables were analyzed using Spearman's correlation coefficients.

RESULTS

Stool samples from 327 individuals aged 11-65 years identified 41 (12.5%) who were geohelminth positive. The number of eggs g⁻¹ of stool (EG/GS) ranged from 2-1543 indicating a mild level of infection. The geohelminth infected individuals had a significantly higher level of IL-10 and significantly lower TC and FBG levels compared to the control group (p<0.05).

Individual subject which infected by geohelminth, IL-10 level was higher than control group (median 2,4 pg mL⁻¹ vs. 2,3 pg mL⁻¹) and the difference was significant (p = 0.001, Fig. 1a). Conversely, geohelminth infected subjects showed the lower level of TC than control group (median 163 mg dL⁻¹ vs. 181 mg dL⁻¹) and the difference was significant (p = 0.001, Fig. 1b). The level of FBG in infected subjects were lower than control group (median = 80 mg dL⁻¹ vs. 89 mg dL⁻¹) but the difference was not significant (p = 0.004, Fig. 1c).

Spearman's correlation *coefficients* analysis discovered significantly negative correlations between EC/GS and TC levels, between IL-10 and TC level and between IL-10 and FBG level in infected geohelminth subjects. There was no correlation between EC/GC and IL-10 level and no correlation between EC/GS and FBG level (Table 1).

DISCUSSION

This study found that the geohelminth positive group had a higher IL-10 level than the control. In line with a previous study using a different methodology that also found elevated IL-10 levels with geohelminth infections¹⁹. A number of previous studies have measured the *in vitro* production of IL-10 in both Peripheral Blood Mononuclear Cells (PBMC) and whole blood, measuring spontaneous IL-10 production and production after stimulation with *Ascaris lumbricoides* antigen^{27,28} or *Trichuris trichiura* antigen²⁹.

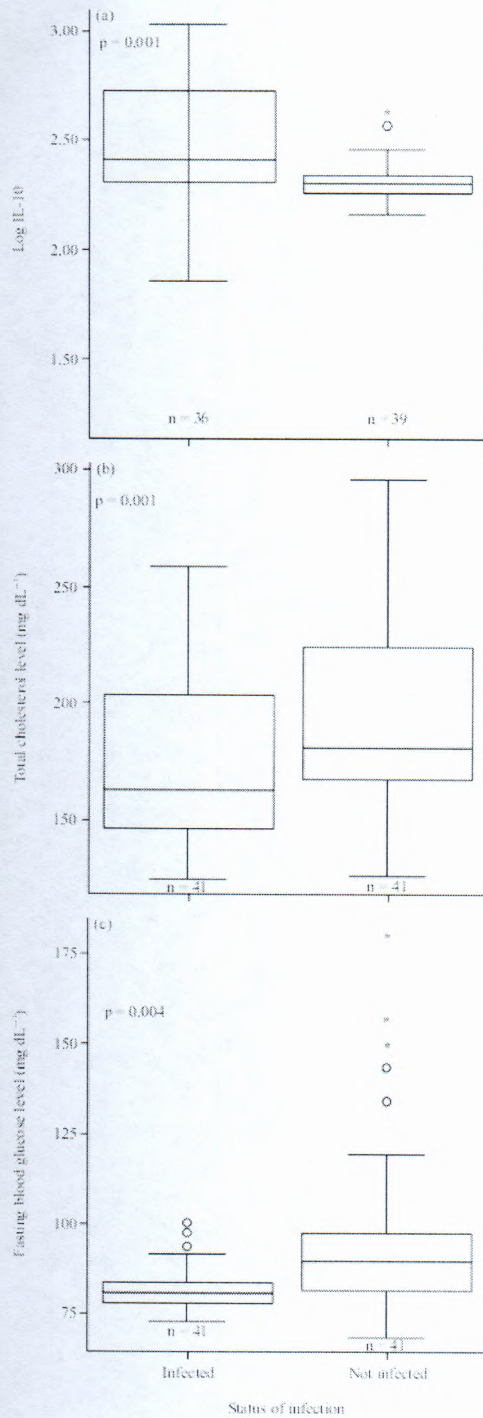


Fig. 1(a-c): Differences in (a) IL-10, (b) TC, (c) FBG Levels according to infection status

Box plots show median values (horizontal center line), inter-quartile range (box margins) and 95% confidence intervals (bars)

The production of cytokines *in vitro* does not necessarily reflect *in vivo* levels of IL-10 as *in vivo* cytokine levels are influenced by other factors³⁰. In this present study, some conditions that affected IL-10 levels were excluded. Sanchez *et al.*³⁰ used a similar IL-10 measurement method to this study but found no association between IL-10 levels and geohelminth infections in Honduras children. This may be due to the age difference of the study subjects. In this study, the subject age ranged from 11-65 years, where the immune system is fully developed, while the subjects of Sanchez's research were younger, aged 7-15 years. After age 11 the intensity of *A. lumbricoides* infection decreases and levels of Th2 cytokines (IL-9, IL-10 and IL-13) increase, indicating that the Th2 response to helminth infection is associated with immune system maturity along with lower infection intensities²⁸.

In this study, no significant correlation was found between EC/GS and IL-10 levels. This may be because all of the positive subjects in this study had mild rates of infection. Even so, these were sufficient to result in a significant difference from IL-10 levels in the control group.

TC levels in geohelminth-infected individuals were lower than in the control group ($p < 0.05$) and there was a significant negative correlation EC/GS with TC levels. This condition explained that geohelminth absorbs cholesterol from the host digestive tract for growth and for egg production³¹. However, extracts produced from helminth have also been found to reduce cholesterol levels when introduced into mice. This indicates that metabolites associated with helminth can trigger an immune response in the host³².

In this study, FBG levels in geohelminth infected individuals were significantly lower than in the control subjects. There was also a significant negative EC/GS correlation with FBG levels. The effect of nutrition in this study was excluded by selecting controls with matched BMI and age. Madden *et al.*³³, found a decrease in glucose absorption in the intestinal epithelium of mice infected with nematodes. Although the underlying mechanism is unclear, a relationship with the Th2 immune response to nematodes is strongly suspected. Morimoto *et al.*³⁴ have shown significant increases in levels of IL-10, IL-4, IL-13 and accumulation of activated macrophages in the submucosa of mouse small intestines infected with *Heligmosomoides polygirus* (Hp).

There was a negative correlation between IL-10 and TC and FBG in this study further strengthens support for the role of Th2 especially IL-10 to low cholesterol and blood glucose levels in geohelminth infections. Research with animal models has demonstrated that administration of IL-10 increases insulin sensitivity³⁵.

All positive subjects in this study contained eggs of helminth with light infection level but the effect has showed significant difference of IL-10 level between infected subjects with control group. There was no correlation between EC/GS with IL-10 level ($p>0.05$), it was probably caused by the increase of IL-10 level, not by the number of eggs. But, it was related to presence of geohelminth in intestine. Therefore, light infection of geohelminth is not proper to eliminate, considering the beneficial.

CONCLUSION

This study showed that geohelminth infections increased IL-10 levels and lowered total plasma cholesterol and fasting blood glucose levels in subjects with mature immune systems. There was a negative correlation between IL-10 levels with total plasma cholesterol and fasting blood glucose levels. This adds to the growing evidence that demonstrates the positive effect of mild geohelminth infections on metabolic syndrome factors and indicates that total elimination of such parasites may not be in the best interests of a community.

SIGNIFICANCE STATEMENT

This study was conducted in endemic helminth areas which focused to adolescents and adults with mature immune system. This study discovered positive that there was a positive effect from geohelminth infection to the decrease of cholesterol level in blood that can be beneficial for metabolic syndrome prevention. This study will help the researcher to uncover farther the role of IL-10 in geohelminth infection. The elevation of IL-10 seems not determinable by a number of eggs but the longer-running of infection. Thus, the theory of geohelminth infection beneficial could be proposed.

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