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Original Article

Seroprevalence of *Strongyloides stercoralis* Infection in Patients with Hyperlipidemia: A Case-Control Study

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Abstract

Background: *Strongyloides stercoralis* is one of the soil-transmitted helminths (STH) in tropical and subtropical regions. The role of lipid profile has been investigated in the survival of larval stages of helminths, but there is limited information about the role of lipid profiles and strongyloidiasis. Hence, we aimed to investigate the seroprevalence of *S. stercoralis* infection in patients with hyperlipidemia is compared with the non-hyperlipidemia.

Methods: In 2023, participants were selected from the laboratory of Porsina Hospital in Guilan Province, northern Iran and their lipid profiles including TG, CHOL, LDL, HDL, and VLDL were measured. They were divided into two groups of case and control and matched based on sex and age. *S. stercoralis* Ab (IgG) was measured by ELISA methods, using the NovaTec kit. Finally, statistical analysis was performed.

Results: Each case and control group consisted of 105 participants, from 13 to 80 years old. 56.66% were female and 43.33% were male. The sero-prevalence of *S. stercoralis* was found 4.76% in the case group compared to 0.95% in the control group. We found an association between TG fall and VLDL with sero-prevalence of *S. stercoralis* in hyperlipidemia group (P= 0.034), but other lipid profiles did not show a significant association. A significant relationship was found between contact with dogs and sero-prevalence of *S. stercoralis* (P=0.001).

Conclusion: The sero-prevalence of *S. stercoralis* in the case group was 5 times higher than the control group. A significant association between TG and VLDL fall with *S. stercoralis* Ab (IgG) was observed, but future studies with more sample sizes are suggested to investigate the anti-atherogenic effect of *S. stercoralis*. Also, a genetic assessment of *S. stercoralis* and the host (humane and dogs) is recommended to research zoonotic potential in epidemic areas.

Introduction

Strongyloidiasis is a disease caused by infection with the *Strongyloides stercoralis* nematode (1). *S. stercoralis* is a part of the soil-transmitted helminths (STH) that are endemically found in tropical and subtropical regions (2). It is estimated that more than 370 million people are infected with this parasite worldwide (1,3). The northern (Guilan and Mazandaran provinces) and southern (Hormorzgan and Khouzestan provinces) regions of Iran are endemic regions of strongyloidiasis (4,5). However, there are reports of infection with this parasite in other regions of Iran as well (6,7).

S. stercoralis contamination occurs when the skin is penetrated by the filariform larva. Female worms of this parasite can parthenogenesis and due to the unique cycle (8) of this parasite in individuals with immunodeficiency, the parasite burden increases significantly in these individuals which can cause hyper infection syndrome and disseminated disease which results in extensive tissue invasion. In this situation, a delay in treatment may result in the death of the patient (9–11).

In general, most patients with chronic strongyloidiasis do not show any clinical symptoms (4). However, patients with underlying conditions such as diabetes, chronic liver diseases, cancer, and kidney failure, patients with heart problems, rheumatic disease, colitis, bone marrow transplantation and factors that can cause immunosuppression such as HTLV-1 might show some symptoms (4,12). Recent studies have shown a positive association between contamination by strongyloidiasis and diabetes type II and it has been reported that the probability of disseminated disease caused by strongyloidiasis is higher in patients with diabetes type II compared to non-diabetic patients (13). The diagnosis of strongyloidiasis in diagnosis laboratories depends on parasitological methods (8), but serological tests are used in epidemiological studies and screenings (14,15).

Lipid profile play a role in many vital functions of the body, including organizing organization, dynamics, function, and classification of the cell membrane, providing the main source of energy and participating in the synthesis of steroid hormones (16). The lipid profile of the membrane plays an important role in the transmission of pathogens and messages into the host's cell (17). Different types of blood lipids are cholesterol, triglycerides, and lipoproteins such as High-Density Lipoprotein (HDL), Low-Density Lipoprotein (LDL), and Very- low-density Lipoprotein (VLDL), recent studies have demonstrated that an elevated level of HDL, LDL and total cholesterol is present in patients with parasitic infections (18). *Schistosoma mansoni* metabolism studies have shown that these parasites decrease the total blood cholesterol concentration. They affect atherogenic foods by modulating the host's lipid metabolism and inducing a decrease in total blood cholesterol concentration (17). Also, there has been a report about the decrease of serum lipid and the negative correlation between the production of eggs and larvae and the amount of HLD in the patients infected with strongyloidiasis, hookworm and *Trichuris* spp. (19). Hence, cholesterol might play a role in worm infections by contributing to the survival of the worms in the host's tissues (19). A study in Brazil examined the lipid factors in alcoholic and non-alcoholic patients and concluded that strongyloidiasis has an anti-atherogenic effect (20).

The WHO started a control program to reduce the adverse effects caused by worms in soils, including *S. stercoralis*, in 2008. The results of this program have shown a significant decrease in the effect of worms in endemic regions (21). Given the fact that strongyloidiasis is also endemic in Iran, it is important to identify the factors related to this nematode.

Considering that there is limited information regarding the relationship between strongyloidiasis and lipid profile, we aimed to investigate the prevalence of *S. stercoralis* Ab (IgG) in hyperlipidemia patients for the first time in Iran.

Materials and Methods

This study is a case-control study conducted in 2023. Blood samples were taken from the patient's referred to Porsina Hospital laboratory centers, under the supervision of the Medical School of Guilan (Rasht), Northern Iran with written consent, filling up questionnaires, and recording clinical symptoms. After 12–14 hours of the initial data collection, participants' blood samples were collected. Then clotting, the blood samples were centrifuged at 2500 rpm for 5 minutes.

Biochemistry tests included TG, CHOL, LDL, HDL, and VLDL were measured daily using a chemistry analyzer Alpha Classic-AT++ and biochemistry kits from the MAN Company. The Normal range of TG is <150 mg/dl and CHOL is <200 mg/dl for adults or <170 mg/dl for children (22). Also, the normal range for HDL in men and women is < 60 mg/dl and < 50 mg/dl with low risk,45-60 mg/dl and 35-50 mg/dl with medium risk, and >45 mg/dl and > 35 mg/dl with high risk. On the other hand, for LDL in men and women, 100-119 mg/dl are considered as low risk, 130-150 mg/dl as medium risk and 160 mg/dl < as high risk . The normal range of VLDL is <30 mg/dl (22). Based on the results, participants were categorized into two groups: hyperlipidemia (case) and non-hyperlipidemia (control).

In both groups to be included, all risk factors such as diabetes, chronic liver diseases, cancer, kidney failure, heart problems, rheumatism, colitis, bone marrow transplantation and some diseases such as HTLV-1 infection, HIV and hepatitis B and C must have been absent. Also, the case and control groups were matched based on age and gender.

Then, serum samples were sent to the Diagnostic Laboratory of Strongyloidiasis in the School of Public Health Tehran University of Medical Health, considering standard conditions for performing ELISA test of strongyloidiasis. These samples were maintained in a -20-degree refrigerator.

Anti- *S. stercoralis* Ab (IgG) test using NOVATEC (Novalisa, NovaTec, Germany) manufactured by a German company with a sensitivity of 89.47% (95% confidence) and specificity of 94.12% (95% confidence) was used to measure IgG antibody levels for *S. stercoralis*. The tests were carried out according to the instructions provided with the ELISA kits. Optical Density (OD) was measured at a wavelength of 450 nm using ELISA reader (BioTek-ELx 808) and anti- *S. stercoralis* antibody concentrations were calculated based on kit instructions. The serology test results were interpreted as follows:

11 NTU: Positive serology result

9-11 NTU: Doubtful

< 9 NTU: Negative

It is important to note that positive test results were confirmed with two repetitions, with readings exceeding 11 NTU. Patients with suspicious serology titers were invited for follow-up blood sampling during the subsequent 3-4 weeks. If a patient refused or did not cooperate, their sample was excluded from the study.

Data Analysis

Data analyses were performed via Stata Version 17. Categorical variables were represented using frequencies. To assess the association of variables with strongyloidiasis, the exact Chi-square test was employed. A *P*-value less than 0.05 was considered statistically significant

This project was approved by Tehran University of Medical Sciences with the ethical code: IR.TUMS.SPH.REC.1402.120, and the participating people cooperated in this study with informed consent.

Results

Overall, 210 people participated in this study categorized into hyperlipidemia (n=105) and non-hyperlipidemia (n=105) groups based on their laboratory results. Their age ranged from 13 to 80 years old (Fig. 1).

In the hyperlipidemia group, 6.7% of females and 2.2% males were positive for *S. stercoralis* Ab (IgG). However, in the normal group, only 2.2% females and 0% males had *S. stercoralis* Ab (IgG). Therefore, the seroprevalence of strongyloidiasis was 4.76% and 0.95% in the case and control groups, respectively.

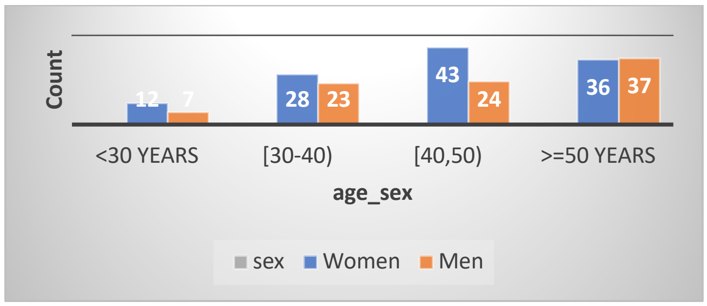

Fig. 1: Age grouping of all participants in this study based on gender

In the hyperlipidemia group, 2 (2.3%) out of 87 individuals with high TG and VLDL and 3 (16.67%) out of 18 individuals with low TG and VLDL were *S. stercoralis* Ab (IgG) positive. Also, 4 (5.19%) out of 78 individuals with high CHOL were *S. stercoralis* Ab (IgG) positive. As well as, out of 61 individuals with a low risk of LDL, 32 individuals with medium risk and 12 individuals with a high risk, 3 individuals (4.92%), 2 individuals (6.25%) and zero individuals respectively were *S. stercoralis* Ab (IgG) positive. Finally, out of 17 individuals with a low risk of HDL, 39 individuals with medium risk and 49 individuals with a high risk, 1 individual (5.88%), 2 individuals (5.13%) and 2 individuals (4.08%) respectively were *S. stercoralis* Ab (IgG) positive. So, the serum prevalence of *S. stercoralis* Ab (IgG) in the hyperlipidemia group was high TG (2.3%), and high CHOL (5.19%). However, a significant relationship between TG falls and VLDL with strongyloidiasis in hyperlipidemia (P=0.034) was observed. Other lipid profiles did not have a significant relationship with the seroprevalence of strongyloidiasis (Table 1).

Table 1: Relationship between blood lipid profiles and the seroprevalence of *S. stercoralis* infection in the case (hyperlipidemia) and control (non-hyperlipidemia) groups

Parameter	***Case (Hyperlipidemia) (n=105)***			***Control (non-hyperlipidemia) (n=105)***	
	Infected (n = 5) X ± SE	Uninfected (n = 100) X ± SE	P-value	Infected (n = 1) X ± SE	Uninfected (n = 104) X ± SE
TG (mg/dL)	170 (66.9)	247.8 (127.7)	0.034	100	121.4(18.3)
TC (mg/dL)	234.2 (47.2)	226.6(37.6)	0.592	158	159.7(25.5)
HDL-C (mg/dL)	44.4 (7.95)	43.7(7.8)	1	38	41.7 (7.4)
LDL-C (mg/dL)	124 (19.7)	129 (23.1)	0.998	91	95.7(20)
VLDL-C (mg/dL)	34 (13.3)	48.3(19.8)	0.034	20	26.6(6.3)

Abbreviations: TC: Total cholesterol, HDL-C: High density lipoprotein-cholesterol, LDL-C: Low- density lipoprotein cholesterol, VLDLC: very low-density lipoprotein; TG: triglyceride

The relevant clinical symptoms were digestive, cutaneous, and respiratory symptoms. Out of the 6 participants with strongyloidiasis, 2 patients had digestive symptoms and one patient had a skin rash and respiratory symptoms simultaneously, but they did not show a significant relationship with strongyloidiasis (*P*= 0.22). In total, 8 participants had a history of contact with animals, out of which 2 of them had contact with dogs. These two participants were in hyperlipidemia group and had *S. stercoralis* Ab (IgG) and a significant relationship between seroprevalence of strongyloidiasis and contact with dogs was observed (*P*=0.001) (Table 2). In addition, no significant association was detected between vegetable cleaning and strongyloidiasis (*P*= 0.61).

Table 2: The relationship between contact with dogs and seroprevalence of *S. stercoralis* infection in the case group (hyperlipidemia)

Contacted with Dogs	*Ab (IgG) S. stercoralis*		*Total*	*P-value*
	No	*Yes*		
No	100	3	103	
Yes	0	2	2	0.001
Total	100	5	105	

Discussion

Strongyloidiasis is a neglected, endemic disease in tropical and subtropical regions of the world (1). The outcome and symptoms of strongyloidiasis patients vary between patients, from having no clinical symptoms to death (8). Some studies have established a relationship between strongyloidiasis and immunosuppressive diseases (12,23).

Lipid profiles play a vital role in the human body. Parasites may enter the cell through the cholesterol present in cell walls (16,17). In experimental studies, a hypothesis has emerged that cholesterol plays a part in the larvae of parasitical worms and affects the pathogenesis of the host (18). Numerous studies investigated the relationship between different lipid factors and *Toxoplasma gondii* (24), *Giardia lamblia* (25) and *Trichomonas vaginalis* (26) but limited studies aimed to assess the relationship between lipid profiles and strongyloidiasis. Hence, this study aimed to investigate this relationship in a sample of patients from an endemic region.

In this study, the prevalence of *S. stercoralis* was 4.76% and 0.95% in the case and control groups, respectively. In recent years, studies have been conducted in northern and southern regions of Iran using serological methods. Kalantari et al utilized ELISA method and reported that 25.6% of diabetic patients had strongyloidiasis (27). In another study, 32% of leptospirosis patients had *S. stercoralis* Ab (IgG) (28). 8.7% of the patients with immunodeficiency in Khouzestan Province were positive for *S. stercoralis* Ab (IgG) (14). Higher rates of strongyloidiasis in the aforementioned studies might be due to the fact that these studies included participants with comorbidities. On the other hand, our study excluded patients who had any comorbidities.

In this study, the prevalence of strongyloidiasis in the case group was 5 times more than in the control group. So, the highest prevalence of *S. stercoralis* Ab (IgG) positive in the hyperlipidemia group was related to individuals with TG, and VLDL normal (16.67%), high CHOL (5.19%), moderate risk of LDL (6.25%) and low risk of HDL (5. 88%). However, there was a significant association between a decrease in TG fell and VLDL with strongyloidiasis in the hyperlipidemia group (*P*= 0.034). Although 4 out of the 5 participants with strongyloidiasis had high cholesterol levels, no association was found between strongyloidiasis and cholesterol, HDL, and LDL. Weidermann et al. (1991) investigated the serum lipid levels of patients with strongyloidiasis, hookworm, and *Trichuris* spp and reported that there is an inverse relationship between the number of worm eggs and HDL levels. They attributed this relationship to a possible role of cholesterol in the survival of the larva in the host and pathogenesis (19). In another study in patients with *Trichinella spiralis* an increase of LDL to VLDL ratio and decrease of HDL-C has been reported (29).

Elizabeth et al. (2017) studied lipid factors, peroxide 1 and cortisol on 276 participants, who were divided into alcoholic and non-alcoholic groups. Based on their findings, in the alcoholic group, the level of TG, LDL-C and VLDL-C were significantly lower in participants with *S. stercoralis*, compared to participants without it. High levels of HDL-C, low levels of LDL-C, VLDL, TG and the activity of PON-1 showed an anti-atherogenic pattern (20). The results of the aforementioned study are in line with our study. On the other hand, changes in lipid plasma levels have been observed in patients with parasitic disease. Hence, a decrease in total cholesterol and triglyceride levels has been reported in patients with *Schistosoma mansoni* (30). Thus, a hypothesis is formed that parasitic infections may have an anti-atherogenic effect on the host's body.

In this study, skin, digestive and respiratory symptoms were the clinical symptoms of patients with strongyloidiasis. Despite having skin symptoms, none of the patients had larva currens which is in line with prior literature (8). Eosinophilia was observed in 6 to 10% of participants with strongyloidiasis. There was no significant association between strongyloidiasis and eosinophilia, education, job, and vegetable cleaning. Conversely, contact with dogs and strongyloidiasis demonstrated a significant association (*P*=0.001). It should be noted that in the prior studies conducted in Iran and other regions on *Cox*1 genes of *S. stercoralis*, genetic similarity and common haplotypes had been observed between isolated of *S. stercoralis* from humans and dogs (31,32), which demonstrates the zoonosis importance of this parasite and showcases the necessity of further research in dogs and humans in endemic regions.

Given the fact that a significant difference has been shown between strongyloidiasis and some diseases such as HTLV-1 and type 2 diabetes, have been reported in the endemic countries, this study serves was as pilot (primary) study in Guilan, which is an endemic region of strongyloidiasis in Iran to establish the relationship between hyperlipidemia and strongyloidiasis. We observed that the prevalence of strongyloidiasis was 5 times more in the hyperlipidemia group compared to the non-hyperlipidemia group. It should be noted, because the prevalence of *S. stercoralis* Ab (IgG) was low, we were not able to investigate the odds ratio (OR) of hyperlipidemia in increasing the chance of contracting strongyloidiasis.

Conclusion

The seroprevalence of *S. stercoralis* was 5 times more in the case group, in comparison to control group. Also, a significant association was observed between the decrease in TG fall and VLDL levels with strongyloidiasis in the hyperlipidemia group. Other lipid profiles did not demonstrate a significant association with strongyloidiasis. So, future studies with larger samples are suggested to investigate the atherogenic effect of *S. stercoralis* and the influence of lipid profiles on the chance of developing strongyloidiasis. Additionally, a significant relationship between contact with dogs and strongyloidiasis was observed. Hence, genetic assessment of *S. stercoralis* and the host (humane and dogs) are recommended to research about zoonotic potential in epidemic areas of strongyloidiasis.

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Conflict of Interest

The authors declare that they have no competing interest.

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