1. Introduction

Intestinal helminthiasis are mainly geo-helminths, which are soil transmitted parasitic nematodes that infect many animals, including humans, and are spread by faecal contamination of soil, foods and water [1]. Intestinal parasitic infections are distributed throughout the world. Ascariasis, hookworm infection and trichuriasis are among the most common infections in the world [2].

Geo-helminth infections are the most prevalent and persistent of all childhood infections and most individuals living in endemic areas are infected at some time during their lives and many are infected continuously from soon after birth into adulthood.
Ascaris lumbricoides, Trichuris trichiura, and Ancylostoma duodenale cause the most prevalent infections. Infection with A. lumbricoides and T. trichiura are acquired at an early age reaching a peak in prevalence and intensity between 5 and 15 years of age. Infections with A. duodenale tend to be delayed until the child is able to walk, and peak prevalence may occur later [3].

Helminthes are responsible for some of the most devastating and prevalent diseases of humans, threatening the lives of nearly one-third of the worldwide human population leading to more than 2 million deaths annually. Habitats of parasites are extremely varied and common parasites of man (helminthes) normally inhabit the intestine, blood, liver, lungs, brain and muscle [4]. Many species of parasites have complex life cycles involving developmental stages that live in soil or water, or use various kinds of intermediate hosts, including vertebrates and invertebrates and cold and warm-blooded animals. In such varied environments, parasites have become adapted to using/tolerating widely differing oxygen, carbon dioxide, hydrogen ion concentrations and temperatures [4]. Their nutritional requirements and their means of obtaining and utilizing the nutrients required for growth, motility and reproduction are also varied. Five species cause widespread disease in humans, these include: Ascaris lumbricoides, Trichuris trichiura, Ancylostoma duodenale and Necator americanus, Strongyloides stercoralis [5].

Cholesterol is a major constituent of eukaryotic membranes and plays a crucial role in cellular membrane organization, dynamics, and function. It is often found distributed non-randomly in domains in membranes [6]. Recent observations suggest that cholesterol exerts many of its actions by maintaining a specialized type of membrane domain, termed “lipid rafts” in a functional state. Lipid rafts are enriched in cholesterol and sphingolipids, and have been thought to act as platform through which signal transduction events are coordinated and pathogens gain entry to infect host cells [7].

Many scientists have observed changes in lipid profile in subjects infected with helminthes, for instance, in-vivo study by Wiederman et al., [8] showed decreased serum lipid levels in the Shipibo population (Peru). They also reported a significant inverse correlation between worm egg excretion and high density lipoprotein – cholesterol (HDL – C) levels in hookworm, Strongyloides and Trichuris infected patients but not in Ascaris infected cases. In 1990, Biadun, [9] observed that during larval ascariasis, the metabolism of lipids is significantly disturbed; also, decreased levels of total cholesterol (TC), high density lipoprotein – cholesterol (HDL – C), and triglycerides (TG) were observed in guinea pigs. The mechanisms underlying the observed association between intestinal worm load and HDL reduction are not completely understood and may include reduced HDL synthesis in the gut wall due to inflammatory toxic irritation. Hence, cholesterol may have a role in pathogenesis by helping the larvae to survive in the host tissue [8].

Relationship of serum cholesterol levels in man infected with parasites has drawn the attention of various workers. Intestinal helminthes infections are most prevalent and persistent of all childhood infections [3]; however, detailed reports on the evaluation of lipid profile in children infected with intestinal helminthes are lacking in this community. To examine the mechanisms underlying the association between intestinal worm load and lipids profile. Thus, this work is designed to determine the plasma lipids and lipoproteins in intestinal helminthiasis among primary school children in Osogbo, Osun State Nigeria.

2. Materials and Methods

2.1. Study Area/Subject Selection

The study was carried out in three Primary Schools; (St. Michael, All Saint School ‘A’ and ‘B’, Primary) all located in Osogbo metropolis, the State Capital of Osun State, Nigeria. A total of three hundred and twelve (312) primary school children aged between 9 – 13 years were selected from the three primary school in Osogbo metropolis

2.2. Ethical Approval

Ethical clearance and approval was obtained from Osun state Ministry of Health and Ethical Committee of Ladoke Akintola University of Technology Teaching Hospital, Osogbo, Osun State. Permission was sought from the Local Education Authority, Olorunda Local Government and the heads of the schools were then duly informed of the study after, which an appointment was made for sample collection. Informed consent from both the children and their parents were also sought and obtained before the commencement of the study. Subjects were included in the study if they had obtained consent from their parents and are pupils in primary three (3) up to primary six (6) as recommended by World Health Organization Genera WHO [10].

2.3. Sample Collection

2.3.1. Stool Sample Collection

The participants were given stool receptacle (Universal Container) on the eve of the day of examination with specific instruction to collect it in the morning. The containers were labeled with the specific assigned identification numbers for each subject.

2.3.2. Blood Sample Collection and Preparation

The children were instructed not to eat in the morning before coming to school in other to obtain an overnight fast of about 12hours. 5ml of venous blood sample was obtained by venous puncture (non-traumatically) of antecubital vein with sterile needle and syringe into EDTA anticoagulant bottle. The blood samples were spun with a bench centrifuge (Surgiﬁeld SM – 2) at 4,000rpm for 10 minutes. Plasma was separated into plain bottles immediately after collection and stored at – 20oc until time for analysis.

2.3.3. Stools examination

Each of the faecal samples obtained was examined using direct faecal smear (direct level saline preparation and iodine preparation) and super saturated sodium chloride solution for ova, larva and/or cyst of parasite as described by WHO [10] Monica [11] and Hassan et al [12].
2.4. Estimation of Plasma Total Cholesterol

Total cholesterol concentration was determined by the enzymatic method of Allain et al., [13]. The concentration of cholesterol in the sample was measured at 520 nm spectrophotometrically following the procedure of Ochei and Kolhatkar [14].

2.5. Estimation of Plasma High Density Lipoprotein (HDL) – Cholesterol

The HDL-Cholesterol was determined using Randox test kit [15,16]. The absorbance of the samples, control and the standard respectively were read against the reagent blank using a spectrophotometer at 545nm.

2.6. Estimation of Plasma Triglycerides

The Plasma Triglycerides were determined using glycerol phosphate oxidase - Peroxidase Method [17,18]. The intensity of the coloured compound formed is measured at 545nm using a spectrophotometer.

2.7. Estimation of Plasma Low Density Lipoprotein (LDL) – Cholesterol

The low density lipoprotein – cholesterol concentration (LDL-C) was calculated from the total cholesterol concentration (TC), the high density lipoprotein – cholesterol concentration (HDL-C) and the triglycerides concentration (TG) in accordance with Tietz [19] as follows:

\[ \text{LDL-C} = \frac{\text{TC} - \text{TG}}{2} - \text{HDL-C} \]

2.8. Statistical Analysis

The SPSS (Statistical Package for Social Science) software package version 16.0 was used for the statistical analysis. Values obtained from the study expressed as mean ± standard deviation were compared using the independent Student’s t-test and significance was measured at P<0.05. The relationship between variables were determined using the Pearsons product moment correlation coefficient and significance was measured at P<0.05 or P<0.01.

3. Result

A total of Three hundred and twelve (312) stool and blood samples were obtained of which one hundred and fifty-two (152) were males while one hundred and sixty (160) were females, aged between 9 – 13 years.

128 (41.0%) of the 312 stool samples examined were positive for helminthes of which 72 (47.4%) were males while 68 (42.5%) were females. All the eggs encountered belonged to two species, A. lumbricoides and T. trichuris. The egg of the A. lumbricoides was the most prevalent as it was encountered in 112 (35.9%) participants while the egg of T. trichuris was found in 8 (2.6.3%) participants. Co-infection of A. lumbricoides and T. trichuria was only observed in 12 (3.8%) participants (Table 1).

### Table 1: Prevalence Of Helminth Eggs Examined Among The School Children In Osogbo, Nigeria

<table>
<thead>
<tr>
<th>Species</th>
<th>No. Examined</th>
<th>No. Positive</th>
<th>% Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. lumbricoides</td>
<td>312</td>
<td>112</td>
<td>35.9</td>
</tr>
<tr>
<td>T. trichuria</td>
<td>312</td>
<td>4</td>
<td>1.3</td>
</tr>
<tr>
<td>Ascaris</td>
<td>312</td>
<td>12</td>
<td>3.8</td>
</tr>
<tr>
<td>and T. trichuris</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>312</strong></td>
<td><strong>128</strong></td>
<td><strong>41.0%</strong></td>
</tr>
</tbody>
</table>

Key:

- \( A. \text{lumbricoides} = \) Ascaris lumbricoides
- \( T. \text{trichuria} = \) Trichuris trichuria

There were significant decreases in the mean concentration of plasma total cholesterol and high density lipoprotein cholesterol in the infected subjects when compared with the controls (p<0.05). However, the mean concentrations of plasma triglycerides and low density lipoprotein cholesterol were not significantly different in infected subjects when compared with non-infected participants (p>0.05) (Table 2). Using the Pearsons product moment correlation coefficient (Table 3), there was a negative relationship between the mean concentration of plasma total cholesterol and egg count per gram of stool (p<0.05), and HDL-C and egg count per gram of stool (p<0.05) of the parasitic group. Similarly, there was a negative relationship between the mean concentrations of plasma low density lipoprotein – cholesterol and total cholesterol (p < 0.01), and high density lipoprotein-cholesterol (p< 0.01).

### Table 2: Comparison Of The Mean Concentration Of Plasma Lipid And Lipoproteins In Infected And Control Subjects

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group</th>
<th>Number</th>
<th>Mean ± S.D</th>
<th>t-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC</td>
<td>Test</td>
<td>128</td>
<td>2.23±0.53</td>
<td>0.57</td>
<td>0.014</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>184</td>
<td>2.54±0.52</td>
<td>5.48</td>
<td>0.01</td>
</tr>
<tr>
<td>HDL-C</td>
<td>Test</td>
<td>128</td>
<td>0.57±0.33</td>
<td>0.89</td>
<td>0.98</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>184</td>
<td>0.69±0.44</td>
<td>0.59</td>
<td>0.22</td>
</tr>
<tr>
<td>TG</td>
<td>Test</td>
<td>128</td>
<td>1.42±0.57</td>
<td>3.60</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>184</td>
<td>1.34±0.72</td>
<td>0.59</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Key:

- \( \text{TC} = \) Total cholesterol
- \( \text{HDL-C} = \) High density lipoprotein cholesterol
- \( \text{TG} = \) Triglycerides
- \( \text{LDL-C} = \) Low density lipoprotein cholesterol
- \( \text{S.D} = \) Standard deviation

p<0.05 is significant; p>0.05 is not significant.
Table 3: Correlation of Physical And Biochemical Parameters In The Study.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>EC/GS</th>
<th>Age</th>
<th>Sex</th>
<th>TC</th>
<th>HDL-C</th>
<th>TG</th>
<th>LDL-C</th>
</tr>
</thead>
<tbody>
<tr>
<td>EC/GS</td>
<td>1.000</td>
<td>0.084</td>
<td>-0.017</td>
<td>-0.288*</td>
<td>-0.227*</td>
<td>-0.156</td>
<td>-0.038</td>
</tr>
<tr>
<td>Age</td>
<td>-0.017</td>
<td>1.000</td>
<td>0.041</td>
<td>0.067</td>
<td>0.020</td>
<td>0.087</td>
<td>0.001</td>
</tr>
<tr>
<td>Sex</td>
<td>-0.017</td>
<td>0.041</td>
<td>1.000</td>
<td>0.073</td>
<td>-0.023</td>
<td>-0.096</td>
<td>0.117</td>
</tr>
<tr>
<td>TC</td>
<td>-0.288*</td>
<td>0.067</td>
<td>0.073</td>
<td>1.000</td>
<td>0.109</td>
<td>0.121</td>
<td>0.708**</td>
</tr>
<tr>
<td>HDL-C</td>
<td>-0.227*</td>
<td>0.020</td>
<td>-0.023</td>
<td>0.109</td>
<td>1.000</td>
<td>-0.68</td>
<td>-0.554**</td>
</tr>
<tr>
<td>TG</td>
<td>-0.156</td>
<td>0.087</td>
<td>-0.096</td>
<td>0.121</td>
<td>-0.68</td>
<td>1.000</td>
<td>-0.154</td>
</tr>
<tr>
<td>LDL-C</td>
<td>-0.038</td>
<td>0.001</td>
<td>0.117</td>
<td>0.708**</td>
<td>-0.554**</td>
<td>-0.154</td>
<td>1.000</td>
</tr>
</tbody>
</table>

Key:
* Correlation is significant at the 0.05 level (2-tailed).
** Correlation is significant at the 0.01 level (2-tailed).

4. Discussion

Morbidity due to soil transmitted helminthes has remained a major problem in the study area and other parts of the world[1]. In this study, the overall prevalence rate of 41% was observed. It was shown that A. lumbricoides and T. trichuria were the commonest helminthes in the area with high prevalence of Ascaris than T. trichuria. The higher prevalence of Ascaris agreed with a previous report by [12]. A. lumbricoides is the largest and the most common helminthes parasitizing the human intestine and currently infects about 1 billion people worldwide [20]. The high morbidity due to Ascaris is a reflection of environmental contamination and unsanitary lifestyle in the study area (12). This is a dangerous trend as intestinal parasites have been shown to impact deleterious effect on children especially those of school age [2].

The significant decrease in the mean concentrations of plasma total cholesterol, high density lipoprotein – cholesterol and non-significant decrease in mean concentration of plasma triglycerides; an insignificance increase in the mean plasma concentration of low density lipoprotein – cholesterol were observed in the infected participants compared with the negative participants. This partly agreed with Biadun [9] report whose findings showed decreased levels of total cholesterol (TC), high density lipoprotein – cholesterol (HDL – C), and triglycerides (TG) in guinea pigs. Data from the 1988–1994 National Health and Nutrition Examination Surveys (NHANES) for ages 4 to 19 years showed that the mean total cholesterol concentration was 165 mg/dl or 4.3mmol/L [21]. Age-specific values for mean total cholesterol concentration actually peaked at 171 mg/dl or 4.4mmol/L at 9 to 11 years of age. In comparison with the above report, the mean total cholesterol concentration of the study population were found to be relatively low, most probably due to a low proportion of dietary fat. This study showed decrease plasma cholesterol; this is similar to that obtained by Wiedermann et al [8], who demonstrated decreased serum lipid levels in the Shipibo population. Ascaris has been implicated with nutritional states of the subjects. For instance, Woodruf [22] observed that the presence of Ascaris in children is often associated with poor nutritional states. The poor malnutrition may also account for the decreased plasma cholesterol.

One of the objectives of this study was to determine the mechanism underlying the association between intestinal worm load and lipid profile if any. It was deduced from the study that, there was a negative relationship between egg count per gram of stool (intestinal worm load) and the mean concentrations of plasma total cholesterol (p < 0.05); egg count per gram of stool (intestinal worm load) and high density lipoprotein cholesterol (p < 0.05), which implies that as the intestinal worm load (intensity) increases the total cholesterol and high density lipoprotein – cholesterol decreases. Similarly, there was a positive relationship between LDL-C and TC; LDL-C and HDL-C. This result is partly consistent with earlier report by Wiedermann et al.[8], which showed a significant inverse correlation between worm egg excretion and high density lipoprotein – cholesterol (HDL – C) levels in hookworm, Strongyloides and Trichuris infected patients but not in Ascaris infected cases.

5. Conclusion

In conclusion, this study has shown that intestinal helminthes (Ascaris and Trichuris trichuria) decrease plasma lipids (TC, HDL-C, TG). Furthermore, it showed an inverse relationship between the intestinal worm load and mean concentrations of plasma TC, and HDL-C. Similarly, there was an inverse correlation between mean plasma concentration of LDL-C and TC, and HDL-C. However, further studies regarding the mechanism through which intestinal helminthes increase LDL-C is highly needed as these entire process play role in the liver functionality and hormonal secretion. In view of the considerable morbidity and the public health significance of these parasitic infections, coupled with the fact that children are the future of any nation, it then becomes necessary as a matter of urgency to control these infections in the community. It is therefore suggested that well organized health education should be inculcated in the children and the parents. Also, the Local Government Authorities must also improve on the environmental disposal of human and animal wastes in the city (Osogbo) to overcome the health problems.

Acknowledgment

We are grateful to the Education Secretary, Local Government Education Authority, Olorunda, Igbon, Osogbo for his assistance during the sample collection. We also thank the Headmaster and Headmistress of All Saint School ‘A’ and All Saint School ‘B’, St. Michael School and the children of the above schools especially primary three to primary six. The support of the staff of the ministry of Health and Education of Osun state of Nigeria is also appreciated.

Contribution of authors We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. OAJ, SFF and NAS developed the concept. HAO designed the experiments. MMA and HRO carried out blood samples collection. AAD and SFF prepared the blood samples for and the lipid profile assays. HAO performed parasitological assays, data analysis and prepared the manuscript.
6. References


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