The Egyptian Journal of Hospital Medicine (July 2022) Vol. 88, Page 3906-3910

# Evaluation of Adipokines Among Children Infected with Some Protozoan Intestinal Parasites

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## ABSTRACT

**Background**: Adipokines are molecules produced by adipose tissue that have involvement in the body's energy/metabolic status and inflammation. It has recently been shown that there is a relationship between these molecules and infection with some intestinal parasites. **Objectives**: The current research aimed to assess the effect of *Cryptosporidium* and *Giardia lamblia* on adipokines. **Patients and methods**: This research was conducted in Baghdad from October 2021 to March 2022. The study included 56 children of both sexes, ranging in age from 2 to 15 years. The enrolled children were divided, according their stool examination, into infected by *Giardia lamblia* (n=21), infected by *Cryptosporidium* (n=23) and apparently healthy children (n=12). All participating children were subjected to some biometric measurements (body mass index (BMI), weight for age percentile and height for age percentile) and adipokines assessment (adiponectin, leptin and IL-6). **Results:** A significant increase ($P<0.05$) in adiponectin and leptin were observed for *Cryptosporidium* and *G. lamblia* infections versus the control group. While significant decrease ($P<0.05$) in IL- 6 were noticed for *Cryptosporidium* and *G. lamblia* infections compared to the control group. BMI, weight for age percentile and height for age percentile were not significantly related with each of adiponectin, leptin and IL-6 for infected patients and control groups. **Conclusions:** Leptin and adiponectin secretion, as well as nutrient absorption, can be interfered with by intestinal *Cryptosporidium* and *G. lamblia*. Their precise function in intestinal parasitosis requires further study.

**Keywords:** Adiponectin, *Cryptosporidium*, *Giardia lamblia*, IL-6, Leptin

## INTRODUCTION

Infectious diarrhea is among the important causes of morbidity and mortality in children worldwide [(1)]. The transmission of this kind of diarrhea is increased by contaminated water and poor sanitation and hygiene [(2)]. It is known that many intestinal parasites are associated with infectious diarrhea especially in children [(3,4)].

*Cryptosporidium* and *Giardia lamblia* are among the most common causes of infectious diarrhea especially in children [(5)]. *Cryptosporidium* is an intracellular protozoan parasite that infects epithelial cells in the microvillus border of its host's gastrointestinal tract, producing impairment to the epithelium and disturbing its role, resulting in mild to severe diarrhea and other outcomes [(6)]. *Giardia lamblia* is a flagellated protozoan parasite with a worldwide distribution that has been documented to cause physiologic alterations at the mucosal surface [(7,8)]. Both *Giardia lamblia* and *Cryptosporidium* are spread by the fecal-oral route, with cysts and oocysts being eaten directly or indirectly[(5)].Anorexia, malabsorption, weight loss, malnutrition, and anemia have all been associated to these two parasites [(9,10)]. They are also linked to mucosal surfaces and activate mesenteric lymph nodes, which are located near adipose tissue. As a result, when the lymph nodes are active, the adipocytes begin to respond by secretion adipokines [(11)].

Adipokines (also known as adipocytokines) are cytokines produced by adipose tissue that have a role in the body's energy/metabolic status, inflammation, obesity, and other functions. Adipokines include leptin, adiponectin, interleukin-6, and other factors [(12)]. Some intestinal parasites can cause anorexia by disrupting the release of leptin and adiponectin, as well as affecting the absorption of specific nutrients. Anorexia caused by parasites is an acute phase reaction to infection mediated by cytokine-induced leptin production [(13)].

Many researchers [(14-17)] have surveyed the epidemiology and demographic status of *Cryptosporidium* and *Giardia lamblia* in different populations from different parts of Iraq [(18-20)]. Other researchers looked at their association to nutritional status, anaemia, blood biochemical parameters, and vitamin deficiencies [(20,21)], but little is known regarding their relation to adipokines level, mainly in children. The objective of this research was to evaluate the adipokines among children with intestinal protozoan parasites.

## PATIENTS AND METHODS

### *Study design and patients*

This research was carried out in Baghdad, Iraq, from October 2021 to March 2022 at a number of primary health care centers and hospitals. Fifty six children were enrolled from both sexes. There were 34 (60.71%) males and 22 (39.29%) females with ages ranging from 2 to 15 years old. In the sixteen weeks prior to the investigation, none of them had used anti-helminth or anti-protozoa medicine, and none of them had other infections.

### Biometric measurements

A free-standing portable stadiometer and an electronic weighing device were used to determine height and weight. While their weight was being measured, each participant was asked to remain motionless on the scale. The measurements were taken to the nearest 0.1 kilogram and 0.5 cm. The children were dressed in the bare minimum of clothing and wore no shoes when they were weighed. By dividing the



Received: 10/04/2022
Accepted: 07/06/2022

weight (kg) by the square of height (m), the BMI was calculated. The calculator from https://reference.medscape.com/calculator/ was used to compute weight for age percentile and height for age percentile.

**Collection and examination of stool**

Stool samples were collected from all children using screw cap container. The identification number and collection date were written on each sample. The formalin-ether technique [21] was used to concentrate the samples. By using a sterile pipette, a drop of each deposit was taken and smeared on a glass slide. Another smear was prepared and stained by modified acid-fast technique [22]. All smears were examined by light microscope with ×100 objective. The 56 participants were separated into two groups according to the results of their stool examinations. The first group was considered as patients group (n=44); they had either *G. lamblia* or *Cryptosporidium* infection, while no parasites were detected in the second group which was considered as control group (n=12). The first group was subdivide into *G. lamblia* infected children (n=21) and *Cryptosporidium* infected children (n=23)

**Serum preparation and adipokines analysis**

Blood samples were taken from each participant by venipuncture technique. The samples were placed in gel activator tubes and allowed to coagulate at 25ºC for about half an hour before being centrifuged for 15 minutes at 5500 rpm. Each serum sample was divided into three portions equally. The concentrations of adiponectin, leptin, and IL-6 were evaluated using sandwich enzyme-linked immunosorbent assay (ELISA) kits (BioSource, USA), as directed by the manufacturer's guidelines. All of the children in this study had their samples examined in a 96-well plate. Then plate was read at 450 nm wavelength using ELISA reader.

**Ethical approval**

**An approval of this study was obtained from the University of Baghdad Academic and Ethical Committee (Ref.: CSEC/0222/0024). Informed consent from all the guardians of patients was taken. This study was carried out in accordance with the World Medical Association Code of Ethics (Declaration of Helsinki) for studies involving humans.**

***Statistical Analysis***

The Statistical Analysis System - version 9.1 was used to do statistical analysis on the data. Mann-Whitney test was used to evaluate the significant differences between parasite infected children and control group regarding the means of adiponectin, leptin, and IL-6. As well as Kruskal-Wallis test was used to measure the significant differences regarding the same parameters among *G. lamblia* infected group, *Cryptosporidium* infected group and control group. Pearson correlation coefficient was used to determine the relation between each of BMI, weight for age percentile, height for age percentile with adipokines (adiponectin, leptin and IL-6) in both parasite infected group and control group. Dunn's test was also used to compare each group with each other group). Statistical significance was defined as a P value of less than 0.05. All data were stated using the mean and standard deviation (mean ± SD).

**RESULTS**

Results presented significant increase in serum adiponectin concentration among patients compared to control group. As well as significant increase in serum adiponectin concentrations were noticed in *G. lamblia* infected children followed by *Cryptosporidium* infected children compared to control group. Significant differences (P<0.05) were noticed between *G. lamblia* infected children and *Cryptosporidium* infected group. As well as a significant differences (P<0.05) were noticed between *Cryptosporidum* infected children versus control (Table 1).

**Table 1**- Serum adiponectin level in patients and control groups

| Group | Adiponectin µg/mL (Mean±SD) | P-value | |
|---|---|---|---|
| Patients (44) | 2.61± 0.45 | 0.03* | Z score= 1.87 |
| Control (n=12) | 1.98±0.24 | | |
| *Giardia lamblia* infected children (n=21) | 2.25±0.21[a] | 0.001* | H-test =41.97 |
| *Cryptosporidium* infected children (n=23) | 2.49±0.37[b] | | |
| Control (n=12) | 1.98±0.24[a] | | |

Means with a different small letter in same column significantly different (P<0.05) using Dunn's test.

Results also showed non-significant correlation between adiponectin and each of BMI, weight for age percentiles, and height for age in both of patients group and control group (Table 2).

**Table 2**- Correlation between adiponectin with BMI, weight for age percentile and height for age percentile in patients and control groups

| Variables | Adiponectin | |
|---|---|---|
| | Patients | Control |
| BMI | r= 0.2711<br>p= 0.075 | r= -0.0734<br>p=0.82 |
| Weight for age percentile | r= -0.0534<br>p=0.73 | r= -0.0503.<br>p=0.87 |
| Height for age percentile | r= 0.0898<br>p=0.56 | r= 0.1045<br>p=0.74 |

r=Pearson correlation coefficient

The results of leptin did not differ from the results of adiponectin, as patient group had the highest mean of leptin versus low level in control group. In terms of adiponectin results, statistical analysis revealed a significant difference between the groups. Moreover, the results showed that there were significant differences regarding leptin levels among the 3 groups. The highest was reported for *G. lamblia* infected children followed by *Cryptosporidium* infected children, while control group showed the lowest level of leptin. Significant differences (P<0.05) , regarding the level of leptin, were noticed between *G. lamblia* infected children and control group. As well as a significant differences (P<0.05) were detected between *Cryptosporidum* infected children versus control (Table 3).

**Table 3**- Serum leptin level in patients and control groups

| Group | Leptin ng/mL (Mean±SD) | P-value | |
|---|---|---|---|
| Patients (44) | 8.02±2.11 | 0.001* | Z-Score= 4.9 |
| Control (n=12) | 0.35±0.22 | | |
| *Giardia lamblia* infected children (n=21) | 8.66±2.5[a] | 0.001* | H-test= 25.1 |
| *Cryptosporidium* infected children (n=23) | 7.41±1.8[a] | | |
| Control (n=12) | 0.35±0.02[b] | | |

Means with a different small letter in same column significantly different (P<0.05) using Dunn's test.

Negative but non-significant relation were detected between BMI and leptin concentration in both patients and control. Weight for age percentile was also showed negative but non-significant relation with leptin in both patients and control group. Additionally, height for age percentile showed negative but non-significant relation with leptin in both patients and control (Table 4).

**Table 4**- Correlation between leptin with BMI, weight for age percentile and height for age percentile in patients and control groups

| Variables | Leptin | |
|---|---|---|
| | Patients | Control |
| BMI | r= -0.0923 p=0.55 | r= -0.4197 p=0.175 |
| Weight for age percentile | r= -0.197 p=0.199 | r= -0.1909 p=0.554 |
| Height for age percentile | r= -0.0618 p=0.69 | r= -0.2045 p=0.52 |

r=Pearson correlation coefficient

The results of IL-6 were completely differed from the results of both adiponectin and leptin levels as there was significant decrease of IL-6 for the patients group versus higher level of IL-6 in control group. As well as, it was found that IL-6 levels were significantly different among the 3 groups. Significant decrease of IL-6 was recorded in both *G. lamblia* infected children and *Cryptosporidium* infected children while the control group showed the highest level of IL-6. Significant differences were (P<0.05) noticed between *G. lamblia* infected children and control group. As well as a significant differences (P<0.05) were detected between *Cryptosporidum* infected children versus control (Table 5).

**Table 5**- Serum IL-6 level in patients and control groups

| Group | IL-6 pg/mL (Mean± SD) | (P-value) | |
|---|---|---|---|
| Patients (44) | 5.86 ±1.08 | 0.001* | Z-score=3.9 |
| Control (n=12) | 23.2 ±1.09 | | |
| *Giardia lamblia* infected children (n=21) | 4.71 ± 1.92[a] | 0.001* | H-test=27.98 |
| *Cryptosporidium* infected children (n=23) | 6.89±1.09[a] | | |
| Control (n=12) | 23.2 ±1.09[b] | | |

Means with a different small letter in same column significantly different (P<0.05) using Dunn's test.

The study found non-significant correlation between Il-6 and each of BMI, weight for age percentiles, and height for age in both of patients group and control group (Table 6).

**Table 6**- Correlation between IL-6 with BMI, weight for age percentile and height for age percentile in patients and control groups

| Variables | IL-6 | |
|---|---|---|
| | Patients | Control |
| BMI | r= -0.1714 p=0.26 | r= 0.0637 p=0.84 |
| Weight for age percentile | r= -0.0775 p=0.613 | r= 0.0561 p=0.86 |
| Height for age percentile | r= 0.0138 p=0.92 | r= -0.1909 p=0.55 |

r=Pearson correlation coefficient

## DISCUSSION

Adiponectin and leptin, two adipocytokines that link nutrition and immunology, have emerged as the most abundant adipocyte components [23]. In the current study, there was a significant increase in adiponectin among infected children with *Cryptosporidium* or *Giardia lamblia*. Adiponectin has been shown to regulate glucose and lipid metabolism [24]. Some investigators showed that serum adiponectin levels are adversely linked with blood glucose [25]. *Cryptosporidium* damages portions of the intestinal brush border membrane, which is necessary for the absorption of nutrients such as glucose [26]. Infection with *G. lamblia* causes epithelial barrier failure, which leads to glucose, salt, and water malabsorption as well as a reduction in disaccharidase activity due to a loss of absorptive surface area [27]. So the low level glucose can negatively correlated with high adiponectin level.

Finding of the current study revealed a non-significant relation between BMI, weight for age percentile and height for age percentile with adiponectin levels in both patient group and control group. This could mean that the inflammatory response generated by *Cryptosporidium* and *G. lamblia*, rather than BMI, is responsible for the rise in adiponectin levels [28].

In this study, significant increases in leptin levels were also seen after *Cryptosporidium* and *G. lamblia* infections. This is because intestinal parasites can disrupt the intestinal mucosa, resulting in crypt dilation and shortening of intestinal villi, which can stimulate mesenteric lymph nodes, which further stimulate neighbouring adipose tissues to release leptin [29]. Immunity to several intestinal parasites is aided by leptin [30]. Leptin may stimulate intestinal epithelial regeneration and suppression of apoptosis [31], besides its pro-inflammatory effects on the immune response, it promotes mucin secretion and maintains intestinal structure [32]. Leptin is also an eosinophil survival factor in humans [33]. This finding corroborated previous research, which found that parasite infections such as *Entamoeba histolytica* and *Strongyloides* were linked to higher levels of leptin [13].The finding of this present study also indicated that the level of IL-6 was not associated with parasite infection as it level was showed to be decreased. The absence of correlation found here could be due to intestinal parasites' methods for avoiding detection by the systemic immune response, such as immunological modulation and evasion [34].

Finally, the current study also showed a non-significant relation between BMI, weight for age percentile and height for age percentile with leptin levels and IL-6 in both patients group and control group. Because high BMI results may represent excess adiposity, the BMI is commonly used as a screening tool to predict weight-related health risks. In children, however, BMI does not assess body composition and cannot distinguish between fat and muscle [35]. Therefore, the non-significant relationship between body mass index and both leptin and IL-6 may not be accurate in the current study.

## CONCLUSION

Finally, via altering immunological response, both adiponectin and leptin have a role in intestinal parasite infections. Intestinal parasites (*Cryptosporidium* and *G. lamblia*) can disrupt leptin and adiponectin secretion as well as nutrition absorption. More investigation is needed to determine their precise role in intestinal parasitosis.

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