Wasit Journal for Pure Science

Journal Homepage:
https://wjps.uowasit.edu.iq/index.php/wjps/index
e-ISSN: 2790-5241 p-ISSN: 2790-5233

The Relationship Between Giardia Lamblia Infection with Ghrelin Hormone and Trefoil Factor 3 in Patients in Salah al-Din Governorate

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DOI: https://doi.org/10.31185/wjps.555
Received 15 September 2024; Accepted 22 October 2024; Available online 30 December 2024

ABSTRACT: Giardiasis is considered one of the parasitic diseases affecting humans, caused by a flagellate protozoan known as Giardia lamblia. It is a common gastrointestinal pathogen that can lead to various clinical and other complications. Study aimed to diagnose infection with the *G. lamblia* in children aged (1-12 years) using a direct wet mount, and to determine Giardia lamblia by targeting the TPI gene using PCR, in addition to evaluating the relationship of infection of Giardia lamblia with (ghrelin and TFF3) using ELISA assay. By examining 238 stool samples for children suffering from diarrhea and some intestinal symptoms visiting some hospitals in Salah al-Din Governorate. 34 (14.2%) positive samples were recorded by microscopic examination. 100 fecal samples using the PCR, showed 60 (60%) positive samples for the G. lamblia. The study revealed a highly significant increase in the level of TFF3 a non-significant decrease in the level ghrelin in the serum of patients with infection. Direct microscopic examination of stool samples It has good sensitivity for detecting G. lamblia parasite, and the TPI gene targeted in PCR technology has high sensitivity and specificity for genotyping detection of the parasite.

Keywords: *Giardia lamblia*, PCR, TPI, Ghrelin hormone, TFF3

1. INTRODUCTION

Giardia lamblia, also known as (*Giardia duodenalis* or *Giardia intestinalis*) is the causative agent of giardiasis (1).*G.lamblia* caused a widespread illness in both humans and animals that was recognized As opportunistic, affecting healthy people immunocompetent. According to the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO), *Giardia lamblia* is the eleventh parasite that is transmitted by food in terms of priority (2). Early detection of giardiasis is essential for effectively treating and preventing diseases. The conventional laboratory diagnosis includes microscopic examination of at least three independently collected fecal samples to detect trophozoites or cysts (3). Moreover, *G. lamblia* is categorized into 8 genotypes termed by A, B, C, D, E, F, G and H, according to *triosephosphate isomerase* (*tpi*), *glutamate dehydrogenase* (*gdh*) and *small–subunit (SSU) rRNA* genes (4). Giardiasis presents a significant challenge due to its growing prevalence in the environment. Nevertheless, conventional diagnostic

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techniques continue to exhibit limited sensitivity. Utilizing genetic testing for identifying human pathogens through PCR proves to be a beneficial approach with satisfactory sensitivity in detecting G. duodenalis in human fecal sample (5). The chemical characteristics called biomarkers, which are markers of the physiological, pathological, or pharmacological response to therapeutic intervention, can be objectively checked in fluid and tissue samples (6). Conceptually, biomarkers can have diagnostic, prognostic, or therapeutic value (7). The hormone ghrelin is made from ghrelin Predominantly in the stomach, but also expressed in many other organs including the intestine The pancreas, kidneys, myocardium, hypothalamus, and pituitary gland (8). Trefoil factor 3 or TFF3 are formed in intestinal goblet cells in association with mucin (9).

2.MATERIALS AND METHODS

2.1 Study design and population

The present cross-sectional study was conducted between July 2023 and June 2024. A total of 238 children, aged between 1 day and 12 years, visited the outpatient clinic at a hospital in Salah al Din, Iraq, seeking medical advice. These children presented with various gastrointestinal symptoms, such as weight loss, cramping in the abdomen, diarrhea, and gas. Furthermore, several kids displayed no symptoms at all.

2.2 Fecal samples collection

The sterile cup was used to collect a single stool sample chosen randomly. Both macroscopic and microscopic analysis of the samples were conducted right away. All samples were placed in designated tubes without preservatives and stored at −20 °C until molecular analysis was performed.

2.3 Serum samples collection

90 Serum samples (60 positive for parasite+30 negative controls) were collected from patients and suspected of being infected with *Giardia*, during the collection of stool samples, and stored at -20 °C for later use with the ELISA test.

2.4 Microscopic examination

This method was executed by preparing clean glass slide and two drops of N.S. was put-on one-half slide and a drop of Lugals iodine on the other half, two swabs are taken from stool samples by wood stick from different places of samples (about pin head in size). One of them was put on normal saline and mixed well, while the other swab mixed with Lugals iodine then covered by cover slip and

examined using the magnification power 400 (40 x 10) to record the presence of trophozoites and/or cysts [4].

2.5 Molecular technique

2.5.1 DNA Isolation

DNA was isolated from stool samples Using a (Stool DNA Extraction) kit prepared by the Taiwanese company Geneaid. The concentration and purity of genomic DNA extracted by Nanodrop (Napi, Korea) was measured by reading the absorbance at (260/280 nm) and stored at − 20°C.

2.5.2 *Giardia lamblia* DNA amplification by PCR

The molecular characterization of *Giardia lamblia* loci, specifically the triose phosphate isomerase gene (tpi), is identified through PCR. This gene, tpi, is relatively short in length. Consistency is observed in the primer design used across various research studies. The amplification of the tpi gene was carried out using specific primers, detailed in Table 1(5).

2.5.3 PCR for amplification

This thermocycler was set for four minutes at 94 °C, a temperature that is necessary to break DNA bonds. Denaturation was then carried out for 30 seconds at 94 °C. The perfect primer annealing temperature for the TPI gene is 52 °C for 30 seconds. The final step was to extension at 72 °C for 30 seconds. Finally, a four-minute extension at 72 °C is required. Gel electrophoresis to separate amplified DNA, a 2% agarose gel was utilized, and the DNA was stained with DNA safe dye. The DNA ladder was employed on 110 volts for 60 minutes and viewed by used gel documentation(5).

Table 1. The primer sequence utilized

Primer	Sequence	Size	Reference
tpi gene	Tpi F 5-AAATIATGCCTGCTCGTCG-3 Tpi R 5-CAAACCTTITCCGCAAACC-3	605 bp	(5)

2.6 Human Ghrelin hormone and Trefoil factor 3 ELISA test

90 Serum samples (60 positive +30 negative control) were used to assess Ghrelin hormone and Trefoil factor 3 protein using the ELISA kits (China-BT LAB). Human antibodies percolated through the ELISA plates. We added the samples to the plates and correlated the color development in the substrate solution with the levels of Ghrelin hormone and Trefoil factor 3 protein. The process was stopped by adding a stop solution, and absorbance was measured at 450 nm.

2.7 Statistical analysis

Statistics were tested by IBM SPSS 26.0 and GraphPad Prism (version 8, GraphPad Software Inc., CA, USA). The data was expressed as mean ± SE, and significant results were determined using the T-test ($P < 0.05$).

2.8 Ethics approval and consent to participate

In accordance with the mandates of the Scientific Committee of the Salah al-Din Health Department and the Scientific Committee of the College of Science at Tikrit University, authorization for the research study was issued on 9 July 2023, following the guidelines outlined in administrative directive 3625. Subsequent to informing the parents about the study, a questionnaire was utilized to obtain samples from the children, with parental consent obtained prior to gathering information on each child, including their name, address, and prevalent symptoms.

3. RESULTS AND DISCUSSION

3.1 Diagnosis of *G. lamblia* by direct Examination

The total number of this study was 238, 34 sample (14.285%) test result was positive for microscopic light infection of *Giardia*, Uninfected samples (85.714%). As in Table 2 and Figure 1.

Table 2. diagnosis of Giardia by Microscopic Examination

	Microscopic examination	
Number of positive cases	34	14.285 %
Number of negative cases	204	85.714 %
The total number	238	

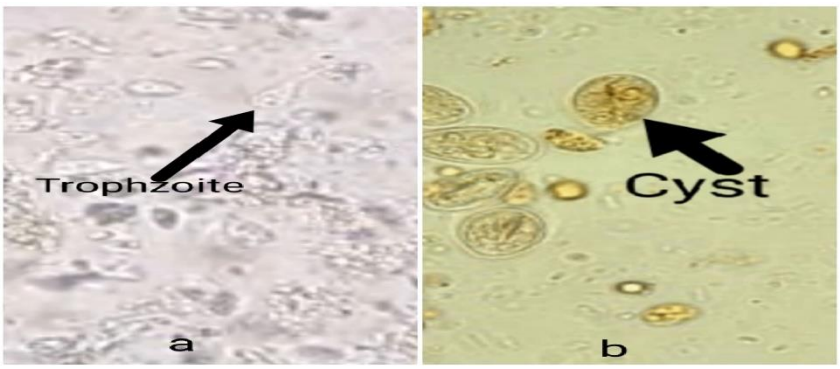

Figure 1 (a) show Trophozoite of by wet smear normal saline (0.9%) 40X (b) cyst by Lugol's iodine (1%) 40X

3.2 Diagnosis of *G. lamblia* specie by PCR assay

PCR diagnosis was performed on 100 samples, of which 34 samples were diagnosed by light microscopy, and 66 samples were negative by light microscopy. only 60(60%) positive samples for DNA from the specific TPI gen. As in Table 3 and Figure 2.

Table 3. diagnosis of *Giardia lamblia* by PCR assay

	PCR assay fore TPI gene	
Number of positive cases	60	60%
Number of negative cases	40	40%
The total number	100	100%

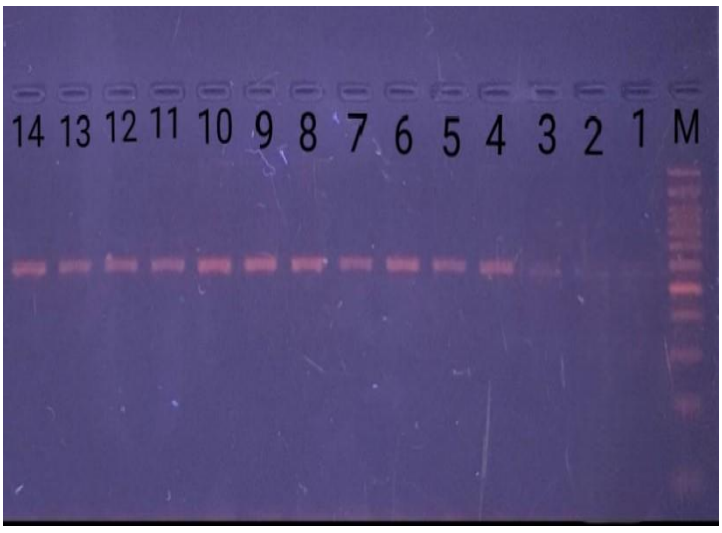

Figure 2. PCR product is subjected to electrophoresis in a gel made of 2% agarose. M: DNA size marker (100 bp ladder) is used to denote the size of DNA. Bands in wells (1-10) indicate the presence of *Giardia lamblia*.

3.3 Human Ghrelin hormone and Trefoil factor 3 ELISA test

3.3.1 Ghrelin hormone

The current study recorded a non-significant decrease in the average concentration of the hormone ghrelin in children's people with giardiasis reached (9.495±0.7246 ng/ml) compared to the control group, which reached (9.898±0.8008 ng/ml) as shown in the table 4 and figure 3.

Table 4. Ghrelin hormone concentration in patients with giardiasis

Ghrelin ng/ml	Patients group N=60	control group N=30	P. Value
Mean ±S. E	9.495±0.7246	9.898±0.8008	0.7317

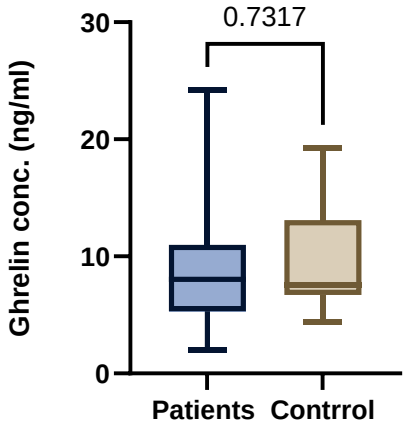

Figure 3. Level of ghrelin in patients' blood serum

3.3.2 Trefoil factor 3

The current study recorded a highly significant increase in the average concentration of Tff3 in children with the disease Giardia (191.5±14.49 ng/ml) compared to the control group (113.5±11.62 ng/ml) As shown in the table 5 and figure 4.

Table 5. Tff3 concentration level in patients with giardiasis

Tff3 ng/ml	Patients group N=60	control group N=30	P. Value
Mean ±S. E	191.5±14.49	113.5±11.62	0.0007*

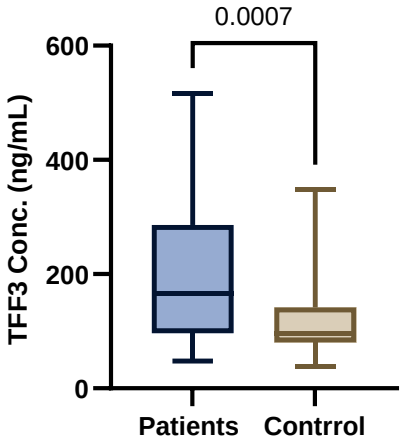

Figure 4. Tff3 level in patients' blood serum

Giardiasis represents the predominant etiology of protozoan infections and diarrheal afflictions on a global scale.Among several demographic categories, children appear as the most vulnerable cohort to Giardia infection (10, 11). Within the scope of this research, Feces specimens were obtained of 238 patients, revealing the incidence of giardiasis of 14.285% by microscopic examination. This percentage is in close agreement with previous prevalence estimates (12) documented in the vicinity of Tikrit, where a prevalence of 14.30% was reported. However, notable inconsistencies exist when compared to the results of disparate investigations, such as investigations conducted in Mosul (13). These studies determined *G. lamblia* infection 1.04%. The variations observed can be attributed to differences in sample size, disparities in environmental circumstances across the studied regions, and various factors including personal hygiene, population density, sanitation standards, geographical positioning, climatic conditions, and socioeconomic standing. Furthermore, the discrepancies are influenced by the level of precision, laboratory expertise, and methodologies employed in the analysis of stool samples. Several investigations have focused on various genetic loci within the genome of *G. lamblia* for molecular characterization, exhibiting differing levels of sensitivity and specificity. The utilization of the TPI gene in this research for the DNA identification of *G. lamblia* was based on its notable genetic homogeneity and polymorphism(14, 15). The presence of G. lamblia genomic DNA was detected in 60 samples out of 100(60%), with 34 samples confirmed positive by microscopic examination. This study is similar to previous studies, In Iraq, Baghdad Governorate, the TPI gene was duplicated in 4 of 7 samples, representing 57.14% (16), and in the city of Diwaniyah, the gene doubled in 27 of 50 samples, a rate of 54% (17). The current study differed from other studies. In Tikrit, the gene was amplified in 6 of 17 samples, at a rate of 35.29% (18). In Najaf,

the gene was amplified in 80 of 100 samples, at a rate of 80% (19). These occurrences are attributed to various factors that could impact DNA isolation, including the presence of some PCR inhibitors in feces, conditions related to Specimen preservation and the particular technique or model of DNA isolation kit used. Furthermore, discrepancies in amplification conditions and the targeted gene may also play a role, alongside the existence of single nucleotide polymorphisms, deletion insertions, and the diversity of Giardia species, all contributing to the generation of inaccurate negative findings. The elevated prevalence of parasite infection is likely a consequence of the substandard economic and residential conditions prevalent in certain regions of the nation. This situation arises due to habitation in inadequately developed areas owing to the absence of healthcare facilities, high population density, and inadequate access to clean water. Furthermore, various areas within Salah al-Din Governorate face service deficiencies resulting from population growth and insufficient provision of health and educational support for the residents. The results of the current study agreed with (20), who indicated a decrease in the level of the hormone ghrelin in people infected with giardiasis reached (33.245 ± 0.847 ng/mL) they compared with the control group (50.102 ± 1.121 ng/mL) and agreed with (21) Which indicated a decrease in the level of the hormone ghrelin in people with giardiasis, reaching (28.85 ±3.2 ng/ml) compared with the control group (81.39 ±3.8 ng/ml), but did not agree with (22) Which indicated an increase in the level of the hormone ghrelin in people infected with giardiasis, as it was reported in children City (85.34 ± 4.79 ng/ml) and rural children (88.24 ± 6.49 ng/ml) compared with a group Control (71.84 ± 14.10 ng/ml). Low ghrelin concentrations in patients with intestinal parasitic infections are thought to compensate This is an increase in the concentration of glucose, hence the relationship between insulin and ghrelin (8).The decreased ghrelin concentration observed in patients in this study is consistent with the suggestion that this may it is the main cause of anorexia in patients suffering from parasitic infections (23). Another possible reason that explains the cause-and-effect relationship, at least in part, is low levels of ghrelin

It may be due to reduced lipid peroxidation in infected people that increased as a result of the parasite (24). The results of the current study agreed with (25), which indicated an increase in the level of Tff3 in people infected with giardiasis reached (0.13907 ± 0.006 ng/ml) in males. In females (0.20530 ± 0.03 ng /ml) compared to the control group (0.078864 ± 0.005 ng /ml) (0.082629± 0.008 ng /ml) respectively, and agreed with (26) Which indicated an increase in the level of Tff3 in people with giardiasis, reaching (29.65 ± 2.45 ng /ml) Compared with the control group (8.60 ± 0.70 ng /ml). The reason for the increased concentration of TFF3 in people infected with the parasite is its

important role in protection and repair the mucous layer of the gastrointestinal tract and cell migration, TFF3 usually increases when the organ is damaged Digestive system(26).

Discrepancies in research methodology, participant selection criteria, and limited sample size could elucidate the conflicting findings observed in our investigation and might constrain its generalizability. Furthermore, there was an ignore in assessing the presence of concurrent virus and intestinal microbial infections, which might have influenced the results, inducing clinical presentations and obscuring the authentic clinical repercussions of G. lamblia infection. More extensive researches on a large-scale population is needed to confirm the connection between the parasite and biomarkers. Subsequent molecular epidemiological investigations across extensive geographical regions are vital for investigating virulence factors, parasite genotypes, and infection sources in the environment. Certainly, conducting genotyping studies at the subpopulation level is indispensable for determining the prevalence and scope of zoonotic transmission occurrences and the relationship between assemblage.

4. CONCLUSION

Giardiasis remains to be a common zoonotic sickness among children in Salah al-Din Governorate, Iraq. The results of our study indicate a high prevalence of Giardia in a portion of the population being studied, and there has been a notable rise in the average concentration of Trefoil factor 3 in patients infected with Giardia and decrease in the average concentration of the hormone ghrelin was recorded in affected patients. These results indicate that it is necessary to strengthen surveillance measures and increase community awareness regarding disease transmission, especially in rural areas. This research explores the molecular epidemiology and evaluates the efficacy and reliability of species-specific primers for Giardia. Highlights the need for further genetic investigations focused on elucidating potential associations between the parasite and other biomarkers.

REFERENCES

[1]. Mostafa DK, Abdulwahhab IG, Ahmed NS. Genetic identification of Giardia lamblia in children for Tikrit city, Iraq. http://dx.doi.org/10.21931/RB/

[2]. Mozer S, Abdulwahhab IG, Al-Azaawie AF. Extraction of the DNA of Giardia lamblia isolated from vegetables and fruits in a simplified way and its diagnosis using Nested-PCR. Journal of Parasitic Diseases. 2022;46(3):771-5. https://doi.org/10.1007/s12639-022-01484-4
[3]. Alharbi A, Toulah FH, Wakid MH, Azhar E, Farraj S, Mirza AA. Detection of Giardia lamblia by microscopic examination, rapid chromatographic immunoassay test, and molecular technique. Cureus. 2020;12(9). https://doi.org/10.7759/cureus.10287
[4]. Al-Ani SF, Al-Dulaimi MF, Al-Fahadawi SM. Detection of genotypes Giardia lamblia (A and B) in human feces of Iraqi patients according to Triosephosphate isomerase (TPI) gene characterization. Biochemical & Cellular Archives. 2020;20(1). DOI : 10.35124/bca.2020.20.1.2015
[5]. Ahmad AA, El-Kady AM, Hassan TM. Genotyping of Giardia duodenalis in children in upper Egypt using assemblage-specific PCR technique. PloS one. 2020;15(10):e0240119. https://doi.org/10.1371/journal.pone.0240119
[6]. Ahsan H. Biomolecules and biomarkers in oral cavity: bioassays and immunopathology. Journal of Immunoassay and Immunochemistry. 2019;40(1):52-69. https://doi.org/10.1080/15321819.2018.1550423
[7]. Dhama K, Latheef SK, Dadar M, Samad HA, Munjal A, Khandia R, et al. Biomarkers in stress related diseases/disorders: diagnostic, prognostic, and therapeutic values. Frontiers in molecular biosciences. 2019;6:91. https://doi.org/10.3389/fmolb.2019.00091
[8]. Erensoy A, Aydin S, Kelestimur N, Kirbag S, Kuk S. The change of ghrelin levels in intestinal parasitic infections. Journal of Medical Biochemistry. 2010;29(1):34. DOI: 10.2478/v10011-010-0004-0
[9]. Wiede A, Jagla W, Welte T, Kohnlein T, Busk H, Hoffmann W. Localization of TFF3, a new mucus-associated peptide of the human respiratory tract. American journal of respiratory and critical care medicine. 1999;159(4):1330-5. https://doi.org/10.1164/ajrccm.159.4.9804149
[10]. Mahdy AM, Surin J, Wan K, Mohd-Adnan A, Al-Mekhlafi MH, Lim Y. Giardia intestinalis genotypes: Risk factors and correlation with clinical symptoms. Acta Tropica. 2009;112(1):67-70. https://doi.org/10.1016/j.actatropica.2009.06.012
[11]. Anuar TS, Nor Azreen S, Salleh FM, Moktar N. Molecular epidemiology of giardiasis among Orang Asli in Malaysia: application of the triosephosphate isomerase gene. BMC infectious diseases. 2014;14:1-12. https://doi.org/10.1186/1471-2334-14-78
[12]. Hasan TAH, Muhaimid AKA, Mahmoud AR. Epidemiological study of Giardia lamblia in Tikrit city, Iraq. Sys Rev Pharm. 2020;11(9):102-6.
[13]. Dhubyan Mohammed Zaki Z. Prevalence of Entamoeba histolytica and Giardia Lamblia associated with diarrhea in children referring to lbn Al-Atheer Hospital in Mosul, Iraq. Archives of Razi Institute. 2022;77(1):73-9.
[14]. Thompson[1] R, Monis P. Variation in Giardia: implications for taxonomy and epidemiology. Advances in parasitology. 2004:69.
[15]. Huey CS, Mahdy MA, Al-Mekhlafi HM, Nasr NA, Lim YA, Mahmud R, et al. Multilocus genotyping of Giardia duodenalis in Malaysia. Infection, Genetics and Evolution. 2013;17:269-76. https://doi.org/10.1016/j.meegid.2013.04.013
[16]. Abbas BM, AL-Saqur IM, Majeed HA. Detection and genotyping of Giardia lamblia in clinical and environmental samples in some regions of Baghdad city. Int J Curr Microbiol App Sci. 2016;5:459-68.
[17]. Al-Difaie RS. Molecular study to detect genotyping of Giardia lamblia from human and cattle feces in Al-Qadisiya Governorate, Iraq. world. 2016;5:6.

[18]. Hasan TAH, Muhaimid AKA, Mahmood AR. Identification of Giardia lamblia genotypes among children in Tikrit City by using nested PCR. Indian Journal of Forensic Medicine & Toxicology. 2021;15(2):1112-7.
[19]. Alhatemi AKS, AlHuchaimi SN, Alshammari MMM, Bashbosh AE, Obaid RF. Phylogenetic analysis of Giardia lamblia using small subunit ribosomal RNA (ssrRNA) gene and triose phosphates isomerase (TPI) gene isolated from Iraqi patients. EurAsian Journal of BioSciences. 2020;14:1127-33.
[20]. Al-Hadraawy SK, Al-ghurabi ME, Al-musawi MM, Alzeyadi M. Ghrelin and melatonin as biomarkers in patients with giardiasis. Biotechnology & Biotechnological Equipment. 2016;30(3):553-7. https://doi.org/10.1080/13102818.2016.1149038
[21]. Al-Nafakh RT, Mubark HA, Al-Asady RA-A. Assessment of Serum Concentration of Ghrelin and Obestatin in Giardia lamblia Infected Patients: A Case Control-Study. Indian Journal of Forensic Medicine & Toxicology. 2021;15(1).
[22]. Hussein ZT. Interrelated impacts of Giardia lamblia, Ghrelin, Cholecystokinin, and Interleukins 6, 11, and 15 on the Digestive system in children. microscope.1:2.
[23]. De Vriese C, Hacquebard M, Gregoire F, Carpentier Y, Delporte C. Ghrelin interacts with human plasma lipoproteins. Endocrinology. 2007;148(5):2355-62. https://doi.org/10.1210/en.2006-1281
[24]. Kılıç E, Yazar S, Saraymen R. Lipid peroxidation level in patients with Blastocystosis. Journal of Inonu University Medical Faculty. 2010;10(1):1-3.
[25]. Toma RS, Al-Hadraawy SK. Trefoil factor3 (TFF3), calprotectin (CALP) and (SIgA) as immunological markers in patients infected with Giardia lamblia parasite. Journal of Pharmaceutical Sciences and Research. 2018;10(9):2221-4.
[26]. Al-halaly ASS, Shubber HWK. Study of Some Immunological Markers of People with Parasitic Infestation and their Relationship to Colorectal Cancer. Medico-legal Update. 2021;21(2).