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Prevalence, risk factors and molecular investigation of Giardiasis among infants in Al-Shamia City, Al-Qadisiya Governorate, Iraq.

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Abstract

The present study aimed at examining the *Giardia dudenale* infection in diarrheal infants who visited the Al-Shamia General Hospital from January 2017 until the end of June 2018. (200) samples of feces were examined for infants aged (1 month-2 years), the samples at the beginning examine by the direct moist smear method and the results showed that 45 samples were positive for infection (22.5%). The study also focused on the impact of some important factors related to children which represent the specific risk factors for the disease, such as age, gender, and nutrition type, the results showed that the lowest percentage was (20.22%) in the age group (1-6 months), while the highest rate of infection was (38.07%) in the age group (1-2 years), and the rate of parasite infection in males and females is somewhat similar, where was (31.69%) and (29.93%), respectively, with the highest rate of infection (39.21%) for infants who rely on artificial feeding and the lowest rate of breastfeeding infants (19.99%). Positive samples were subjected to molecular diagnosis using the most accurate Real-Time PCR technique, the results showed that the parasitic infection rate was (89.42%) positive for microscopic examination. The current study focused exclusively on infants under two years of age in terms of the spread of parasites between this age group using traditional methods and molecular methods together to give an accurate idea of parasite prevalence among the age group under study, It also addressed the risk factors that affect the spread of the parasite *G. dudenale*, because taking precautions to reduce the impact of a particular factor will be reflected positively to reduce the prevalence of the disease because of this parasite of a significant impact on the deterioration of the health and development of children.

Keywords: *Giardia dudenale*, Infants, Real-time PCR, Risk factors.

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Introduction

Giardia dudenale parasite is one of the flagellate that infection different species of animals [1] it has a greater impact on children all over the world, prevalence ranging between 2-5% in industrialized countries, as well as percentage exceed 30% in developing countries [2], it causes Giardiasis disease, its act infection as a result taking the mature cysts of the parasite by mouth in eating food and through oral-fecal contact [3]. This widespread of parasite all over the world is due to some feature the simplicity a life cycle, rapid reproduction and it's don't dependent of carriers of malaria or leishmaniasis [4], so survival for several weeks in humid environments [5], and has virulent factors that inhibit the effectiveness of the immune system of hosts, example cysteine proteinases and an extracellular enzyme [6].

This study showed that Giardiasis in the deterioration of the nutritional status of infants, a random sample of infected infants in the city of Al-Shamia and determine effect of age of patient, gender, and type of feeding as well as area of residence on the incidence of disease, this study considered the first of the city because it specialized in the infection of infants without two years Giardia intestinal parasites and the diagnosis of the use of R-T PCR.

Material and Methods

200 feces samples of patients were collected among the visitors of the Al-Shamia hospital for the period from January 2017 until end of June 2017. A questionnaire was organized for all patient, including the patient's age, gender, feeding as well as the housing area, the samples were then placed in sterilized plastic containers, the samples were examined by the microscopic examination under the light microscope using the direct saline method prepared with the method 0.9% direct normal saline solution [7] and after the completion of the microscopic examination, the samples of positive kept in a temperature of -20to molecular examination, real-time PCR

test was conducted to investigate *G. dudenale* by using *ssurRNA* primers used by Beck et al. [8], the DNA was then extracted from the samples by using a (Stool Genomic DNA extraction) kit supplied by the Korean company Bioneer, according to the company's instructions.

Primers were used specialized for *ssuRNA* genes to diagnosis of the parasite used in Study [9], its prepared by the Korean company Bioneer and examined according to Statistical analysis system SAS Users Guide [10] (Table 1).

Table 1: *Primer used in R-T PCR technique with their nucleotide sequence.*

Primer		Sequence	PCR product size
ssu RNA	F	ACG GGT GAA ACA GGA TGA TCC	73bp
	R	TGA TTG ACA GAG GCG GTC TTG	

Real-time PCR reaction mix was prepared by the AccuPower® 2X GreenStarTMqPCR Master Mix, manufactured by the Korean Bioneer Company and as the company's instruction.

Real-time PCR Thermal cycles have been applied according to the AccuPower® 2X GreenStarTMqPCRmaster mix instructions by calculating the temperature of the Tm primers by the MiniOpticon Real-Time PCR system BioRad, USA device.

The results were analyzed using amplification plot according the Threshold Cycler Number (CT) value, that the sample is positive infection when it exceeds the threshold line, and were analyzed by using the statistical program (SAS, 2012) and Square-X^2 to determine the differences between the prevalence of parasite and the risk factors under probability level of ($p \leq 0.05$) [10].

Results and Discussion

This study showed that 45 samples were positive for *G. dudenale* parasite (22.5%), was higher than recorded by Al-Abodi et al. [11] in Al-Qadisiyah governorate which recorded (22.85%), and higher than [12] in his study in Al-Qadisiyah Governorate when it was examined and recorded (22.2%) of positive infection in age group less than 2 years, and it was also found higher than [9] when examined 926 sample feces for children with diarrhoea ranging less than 12 years and recorded (5.61%). It may also be attributed to differences in different factors such as living standards, personal hygiene, so total number of specimens tested, and differences in sanitation, geographical location, population density, examination methods, accuracy of the examiner and screening methods [9].

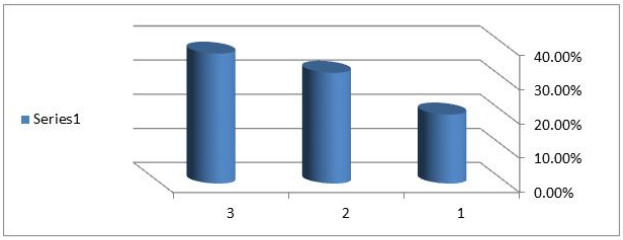
The results showed that the highest percentage of infection with giardiasis was in the age group (1-2 years) (38.07%) while lower percentage in the age group (1-6 months) (20.22%), that agree with the results obtained in Al-Difaie et al. study [11] in Al-Qadisiyah governorate about infection with giardiasis in infants in age group (1-2 years) (25.35%), also higher than the study [13] about the investigation of *Entamoeba histolytica* in the age group (1-2 years) (61.76%) and the lowest percentage in (1-6) months (36.17%), may be this results due to the nature that infants in this age more active and lack of awareness of the rules of hygiene, and practice a habit of placing fingers in mouth, in this age they are eager to learn and taste anything a round them that may be increase probability of infection with disease at this age group, while we do not agree with [14] they recorded the highest percentage of parasitic infection in age group (10-20) years (56.03%), the results of statistical analysis indicated there were significant differences in parasitic infection among age groups under the probability level $p \leq 0.05$ (Figure 1).

The results of the current study referred to no significant differences between male and female infants patients in infection rates with *G. dudenale* under the probability level of ($p \leq 0.05$) with (31.69%) males (29.93%) female, this results agree with [11] when recorded percentage of infection in males (22.51%) and (23.15%) in females, so with [13] Which recorded (53.15%) in male and (50.55%) in female, this results may be due to the similar conditions that infants for both sexes are exposed to (Figure 2).

The results recorded the highest percentage of was in artificially dependent infants (39.21%) Compared with (19.99%) for breast-feeding, which is agree with [11] when recorded infection rate (27.61%) for infants with artificial feeding while (15.71%) for breast-feeding infants, so with [13] when recorded infection rate with *Entamoeba histolytica* in value (59.79%) and (41.09%) for artificial and natural breastfeeding infants respectively, this results may be due to the immunity acquired for breast-feeding compared with the artificial-feeding patients [11], as well as the less care to artificial-feeding and exposure to various contaminants, the statistical analysis of the results referred to the existence of significant differences between the infection rate of giardiasis disease and the type of feeding (Figure 3).

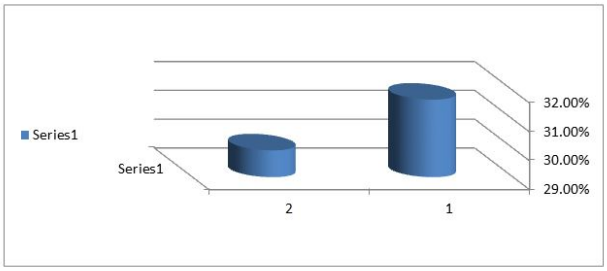
In this study we using R-T PCR technique as an ascertained detection based of the *ssurRNA* gene in *G. dudenale* gene in 45 positive microscopy samples, the results showed that percentage of infection (89.42%), as in Figure 4.

This results is higher than what recorded in the study of Sabanero et al. study [15], which reached to (87.5%) in Al-Qadisiyah governorate when they examined (40) positive samples for microscopic examination for infants using the same methods, so higher than [9] which recorded (73.07%) and study [15] in Egypt when they recorded infection rate (75%), in Menoufia province and (73.9%) and Sharkia province, as well as its higher than recorded by Sadek et al. [16] in Germany with infection percentage of (60.1%) by examining 583 stool samples children with diarrhea, and higher than [17,18] in Egypt when showed the results of the molecular examination according to (tpi) gene and in (42.3%).



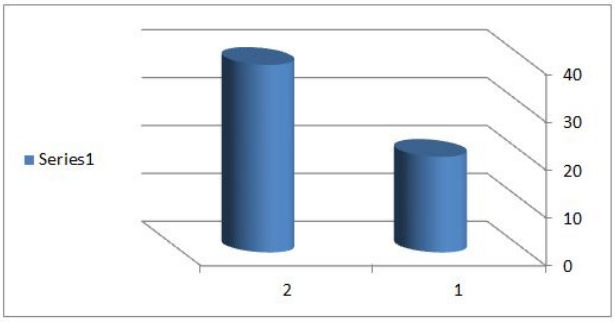
X² calculated=7.321 **X²=Table3.525**

Figure 1. *Infection percentage according to age group of patients.*



X² calculated= 5.016 **X²table= 5.035**

Figure 2. *Shows the relationship between parasitic infections with the gender of infants patient.*



X² table=5.823 **X²calculated=5.107**

Figure 3. *The relationship between infection rate with giardiasis and the type of feeding.*

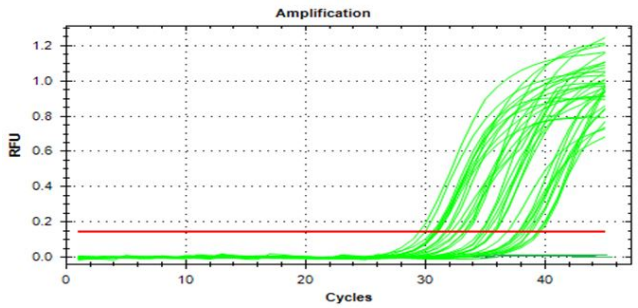


Figure 4. *The amplification plot of the R-T PCR for positive results of the G. dudenale according to ssurRNA gene.*

Conclusion

In conclusion, we can say that the difference in PCR results due to many factors such as differences in the DNA extraction methods and PCR methods, so the amount of parasites found in stool samples, the presence of negative samples because the false in microscopic diagnosis in primary examination and the presence of inhibitory substances in stool samples and may be associated with DNA polymerase enzyme and inhibits its work, the molecular technology used as the most recent and accurate molecular diagnostic techniques.

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