ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Correlation between ABO Blood Group Expression and The Giardiasis Infection in Human.

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ABSTRACT

The current study aimed to determine the total rate of giardiasis infection in humans including infected rate with or without symptoms. Also, the infection rate according to gender was under consideration. The frequency distribution for ABO and Rh blood groups was assessed to seek for the possible relationships with giardias is. The current study was carried in the Microbiology laboratory of Al-Yarmouk teaching hospital/Baghdad/Iraq for the period from the 1st of March till the end of August /2016.The study include of 532 patients (318 male and 214 female) aged 20-45 years old to detect the existence of *Giardia lamblia*in stool samples by microscopic examination using flotation method and staining method (Lugol's iodine and Chromo trope 2R).The blood samples were collected from 130 patients for the purpose of measuring the concentration of IgG and IgM antibodies in serum using Enzyme Linked Immuno sorbent Assay(ELISA)and to determine blood type using ABO and Rh blood group system. The results revealed that the total infection rate was 32.89% with no significant differences according to gender as the higher rate was recorded for males 18.79% compared with females14.09%. For the infection rate according to state, the symptomatic patients recorded 69.7% (59.8% for males and 40.1% for females)while, asymptomatic patients recorded 30.2% (50.9% for males and 49.05% for females),no significant association p=0.27 between gender and the state was observed while, significant differences P<0.0001 for the total symptomatic and the total of asymptomatic was recorded. The mean concentration of IgM and IgG showed significant differences between infected (symptomatic, asymptomatic) and non-infected patients for each of males and females. The overall blood-group frequencies for symptomatic were A(40%) > B (26%)> O (24%) > AB (10%) and for asymptomatic were O (42%) > A (24%) > B (22%) > AB (12%) also higher percentage was observed for Rh positive 98%,96% for symptomatic and asymptomatic respectively, no significant association between ABO P=0.19, Rh P= 1.04blood group and the state of infection was detected. a significant correlation (P<0.01) showed between the total infection rate and the distribution in each of blood group (0.77)and Rh test (0.79). Also a significant correlation (P<0.01) (0.69) and(0.59) was found between ABO and each of symptomatic and asymptomatic infections respectively. The correlations of Rh with symptomatic (0.82) and asymptomatic (0.54) were significant (P<0.01). The overall finding show edhighly prevalent of *G. lamblia* mainly in males. There was a correlation between human blood and Rh types with giardiasis as most blood type A individuals were symptomatic patients while most blood type O were asymptomatic.

Keywords: *Giardialamblia*, Diagnostic methods, Prevalence rate, ABO blood group and Rh

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ISSN: 0975-8585

INTRODUCTION

One of the most common and important hygiene problems worldwide are parasitic infections, particularly in the developing countries[1].The World Health Organization (WHO) reports showed that people worldwide are infected with parasites, among which G.lamblia is the most common[2].Giardiasis is a gastrointestinal infection in humans caused by the protozoan parasite G.lamblia, and considered as a neglected disease in both industrialized and developing countries[3,4].The prevalence of giardiasis is as high as 10% - 50% in the developing countries [5].TheWHO has estimated more than 280 million of human infections occur annually in Africa,America and Asia, [6]. The Centers for Diseases Control (CDC) recorded that in the United States there are more than 2.5 million cases of giardiasis occur each year[7].Infected persons with Giardiasis may be showsymptoms such as diarrhea, with abdominal cramp, anorexia, and weight loss. Approximately $2X10^8$ people have symptomatic giardiasis inAfrica, Latin America and Asia, and about 500,000 new cases are recorded annually[8].On the other hand, asymptomatic persons characterized by a periods with constipation or diarrhea and malabsorption[9].Epidemiology rates for asymptomatic infections according to American Medical Association [10]were about two-third of infected people have no symptoms which leads to difficulty in the eradication and control of disease [11,12] .

Because of the high incedance and disease burden of the infection, Giardiasis exerts a significance public health impact. Infections usually associated with poor personal hygiene, poor water quality and over crowding. Disease variabilityis related with multiple factors such as host age and gender, level of host immunityand nutrition, strain genotype, infective dose and possibly co-infection [13,14].

Fecal-oral rout with the ingestion of the infective stage(cyst) in contaminated food and water consider to be the main source for Giardiasis, transmission also can occur from person-to-person [15].

The detection of G.lamblia depended mainly on traditional method such as microscopic methods including direct smear method, Flotation method and Sedimentation method[16,17,18]. The more sensitive and more specific method focusing on the detection of Giardiaantigen using immune diagnosis through Enzyme Immunoassay(EIA) and Enzyme-Linked Immuno sorbent Assay(ELISA)[19,17].On the other hand ELISA method used for antibodies detection in serum of infected patients, the detection of specific IgG and IgM antibodies in serummay be helpfulin differentiation between patients with recent or acute infection fromthose with previous or treated infections[20].Recentlymolecular diagnosis permit the detection of causative agent depending on gene amplification such as Polymerase Chain Reaction (PCR) method [21].

Many microorganisms , parasites and virus infections associated with the differences in blood group antigen expression of the host. Blood group play a direct role indecrease orincrease host susceptibility to infections through serving as receptors and/or co-receptors and modify the natural immune response to infection.

A series of glycolipids withglycoprotein on the surface of red blood cell constitute blood group antigens ABO and Rh in rhesussystems. This antigen is genetically controlled [22]. Human populations share the same blood group systems with inherited from common ancestor but they differ by their frequencies [23].

The frequency distribution of ABO and Rh blood groups was assessed to seek for the possible relationships with giardiasis in both symptomatic and asymptomatic patients in this study.Investigation of total prevalence rate of G.lamblia infection in humanand the infection rate according to gender in symptomatic and asymptomatic patients was in account.

MATERIAL AND METHODS

Stool samples

During the period from the 1st of march till the end of August /2016 ,532 human stool samples were collected in sterile plastic cups approximately 5 g from patient attended Al-Yarmouk teaching hospital laboratory including 318 sample from male and 214 sample from female aged 20-45 years old, the presence of G.lamblia was observed in both symptomatic andasymptomatic patients.

Identification of Giardia lamblia

Macroscopic examination was done for each stool sample including the observation of the color, odour, consistency (hard, loose or watery), presence of blood , mucus and other foreign bodies.

Microscopic examination involve: direct microscopy by using saline(0.85% of NaCl solution)and 1%Lugol's iodine preparation, examination under 400X magnification to observe the trophozoite sand/or cyst stage of G.lamblia. Also flotation technique which is based on specific gravity was done by using Zinc sulfate solution for cyst purification[24]. Chromotrope 2R stain(Weber's modified trichrome,1g chromotrope 2R (sigma); 0.15g fast green; 0.25g phosphotungstic acid; 3 ml acetic acid; 100 ml distilled water) was used for fecal smear staining.The development of this staining method was obtained in CDC using various components of the Trichrome staining method to differentiate G.lambliacysts and trophozoites from background fecal elements.

Reagents of this stain include Absolute methanol, Chromotrope stain(sigma company), Acid alcohol, ethanol (95% , 100%) and Xylene. The first step of this method require fixation of fecal smear for 1-2 minutes with absolute methanol. The fecal smear then was covered for 90 minutes with Chromotrope stain then, adding acid alcohol for 1 to 3 seconds. The smear was dipped in 95% of ethanol followed by exposure to 100% of absolute ethanol for 3 minutes and transferred to xylene for 10 minutes. Slide was kept to dry in air and mounted with coverslip using Canada balsamas mounting media . Examination under microscope was done using higher power (40X) o. G.lamblia appear with light brownish to grey color [25].

Blood samples

Approving obtained from130 patients to withdraw 3 ml of bloodsample.These samples divided into three categories of 50 samples belonging to both symptomatic and asymptomatic patients (included 25 samples of both males and females)and 30 sample fornon-infected persons(control) including 15 sample for both males and females. Blood was collected with a sterile, disposable plastic syringe.

ABO and Rh test

Four drops were usedFor blood grouping and Rh factor examination by the slide method depending on antigen-antibody agglutination reactionthe blood group that showed agglutination were considered to be positive for a particular blood grouping reagent(26).

Enzyme-Linked Immunosorbent Assay (ELISA)

The rest from the blood sample was placed in Gel and Clot Activator Non Blood Collection Tube, serum was separated by centrifuge 3000 rpm for 5 minutes and stored in eppendorf tube at -20C^0 until use for ELISA test. According to the manufacturer's instructions the antibodies concentration of IgM and IgG in symptomatic and asymptomatic patients respectively was obtained and it was Compared with the concentration found innon-infected patients serum.(Epitope Diagnostics /Catalog No: KT-846 for IgG andKT-847 for IgM)

Statistical analysis

The statistical analyses for all obtained data in the current study were undertaken using SPSS program version 21. The prevalence rate and the degree of association between blood type and the diseases were tested by using of the Chi-square test. The correlation coefficient was estimated. One way ANOVA was used in analysis of IgM and IgG and the differences among means were compared by using LSD. Probability of occurrence by chance is significant if $P< 0.05$.

RESULTS

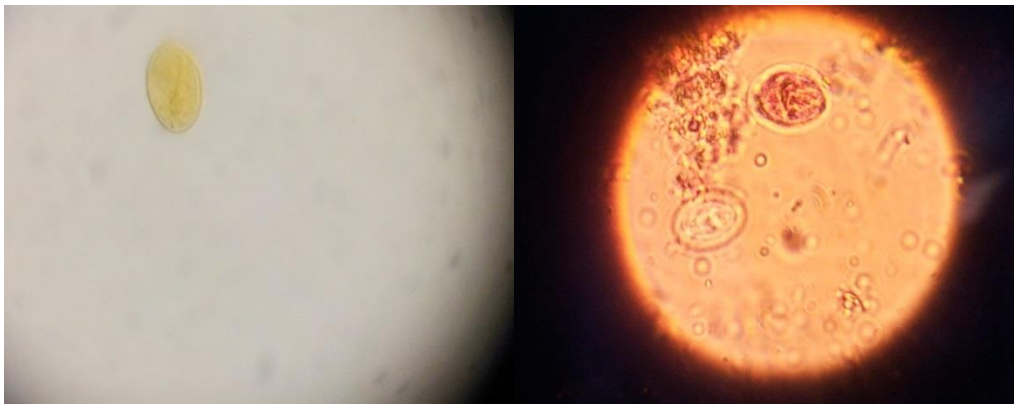
Data analysis revealed that the prevalence of G.lamblia among 532 stool samples examined under microscope was 32.89%, G.lamblia presence was observed in 100 stool samples of a total 318examined samples for males with higher infection rate 57.14% of all positive samples and 18.79% of all total samples. On

the other hand, of a total 214 stool samples for females, G.lamblia was detected in 75 sample with 42.85%, 14.09% as infection rate of all positive and total samples respectively. Significant differences was observed according to gender.(Table 1,Figure A,B)

Table 1: Prevalence of Giardia lamblia

Gender	No. of examined samples	No. of positive	Prevalence of positive %	Prevalence of total %
male	318	100	100/175(57.14)	100/532(18.79)
female	214	75	75/175(42.85)	75/532(14.09)
total	532	175	175/175(100)	175/532(32.89)

*Chi-square =0.75 P=0.38 Significant



A-Giardia lamblia cyst stained with Lugol's Iodine B-Giardia lamblia cyst stained with chromotrop2R

In the present study most samples that diagnosed with G.lambliawere among symptomatic patients122(69.7%) including 73(59.8%) for malesand 49(40.1%) for females while, asymptomatic patients showed 53(30.2%) with 27(50.9%) for male and 26(49.05%) for females. No significant differences were detectedbetween gender and the state.

The infection rate of human giardia was detected in both symptomatic and asymptomatic patients withevidence that theasymptomatic cases represent a major proportion of the total infections rate.(Table 2)

Table 2: The prevalence of Giardia lamblia in symptomatic and asymptomatic patients

State	Male	Rate %	Female	Rate %	Total	Total Rate %
symptomatic	73	73/122(59.8)	49	49/122(40.1)	122	122/175 (69.7)
asymptomatic	27	27/53(50.9)	26	26/53(49.05)	53	53/175 (30.2)
Total	100		75		175	

*Chi-square = 1.19 p=0.27 No significant association between gender and the state
Chi-square =27.20 P<0.0001 for the total symptomatic and the total of asymptomatic

In order to determine the acute and chronic cases for giardiasis in patients, the concentration of Ig M and Ig Gantibodies were obtained in serum using ELISA method. Table 3 showed the mean concentration of IgM and Ig Gantibodies for 50 symptomatic and asymptomatic patients in compered with the mean concentration for 30 non infected persons. Statistical analysis revealed significant differences($p<0.05$) between the mean concentration of IgM for 25 male 4.22 µ /ml , 25 female 4.41 µ /ml and the concentration detected in 15 of non infected male and female 1.80 µ /ml, 1.50 µ /mlrespectively. The same results was observed for IgG antibody measurement in which the higher mean concentration recorded in 25 male 21.46 µ /ml in compere with 15 non infected males 3.51 µ /ml. Also, the 25 infected females showed a mean concentration for IgG 21.45 µ /ml, while the mean concentration 3.68 µ /ml was recorded for 15 non infected females.

ISSN: 0975-8585

Table 3: The concentration of IgMand IgG antibodies in serum of acute and chronic infected human with Giardialamblia(mean ± standard error)

State	No.of samples	Sex	Mean of IgMconcentration µ /ml and standard error
Infected with symptoms	25 25	Male female	4.22± 0.38 A 4.41± 1.07 A
Non infected	15 15	Male female	1.80± 0.70 B 1.50± 0.53 B
State	No.of samples	Sex	Mean of IgGconcentration µ /ml and standard error
Infected without symptoms	25 25	Male female	21.46± 1.01 A 21.45± 3.9 A
Non infected	15 15	Male female	3.51± 0.30 B 3.68± 1.82 B

Different capital letters denoted significant differences ($p<0.05$) between infected and non-infected individuals

Fifty blood samples (25 male,25 female)were obtained from patients for each of symptomatic and asymptomatic for blood group test. The results indicate that for symptomatic patients higher rate 40% was associated with type A,followed by patients have type B (26%) and type O (24%) with less rate for type AB (10%) while, higher rate of type O was observed in asymptomatic patients (42%) followed by type A, B and AB 24%,22% and 12% respectively. No significant differences was obtained between symptomatic and asymptomatic patients according to ABO and Rh test, the results showed that 98% have Rh + with only 2% have Rh- in symptomatic in compared with asymptomatic that recorded 96% for Rh+ and 4% for Rh-.(Table 4)

Table4: Blood group distribution among Symptomatic and asymptomatic patients

Blood group	Symptomatic	%	Asymptomatic	%	Total %
A	20	20/50(40)	12	12/50(24)	32
B	13	13/50(26)	11	11/50(22)	24
AB	5	5/50(10)	6	6/50(12)	11
O	12	12/50(24)	21	21/50(42)	33
total	50		50		100
Rh test					
Rh +	49	49/50(98)	47	47/50(94)	96
Rh-	1	1/50(2)	3	3/50(6)	4
Total	50		50		100

*Chi-square 4.71 p=0.19 No significant association between blood group and type of infection
*Chi-square of Rh test =1.04 p=0.30 No significant association between Rh group and type of infection
Chi-square=9.00 P=0.02 for the differences among blood group in symptomatic
Chi-square=9.30 P=0.02 for the differences among blood group in asymptomatic
Chi-square=46.08 P<0.0001 for the differences between Rh group in symptomatic
Chi-square=38.72 P<0.0001 for the differences between Rh group in asymptomatic

This study is the first onein Baghdad to find if there is a relationship between the type of blood group of infected human and the state of infection (symptomatic, asymptomatic). The pearson correlation coefficient was estimated.

Results indicated that correlation coefficient (r)recorded a significant (P<0.01)betweentotal infectionrateand the distribution in each of blood group 0.772 and Rh test 0.798 respectively.The correlation between the type of blood group and infection was 0.696 for ABO. 0.822 for Rh and on the other hand, infection without symptoms showed 0.596, 0.542 for both ABO and Rh respectively.

ISSN: 0975-8585

In table 5, the distribution of blood group according to gender showed no significant differences between symptomatic and asymptomatic patients.Type A was the higher percentage 40% in the symptomatic for each of male and female as well as typesA recorded 24% for both males and females in asymptomatic patients. Type B percentage was 20% for both symptomatic and asymptomatic males in compared with females that showed 32% for symptomatic and 24% for asymptomatic. The percentage of type AB in males and females for symptomatic, asymptomatic patients were 12%,8%; 8%, 16% respectively. Asymptomatic patients showed higher percentage for type O in both males 48% and females 36% while the percentagein symptomatic malesand females was28%and 20% respectively.

Table 5: Bloodgroup distributionin symptomatic and asymptomatic patients according to gender

Blood group	Symptomatic				Asymptomatic			
	male	%	female	%	male	%	female	%
A	10	40	10	40	6	24	6	24
B	5	20	8	32	5	20	6	24
AB	3	12	2	8	2	8	4	16
O	7	28	5	20	12	48	9	36
Total	25	100	25	100	25	100	25	100

*Chi-square=1.22 P=0.74 to measure the association between gender and blood group in symptomatic
*Chi-square=1.18 P=0.75 to measure the association between gender and blood group in asymptomatic
Chi-square=4.28 P=0.23 male symptomatic
Chi-square=5.88 P=0.11 female symptomatic
Chi-square=8.44 P=0.03 male asymptomatic
Chi-square=2.04 P=0.56 female asymptomatic

Results in table 6 revealed that Rh positive was observed in most participants, males percentage was 96% (24/25) in both symptomatic and asymptomatic while, females showed 100% for symptomatic and 92% (23/25)for the asymptomatic. On the other hand the proportion of the Rh negative individuals affected with giardiasis was 4% (1/25) in symptomatic, asymptomatic malesand was 8% in asymptomatic females. Significant differenceP<0.05 in the frequency of Rh factorbetween gender and blood group was recorded for symptomatic population.

Table 6: Rh test according to gender

Blood group	Symptomatic				Asymptomatic			
	male	%	Female	%	male	%	female	%
Rh +	24	96	25	100	24	96	23	92
Rh-	1	4	–	0	1	4	2	8
Total	25	100	25	100	25	100	25	92

*Chi-square=1.02 P=0.30 to measure the association between gender and blood group in symptomatic
*Chi-square=0.35 P=0.55 to measure the association between gender and blood group in symptomatic
Chi-square=21.16 P<0.0001 male symptomatic
Chi-square=25.00 P<0.0001 female symptomatic
Chi-square=21.16 P<0.0001 male asymptomatic
Chi-square=17.64 P<0.0001 male asymptomatic

ISSN: 0975-8585

DISCUSSION

The higher total infection rate 32.89% for giardiasis recorded in this study was associated with many factors such as high resistance of the infective stage (cysts) to environment factors, the low infected dose (10cyst), modes of transmission (mainly inadequate clean water) and poor personal hygiene [27, 28].

Nearly closely result in the total prevalence rate was observedin Egypt, since the results of a study conductedby Forondaetal[29] revealed 34.6% as giardiasis infection rate. The CDC recorded that indeveloping countries the infection rate of giardiasis was 33%[30]. The difference in the results can be attributed to the number of samples examined, methods for detection, environmental differences and host immunity conditions [31].

According to patients gender, the results showed that sex of the patient had no significantly ($p<0.05$) influence on the total infectivity rate with the parasite and this has been confirmed by the results of some previous studies [32, 33]. A study done on the prevalence rate of G.lamblia by Amjed[34]showed males were recorded high prevalence 2.18% than females 1.51%.Another study documented the total prevalence rate of G.lamblia in 756 examined stool sample was 148(19.57%) and males recorded higher rate 20.31% than female 18.11% [35].

Analysis of the results showed that males infection rate 18.79% was higher than females 14.09% .The reason may be due to the difference in the chance of exposure to the parasite between males and femalesthrough transmission modes such as food and water supplies, and other effected factors such as community hygiene, sanitation, reported household pet contact especially cats, dogs and cattle. Recently, studies focus on sex-associated hormones which maymodulate immune responses and consequently affectedthe susceptibility to parasitic infection.[36,37].

On the other hand,disapproval was observed with the results of several studies in that females recorded higher prevalence rate than males[38, 39,40,41].

According to American Medical Association, approximately two hundredmillion person in Asia, Africa, and Latin America have symptomatic giardiasis [42].while about two-third of infected people have no symptoms (asymptomatic infections) which leads to difficulty in the eradication and control of disease [11,12].Although the proportion of infected persons without symptoms less than the proportion of persons with symptoms, asymptomatic cases of Giardia represent a major proportion of the total cases of infection and so giardiasis mostly occurs sporadically.In a study conducted in northern Ghana,G.lambliawere observed more than twice as frequently in asymptomatic individuals 12(9.7%) than in symptomatic individuals 9(3.7%) [43].Also the American Academy of Pediatrics revealed that asymptomatic infection is common, approximately 50% to 75% of infected people[44]. The differences in the severity of infection due to host defenses against Giardiawhich can be classified into two categories– non-immunological responses and immunological responses [45].

There has been increasing evidence that blood groups have a possible function and biological role in disease association including bacterial, viral or parasitic infections.

The red blood cells and some human cells and tissues showed the expression of ABO antigens(A, B and H) which are complex carbohydrate molecules.So, RBCs may be used as a tool in the detection of the causative microorganisms for diseases[46,47,48,49]. These carbohydrate consider to be asreceptors for ligands, viruses, bacteria and parasites, enzymes,adhesion molecules and structural proteins [50]. Thisblood antigens may bepresence or lack in some blood groups causing changes in blood cells membrane , functionally and morphologically [51].

Results of data analysis of this study showedno significant association between the ABO and giardiasis infection this may be related to the level of immunoglobulin in the serum of individual, type A was more susceptible than O,B,AB in symptomatic patients while, most of the asymptomatic patients showed O type. Only a few studies have investigated the information on the association between giardiasis and blood groups. In Egypt, a studies concluded that blood group A was more susceptible to giardiasise specially asymptomatic

form[52,53] .Al-Taieet al [54] recorded that there was no correlation between ABO and Rh blood group and giardiasis infection. Our findings were contrary to another study conducted by Ayeleetal[55].

CONCLUSION

Giardiasis infection in Baghdad still recordeda high rate in individual without any outbreak documented. Gender had a role in the incidence of infection as males were the most infected in comparison with females.

ABO may influence the risk of giardiasis by unknown mechanisms. It is now clear that ABO blood types and Rh are not the exact cause of diseases but they affect susceptibility and resistance to disease and health.Most symptomatic patients showed A blood type while, O blood type was the higher in asymptomatic patients.

RECOMMENDATIONS

More Studies on the association of G. lamblia genotypes with the spectrum of symptoms or asymptomatic giardiasis are required. Additional testing is needed to determine the extent of association of the blood groups genotypes with giardiasis infection. Persons with high risk blood types must be screened in order to modify their lifestyles and health behavior to prevent or control the infection.

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