

Toxoplasmosis in Relation to Entameba Histolytica and Giardia Lamblia Infection

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Abstract

This study was carried out in Baghdad , on 150 individuals (82 females, 68 males) , were divided into four groups on the bases of parasitological and serological findings : group I : Control healthy Individuals : negative for toxoplasmosis and E . histolytica and G. lamblia (20, 13.3%)group II : positive for toxoplasmosis alone (30 , 20%), group III: positive for E histolytica ,and G. lamblia (63 , 42%), group IV: positive for toxoplasmosis , E . histolytica and G . lamblia (Mixed infection 37 , 24.7%) .

The results of this study showed highly significant increase in serum levels of interleukin -12 (IL- 12) in all groups especially in group IV , followed by group II .The serum level of Interferon –gamma (IFN- γ) showed also significant increase(P <0.05)in group IV, followed by group III , but nonsignificant (P >0.05) in group II .

There was also significant increase in serum concentration of IgG , IgM, and IgE in group IV , but nonsignificant level was recorded in IgE of group II .

When comparing group II with the group IV , there was significant difference in serum levels of IL-12 , IFN- γ , IgG , IgM and IgE .

It was concluded that mixed infection with these parasites could provide a strong synergistic action to induce both cell mediated and humoral immunity .

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Introduction

Toxoplasmosis is a zoonotic disease caused by an obligate intracellular parasite, Toxoplasma gondii, which is transmitted by ingestion either of the tissue cyst stage found in the meat of infected animals or the oocyst stage, released in the feces of infected cats or transplacental transmission from an infected woman to her fetus[1]. Toxoplasma gondii infects a large proportion of the world population. Individuals at risk include fetuses, newborns and immunologically impaired individuals [2].

Toxoplasmosis is mostly diagnosed in the laboratory by serological tests which were greatly improved in the current decade

[2].High T gondii seroprevalence has been found in many countries including Iraq [3-5]. In endemic areas, Toxoplasmosis is mostly associated with other intestinal protozoal parasitic infection especially Entamoeba histolytica and Giardia lamblia [6]. Both parasites are quite common causes of diarrhea in tropical and subtropical countries. E. histolytica causes a disease called, amebic dysentery while G. lamblia causes a diseases called giardiasis. Amebic dysentery is characterized by abdominal pain (tenesmus), feces with blood and mucus, while giardiasis presents abdominal Cramps, flatulence, diarrhea with fatty but never contain blood. E. histolytica may cause also extra intestinal amebiasis through hematogenous routes

(liver and brain abscess) .Most infected people may show no symptoms (asymptomatic) but still carrying the parasite stages in their intestine .[7].Transmission of both parasites are similar, namely through ingestion of infective cyst with the contaminated food or water⁽⁶⁾ .

E. histolytica and G. lamblia infections may occur separately but sometimes occurring together as a mixed infection in the same patient. The infections are worldwide in distribution[6].

It was found that E. histolytica and/or G. lamblia can induce the immunological reactions that producing a wide range of cytokines such as interferon- gamma (IFN- γ), tissue necrotic factor- alpha (TNF- α) and interleukin- 12 (IL-12) as well as producing different types of serum immunoglobulins.[8-9]

In last few years, some studies were carried out, showing the effects of E. histolytica and/or G. lamblia infections on some gastrointestinal bacterial infections [8, 10].

The aim of this study is to explore the relationship between toxoplasmosis and E. histolytica and/or G. lamblia infections through determination of IL-12 , IFN- γ and serum immunoglobulins.

Materials and Methods

Study subjects :

This includes 150 individuals (82 females and 68 males) ,their ages ranged from 16-62 years in males, and from 15-55 years in females, were attending to medical city(Baghdad) for medical care and for check up , from the beginning of March to the end of July (2010). All the participants were asked special questionnaire regarding : age ,sex ,occupation , residence , socioeconomic state , any signs of disease , treatment or drug received .

Blood collection :

Blood (5 – 10 ml) was taken from each individual and serum was separated and stored at -20 C° for laboratory investigation .

Stool collection :

Stool specimens were collected in clean dry containers.Each specimens was divided into three portions , the first for direct normal saline preparation , a second one was for Lugol's iodine ,the third for flotation method.

Methods

Parasitological Stool Examination :

a- Macroscopical Stool Examination :

Each fresh stool specimen was examined visually to look for consistency , color , presence or absence of mucus , blood , worms or any other abnormalities .

b- Microscopical Stool Examination : This includes :

1-Direct Normal Saline Preparation :

A small portion of stool was mixed with one drop of normal saline on a glass slide then covered with glass cover slip . Each slide was examined by microscope under low power (X 10) and then under high power (X 40) to detect presence of helminth ova and cyst, trophozoites of protozoa[6].

2-Lugol's iodine preparation method :

This was done by mixing a small portion of stool with one drop of Lugol's iodine on a glass slide , then covered with cover slip ,examined by microscope using low power (X10) and high power (X40)[6].

3-Zinc Sulphate Centrifugal Flotation :

This procedure allowed the detection of the parasite (cyst , ova) that might be missed by using only the direct examination [6].

Immunological Examinations :

a-Determination of Anti- Toxoplasma Abs :

By using Latex agglutination test (LAT), Biokit (Spain), result evaluated visually by clear agglutination. If serum contains more than 10 IU/ml of Toxoplasma Abs, it is considered positive . [10]

b-Determination of Serum Level Of IFN-Gamma :

By using IFN- γ immunoassay kit (Immunotech. , France) using ELISA technique .

c-Determination of Serum Level Of IL-12 :

This was done by using IL-12 enzyme immunoassay kit (Biosource ,Belgium) using

ELISA technique .

d-Determination of serum level of Immunoglobulins (IgG ,IgM) :

(Biomaghreb , Tunisia) :Quantitation of serum immunoglobulins of the study groups was carried out by Single radial immunodiffusion (SRID) test (Mansini method)

e-Determination of serum level of IgE :

(Biomaghreb , Tunisia) by total IgE microplates ELISA kit is a two site enzyme-linked immunosorbent assay for the quantitative determination of IgE .

Control group:

Those persons who gave negative result of Latex test(free of toxoplasmosis)as well as negative results of parasitological stool examination (free of protozoan and helminth parasites) with no any clinical symptoms ,were considered as healthy control group . This includes 20 individuals (12 females and 8 males), their ages range from18-52 years .

Statistical analysis :

The statistical analysis was performed by using SPSS (Statistical package for social Science) program –version-18 according to (Sorlie 1995) . Student s' t test & Chi-square (χ^2 test) were used in evaluation of the differences. P value of any test was considered significant when $P < 0.05$.

Results

The study revealed that 36.7% was infected with E histolytica and 14% with G lamblia and 16% with both parasites (mixed infection) .(Table 1) .

The majority of infection occurred in males (52.7%) at the age 30-39 years, and in females (57.1%) at the age < 20 years, in E. histolytica and G lamblia respectively . There was no relationship between infection with E. histolytica , G lamblia and the sex or ages of the individuals ($P > 0.05$).Table (2) shows the seroprevalence of toxoplasmosis. Out of the total 150 individuals , there was 44.6% seropositive (67/ 150) persons of both sexes . The highest seropositive rate occurred in females (72.2%)at the age 20-29 years then declined at the age category ≥ 40 years (40%) . There was an association between gender , age , and the seropositive results for toxoplasmosis in this study($P < 0.05$) The study groups were divided according to their serological and parasitological results into four groups :GroupI:Control healthy individuals :negative for toxoplasmosis and negative for E .histolytica and G. lamblia (20, 13.3%),GroupII:positive for toxoplasmosis alone (30, 20%). GroupIII: positive forE .histolytica and/or G.lamblia(63,42%),GroupIV positive for toxoplasmosis and E .histolytica and G. lamblia (Mixed infection) .The E.histolytica infected subgroup or G. lamblia infected subgroup when compared each one with the control,there was no significant difference, so both subgroups was considered as one group (group III) . Table (3) reveals more infected females than males in all groups especially in groupIV (Mixed infection) (72.9%).The highest infection rate appeared at the age 20-29 years (54.1%) in group IV, followed by the age <20 years (40%) in groupII Age & gender showed direct association with the infection rate in study groups ($P < 0.05$) Table (4) reveals a significant increase in serum level of IL-12 in all groups when compared with the control ($P < 0.05$) . However the serum level of IFN- γ also increased significantly in groups III and IV, in comparison with the control group ($P <$

0.05)but showed nonsignificant difference ($P > 0.05$) in group II .

Table (5) shows rapid and high elevation in serum level of IgG in all study groups especially in group IV (2870 mg / dl) followed by group II (1850 mg/dl) and group III, (1452.3 mg/dl). Those elevations were significant when compared with the control ($P < 0.05$) .The IgE also showed a significant increase ($P < 0.05$)in their serum level which was:159.75 IU/ml, 138 IU/ml , in group IV and in group III respectively , but showed

nonsignificant increase ($P > 0.05$) in group II. Similarly, IgM showed increase in its'serum

level but to less extent than IgG and IgE : 150 mg/dl in group II ($P < 0.05$), 200.7mg/dl in group IV($P < 0.05$),,and 200.5mg/dl in group III ($P < 0.05$) .

The most remarkable finding in this study was that the group IV(mixed infection group) contained the highest and most significant serum levels of all immunological parameters (the cytokines and the immunoglobulins) .

Table (1): Detection rate of Entameba histolytica and Gairdia lamblia in relation to sex and age.

| Gender | <u>E . histolytica</u> | | | | | <u>G lamblia</u> | | | | | Both parasites (mixd infection) | | | | |
|--------------|------------------------|------|----------|------|-------|------------------|------|----------|------|-------|------------------------------------|------|----------|------|-------|
| | Positive | | Negative | | Total | Positive | | Negative | | Total | Positive | | Negative | | Total |
| | No. | % | No. | % | No. | No. | % | No. | % | No. | No. | % | No. | % | No. |
| Female | 26 | 31.7 | 56 | 68.3 | 82 | 12 | 14.6 | 70 | 85.4 | 82 | 13 | 15.9 | 69 | 84.1 | 82 |
| Male | 29 | 42.6 | 39 | 57.4 | 68 | 9 | 13.2 | 59 | 86.8 | 68 | 11 | 16.2 | 57 | 83.8 | 68 |
| Total | 55 | 36.7 | 95 | 63.3 | 150 | 21 | 14 | 129 | 86 | 150 | 24 | 16 | 126 | 84 | 150 |
| Age in years | | | | | | | | | | | | | | | |
| <20 | 10 | 38.5 | 16 | 61.5 | 26 | 11 | 23.4 | 36 | 76.6 | 47 | 7 | 21.2 | 26 | 78.8 | 33 |
| 20-29 | 12 | 27.9 | 31 | 72.1 | 43 | 4 | 11.8 | 30 | 88.2 | 34 | 6 | 14.3 | 36 | 85.7 | 42 |
| 30-39 | 24 | 53.3 | 21 | 46.7 | 45 | 4 | 10.5 | 34 | 89.5 | 38 | 4 | 10 | 36 | 90 | 40 |
| ≥40 | 9 | 25 | 27 | 75 | 36 | 2 | 6.5 | 29 | 93.5 | 31 | 7 | 20 | 28 | 80 | 35 |
| Total | 55 | 36.7 | 95 | 63.3 | 150 | 21 | 14 | 129 | 86 | 150 | 24 | 16 | 126 | 84 | 150 |

Table (2) : Seroprevalence of anti Toxoplasma.- gondii antibodies in relation to sex and age.

| Age (year) | Female | | | | | | Male | | | | | |
|------------|--------------------------------|------|----------|------|-------|------|--------------------------------|------|----------|------|-------|------|
| | Latex agglutination test (LAT) | | | | | | Latex agglutination test (LAT) | | | | | |
| | Positive | | Negative | | Total | | Positive | | Negative | | Total | |
| | No. | % | No. | % | No. | % | No. | % | No. | % | No. | % |
| < 20 | 10 | 66.7 | 5 | 33.3 | 15 | 18.3 | 7 | 58.3 | 5 | 41.7 | 12 | 17.6 |
| 20- 29 | 13 | 72.2 | 5 | 27.8 | 18 | 21.9 | 4 | 19.1 | 17 | 80.9 | 21 | 30.9 |
| 30- 39 | 12 | 50 | 12 | 50 | 24 | 29.3 | 5 | 20.8 | 19 | 70.2 | 24 | 35.3 |
| ≥ 40 | 10 | 40 | 15 | 60 | 25 | 30.5 | 6 | 54.5 | 5 | 45.5 | 11 | 16.2 |
| Total | 45 | 54.9 | 37 | 45.1 | 82 | 100 | 22 | 32.4 | 46 | 67.6 | 68 | 100 |

Table (3): Age and Sex distribution in study group.

| Gender* | Control (negative for toxop. & E. hist. & G. lamb.) (group I) | | Positive for Toxoplasmosis alone (group II) | | Positive for E.hist. & G.lamb. (group III) | | Positive for Toxop. & E. hist. & G. lamb.(group IV) | |
|---------------------|--|----------|--|----------|---|----------|--|----------|
| | No. | % | No. | % | No. | % | No. | % |
| Female | 12 | 60 | 18 | 60 | 25 | 39.7 | 27 | 72.9* |
| Male | 8 | 40 | 12 | 40 | 38 | 60.3 | 10 | 27.1 |
| Total | 20 | 100 | 30 | 100 | 63 | 100 | 37 | 100 |
| Age in year* | | | | | | | | |
| <20 | 2 | 10 | 12 | 40 | 13 | 20.6 | 4 | 10.8 |
| 20-29 | 8 | 40 | 8 | 26.7 | 20 | 31.7 | 20 | 54.1* |
| 30- 39 | 6 | 30 | 8 | 26.7 | 12 | 19 | 6 | 16.2 |
| ≥40 | 4 | 20 | 2 | 6.6 | 18 | 28.7 | 7 | 18.9 |
| Total | 20 | 100 | 30 | 100 | 63 | 100 | 37 | 100 |

* significant (p < 0.05)

Table (4): serum concentration of cytokines (IFN- γ and IL- 12) in study groups.

| Study groups | No. | IL-12 Pg/ ml (mean ±SD) | IFN- γ- lu/ ml (mean ± SD) |
|---|------------|--------------------------------|-----------------------------------|
| Control (-ve for Toxoplasmosis, E. hist& G. lamb.) (group I) | 20 (13.3%) | 96.45±30.53 | 1.69± 0.82 |
| Toxoplasmosis alone (group II) | 30 (20%) | 157.95* ±22.33 | 3.85** ± 2.28 |
| E. hist.&G.lamb.(groupIII) | 63 (42%) | 139.42* ± 34.94 | 5.69* ± 2.91 |
| Toxop.&E.hist.&G.lamb (group I V) | 37 (24.7%) | 181.3* ± 31.0 | 8.9* ± 4.77 |

* = significant (p < 0.05)

** = non significant (p > 0.05)

Table (5): Serum concentration of Immunoglobulins (IgG, IgM, IgE) in study groups.

| Study groups | No. | IgG (mg/dl) Mean ± SD | IgM (mg/dl) Mean ± SD | IgE (IU/ml) Mean ± SD |
|---|------------|------------------------------|------------------------------|------------------------------|
| Control (-ve for Toxop. & E. hist. & G. lamb.) (group I) | 20 (13.3%) | 750±152.5 | 67.3± 21.43 | 26.8 ±16.33 |
| Toxoplasmosis alone (group II) | 30 (20%) | 1850* ± 506.37 | 150* ± 45.49 | 33.67** ± 16.13 |
| .E.hist. & G. lamb. (group III) | 63 (42%) | 1452.3* ±518.21 | 200.5* ±58.02 | 138* ± 13.33 |
| .Toxop.&E.hist. & G. lamb.(group IV) | 37 (24.7%) | 2870* ±858.83 | 200.7* ± 72.14 | 159.75* ± 23.86 |

* = significant (p < 0.05)

** = non significant (p > 0.05)

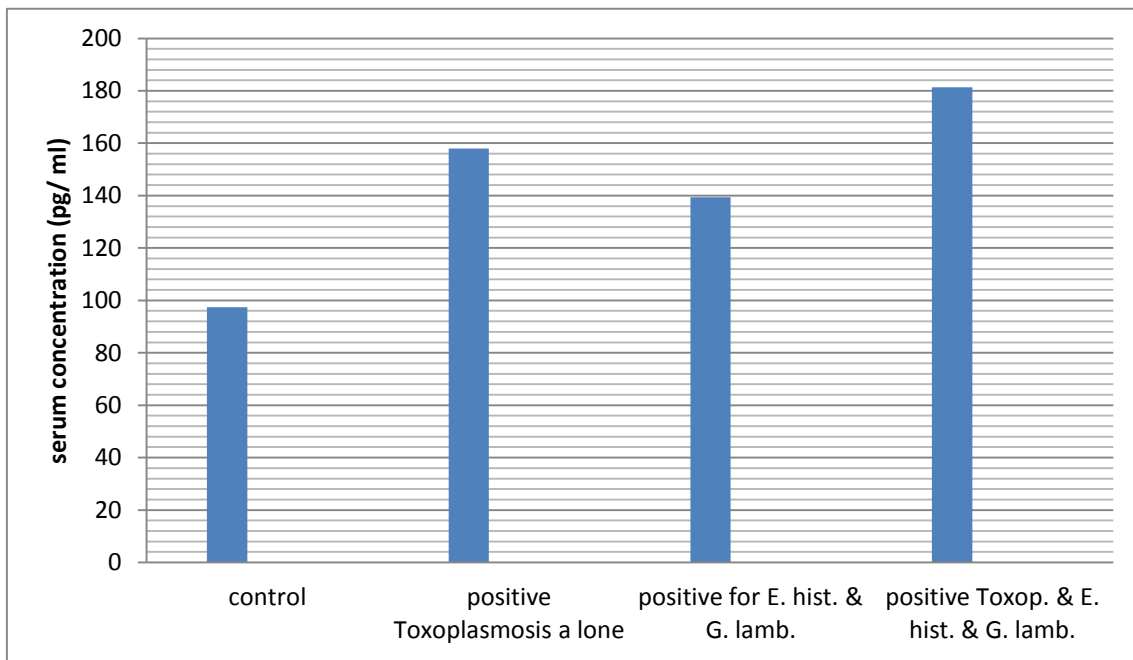


Fig. (1): Serum Concentration of IL- 12 in Study groups.

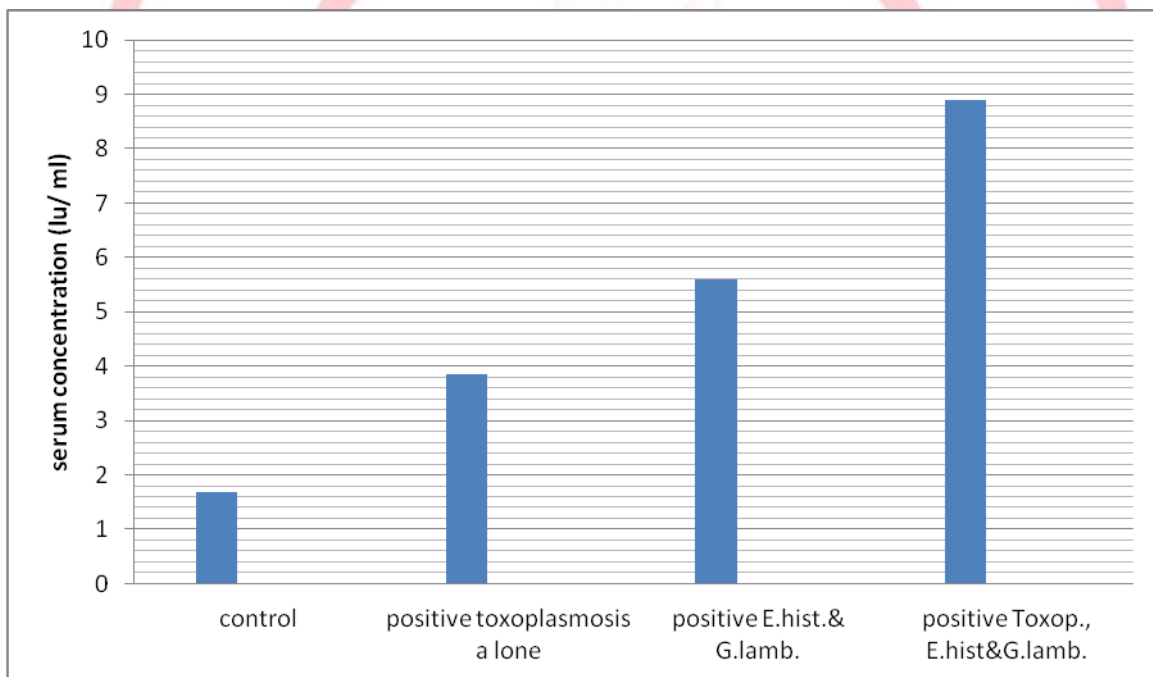


Fig.(2): Serum Concentration of IFN- γ in Study groups.

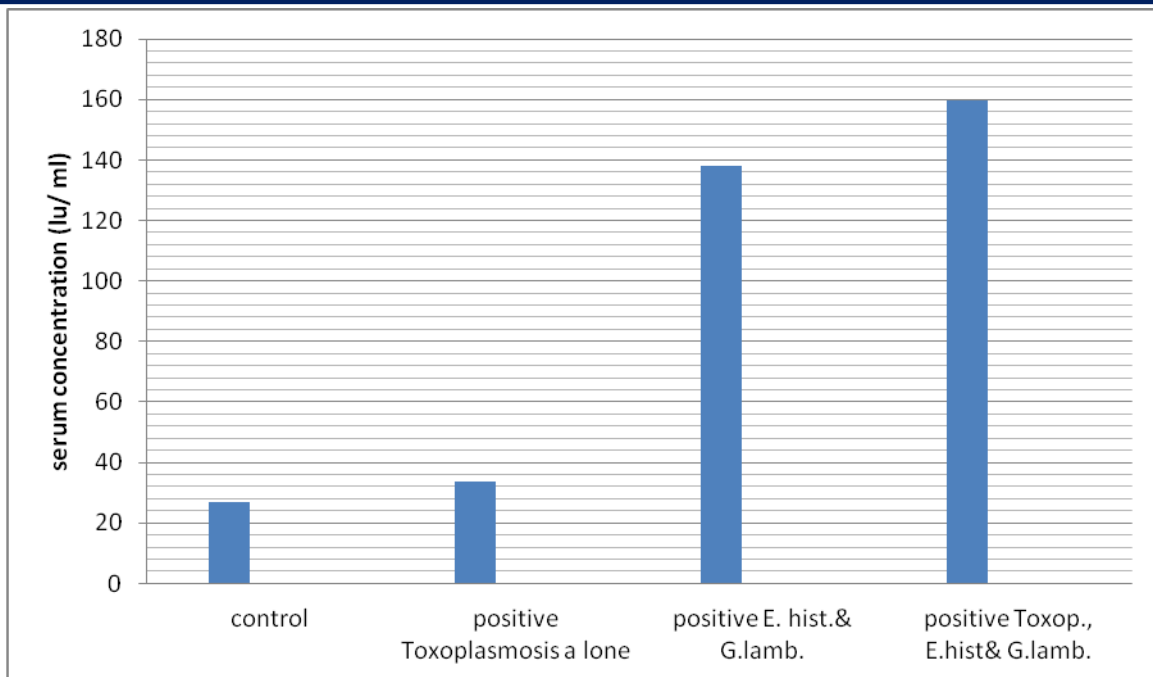


Fig. (3): Distribution of Serum Concentration of IgE in Study groups.

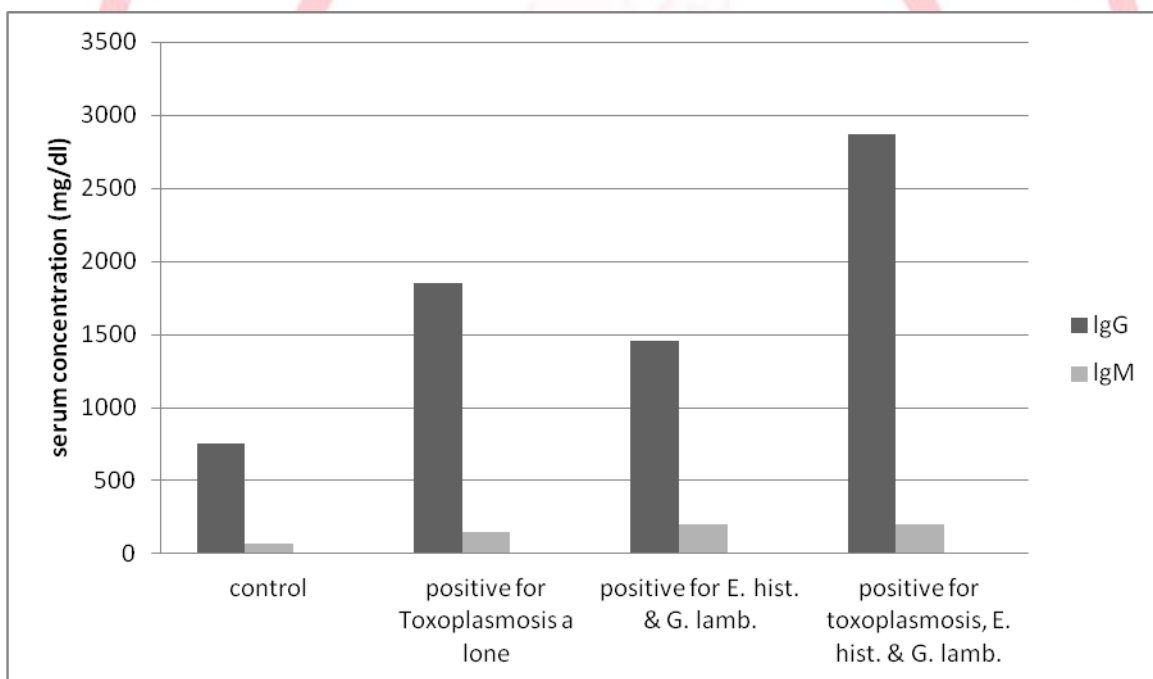


Fig. (4): Serum Concentration of IgG & IgM in Study groups.

Discussion

Parasitological examination of the stool :

This study revealed a prevalence of 36.7% infection with *E. histolytica*, 14% infection with *G. lamblia*, and 16% with both parasites (mixed infection), these are in

general agreement with some investigators [9, 12] but much higher than others [13- 14].

These variations might be due to several reasons such as: the different locations and times of these studies, numbers of the study samples, differences in socioeconomic states of the individuals, as well as some

nonhygienic habits, together with lack of sanitary precautions of the community . The presence of high rate of asymptomatic individuals with E histolytica (70%) in this study might be attributed to the presence of various strains of the parasite, personal immunity , type of nutrition ,or improper treatment. The health authorities in this country must give a great attention to such cases because those persons may act as carriers for stages of this parasite and participate in its transmission through direct contact with the people especially through food handling .

Immunological examinations :

Seroprevalence of Toxoplasmosis :

The higher seropositive result of toxoplasmosis (44.6%), with more females affected (72.2%) in this study, than other researchers(3-4) might be due to more current exposure of females to increased sources of infection which include, the stray cats and raw meat containing bradyzoites .

Serum concentration of cytokines (IL- 12 & IFN - γ) :

Induction of IL-12 by macrophages is a major mechanism in driving early IFN- γ to parasites[15] .The high serum levels of IL-12 and IFN- γ recorded in all groups especially in group IV (mixed infection) which was statistically significant (P< 0.05) may be due to the fact that the infection with mixed parasites induce strong cell mediated immunity (CMI) characterized by a high Th1- cell response[16].Even when group IV compared with the group II(toxoplasmosis alone) would reveal a significant difference (P< 0.05) . This clearly indicated the synergistic action of these mixed parasites in inducing both cytokines .There was a slight nonsignificant increase in IFN- γ in group II, which is similar to results obtained in mice experimentally infected with *Toxoplasma gondii*[17] .It was suggested that elevated cytokines were necessary for

control of G lamblia and E. histolytica infection in mice[16] , However,it was referred that in presence of E.histol. and G.lamb and Helicobacter pylori in the same individual , the cytokines of Th2 – cells decreased regulation of action of Th1 – cells and this leads to reducing H. pylori infection[10] .

Serum Immunoglobulins (IgG , IgM , IgE) :

Many pathogens are acquired by contaminated food, water and invasion of the contribute to initiation of immunity by activation of T& B cell to control this infection[18] .

Activation and estimation of specific serum immunoglobulin has been established as a tool for diagnostic and prognostic purpose⁽¹⁾ .The high serum levels of IgG,IgM and IgE reported in this study are similar to that obtained by some workers(8-9) . It was commonly known that serum IgM increased at the beginning of the infection then decreased after a time , while increase in serum IgG will Persist for months or years . Also IgE increased in allergy , parasitic infection in association with eosinophilia and subsequent release of histamine & serotonin [1]. It was referred that E histolytica can cause more increase in serum level of IgG than G .lamblia and this might be due to the fact that E.histolytica plays a role in killing the intestinal epithelial cells and tissue invasion ,as well as migrates outside of intestine to produce extraintestinal disease (mostly amebic liver abscess) ,All these activities of E histolytica elicit strong local and systemic immune response, while G lamblia, comes in direct contact with enterocytes but not tissue invasion [9] . A surprising finding in this data is that the group IV (mixed infection) revealed the highest serum level of IL-12 ,IFN- γ ,IgG , IgM ,and IgE , this might be explained by a polarized Th2 immune response in this group more than the others .

In **conclusion** of this study is that, the mixed infection with Toxoplasma gondii , Entameba histolytica , and/or Giardia lamblia in the same individual can provide strong synergistic action to produce both cell mediated and humoral immunity .

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