Iraqi Journal of Science, 2021, Vol. 62, No. 3, pp: 801-810
DOI: 10.24996/ijs.2021.62.3.11

ISSN: 0067-2904

# Changes in Serum Levels of Lipid Profile Parameters and Proteins in *Toxoplasma gondii* Seropositive Patients

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Received: 11/7/2020 Accepted: 31/8/2020

**Abstract**

The influence of *Toxoplasma gondii* on some biochemical parameters has lately gained an increasing attention. The aim of this study was to assess the levels of some biochemical parameters in *Toxoplasma* positive and negative subjects. An analytical case–control study was achieved in Baghdad for the period from October 2018 until March 2019. Forty nine females participated in this study, with an age range of 18-55 years. The participants were separated into two groups, namely *Toxoplasma* positive subjects (n=21) and *Toxoplasma* negative subjects (n=28), based on enzyme-linked immunosorbent assay (ELISA). Blood and serum samples were collected from all subjects to evaluate the serum levels of cholesterol, triglycerides, high density lipoprotein (HDL), very low density lipoprotein (VLDL), low density lipoprotein (LDL), total protein, total globulin and total albumin. The results showed non-significant differences between *Toxoplasma* positive and negative subjects for all the parameters, although cholesterol levels were lower (mean 149 mg/dL; range 131.9-165.9 mg/dL) in *Toxoplasma* positive patients as compared to those in *Toxoplasma* negative subjects (161 mg/dL; 146.7-175 mg/dL). In addition, triglycerides levels were lower (160 mg/dL; 123.3-196.8 mg/dL) in *Toxoplasma* positive subjects as compared to the control subjects (165mg/dL; 134.2-195.3 mg/dL). The only significant difference was noticed among subjects with an age range of 26-35 years, where globulin level was significantly higher (p=0.023) in *Toxoplasma* negative subjects as compared to that in *Toxoplasma* positive subjects.

**Keywords**: biochemical parameters, *Toxoplasma gondii*, women

# تغييرات مستوى الدهون و البروتينات المصلية في مرضى الحالة الموجبة بداء المقوسات الكوندية

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**الخلاصة**

زاد الاهتمام مؤخرا بالتأثير الذي يقوم به طفيلي داء المقوسات الكوندية على بعض المؤشرات البايوكيميائية لدى الاشخاص الذين يظهرون فحصا موجبا للطفيلي. هدف هذا البحث الى تقييم بعض المؤشرات البايوكيميائية لدى الاشخاص الموجبين و السالبين للفحص المصلي لداء المقوسات الكوندية. أُجريت هذه الدراسة ضمن الفترة من تشرين الاول لغاية اذار من العام 2019. شملت هذه الدراسة تسعة و اربعون من الاناث اللاتي تتراوح اعمارهن بين (18–55) سنه. تم تقسيم المشاركات في هذه الدراسة الى مجموعتين اعتمادا على الفحص المصلي بتقنية الامتزاز المناعي المرتبط بالأنزيم للتحري عن الاضداد المناعية المضادة

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لداء المقوسات الكوندية. شملت المجموعة الاولى الموجبات للفحص المصلي (العدد=21) بينما شملت المجموعة الثانية السالبات للفحص المصلي (العدد=28). جُمعت عينات الدم و المصل من كل الاناث اللاتي تم شمولهن بالدراسة و ذلك لتقييم مستويات كل من الكولسترول, الدهون الثلاثية, البروتين الشحمي عالي الكثافة (VLDL), البروتين الشحمي قليل الكثافة (LDL), البروتين الكلي, الغلوبيولين و الالبومين الكلي. أظهرت النتائج عدم وجود اي فروق معنوية بين الموجبات و السالبات للفحص المصلي لكل من المؤشرات البايوكيميائية التي تم اعتمادها في الدراسة, على الرغم من ان مستوى الكولسترول كان واطئا 149 (131.9−165.9) ملغم/ ديسيلتر لدى الموجبات للفحص المصلي مقارنة بمستويات اعلى لدى السالبات للفحص المصلي 161(146.7−175) ملغم /ديسيلتر. كما ان الدهون الثلاثية اظهرت مستويات واطئة لدى الموجبات للفحص المصلي 160 (123.3−196.8) ملغم/ديسيلتر مقارنة بمستويات اعلى لدى السالبات للفحص المصلي165 (134.2−195.3) ملغم/ديسيلتر. الفرق المعنوي الوحيد الذي تم ملاحظته في هذه الدراسة كان في مستوى الكلوبيولين للفئة العمرية بين (26−35) سنه حيث كان مرتفعاً و بشكل معنوي (P=0.023) لدى السالبات للفحص المصلي مقارنة بالموجبات.

## Introduction

Toxoplasmosis is an infection caused by a member of the Apicomplexa eukaryotes, namely *Toxoplasma gondii*. Toxoplasmosis is a cosmopolitan infection of humans and other warm-blooded animals. This protozoan parasite is of remarkable public health and economic concern [1]. Infections initiated by *T. gondii* continue to cause major public health problems as they can cause various clinical outcomes, such as abortion, retino-choroiditis, hydrocephalus, mental retardation, and even fatal death and life-threatening encephalitis in people with AIDS, organ transplantation, and immunosuppressive therapy [2, 3]. Globally, it has been estimated that *T. gondii* infection affects 30% to 65% of the entire global population [4]. The sero-prevalence of *T. gondii* infection in humans increases with age, but it does not differ significantly between the sexes and is lower in cold regions [5].

Recently, focusing on Toxplasmosis has increased dramatically in Iraq through several studies [6, 7]. The involvement of serum biochemical parameters, e.g. the levels of cholesterol, triglycerides, high density lipoprotein (HDL), very low density lipoprotein (VLDL), low density lipoprotein (LDL), total protein, total globulin, and total albumin, in humans infected with some parasites has attracted the attention of many investigators [8, 9]. *In-vitro* studies revealed that such parasites can grow in lipid-rich media without serum [10]. The aim of this study is to assess the levels of some biochemical parameters in *Toxoplasma* positive and negative subjects.

## Materials and methods

### Subjects and Study design

This research was an analytical case control study conducted from October 2018 until March 2019 .The population in this study was adult women who attended several hospitals, Primary Health Care centres, and private clinics. Sample collection was carried out by consecutive sampling with 49 participants who matched inclusion criteria and had no exclusion criteria. The inclusion criteria included adult women with age of less than 60 years, whereas exclusion criterion was pregnancy.

### Blood Samples

Five ml of vein-blood was collected from each participant in a sterilized plain tube and left for 25 minutes at room temperature for clotting. The sample was then centrifuged at 2000xg for 12 minutes for serum collection, which was aspirated by using a micropipette, dispensed into a sterile tube, and stored at -20$^{\circ}$C until used for ELISA test.

### Detection of the anti-*T. gondii* IgG and anti-*T. gondii* IgM

Serum samples were analysed for anti-*T. gondii* IgG antibodies with the enzyme immunoassay kit "*Toxoplasma* IgG" (Humman). Result equivalent or higher than 8 IU/ml was considered positive. Also, positive serum samples for anti-*T. gondii* IgG antibodies were screened for anti-*T. gondii* IgM antibodies by the available enzyme immunoassay "*Toxoplasm*a IgM" kit (Humman). Both tests were applied after the instructions available with the kits.

**Biochemical tests**

Triglycerides (TGS), cholesterol, and HDL levels were measured using a traditional enzymatic assay (Linear chemicals, Montgat-Barcelona, Spain). While, levels of LDL and VLDL were calculated using the following equations [11]:

LDL (mg/dL) = Total Cholesterol-HDL – Triglycerides (mg/dL) /5

VLDL (mg/dL) = Triglycerides (mg/dL) /5

Serum levels of total protein and albumin were measured by using the enzyme colorimetric method (Bio kit, Spain). Finally, serum globulin was measured by using protein electrophoresis [12].

**Statistical Analysis**

The results were stated as percentage and mean ±standard deviation (SD). Data analysis was achieved by SPSS 16.0 (SPSS Inc., Chicago, IL, USA).The data were assessed by chi-square test and the Student's t-test (t). P values < 0.05 were considered statistically significant. Kappa value was used to assess the agreement between the IgG and IgM results.

**Results**

This study was designed as a case control study. The patients were divided into two groups according to their anti-toxoplasma IgG and/or IgM results. A substantial agreement between the levels of IgG and IgM was found and the Kappa value was 0.80.

The first group was the *Toxoplasma gondii* positive group (n=20). The second group was *T. gondii* negative group (n=29). The results of cholesterol levels are illustrated in Figure-1. The results demonstrated a non-significant difference of cholesterol level in the serum samples of *T. gondii* positive and *T. gondii* negative groups, although cholesterol levels were lower (149; 131.9-165.9 mg/dL) in *T. gondii* positive group as compared to *T. gondii* negative group (161; 146.7-175 mg/dL) .

The results of triglyceride levels are shown in Figure-2. The results also demonstrated a non-significant difference of triglycerides in the serum samples of *T. gondii* positive and *T. gondii* negative groups, although the levels were lower (160; 123.3-196.8 mg/dL) in *T. gondii* positive group compared with *T. gondii* negative group (165; 134.2-195.3 mg/dL) .

The results also showed a non-significant difference of cholesterol in the serum samples of *T. gondii* positive and *T. gondii* negative women for the different age groups (Table-1).

The results also showed non-significant differences of triglycerides in the serum samples of positive *T. gondii* and negative women for the different age groups.

The results revealed non-significant differences of HDL in the sera of T. *gondii* positive and *T. gondii* negative groups, although the level was slightly higher in *T. gondii* positive women (50.48 mg/dL) compared with *T. gondii* negative women (48.86 mg/dL) (Figure-3). These results were similar to those of serum levels of VLDL, which also presented no significant differences between *T. gondii* positive and *T. gondii* negative women, who showed very similar levels (32.76 and 32.31 mg/dL, respectively) (Figure-4). On the other hand, LDL was significantly decreased in the *T. gondii* positive women (63.6 mg/dL) as compared to *T. gondii* negative women (80.99 mg/dL) (Figure-5).

Globulin level was slightly lower in *T. gondii* positive women (3.01g/dL) compared with *T. gondii* negative women (3.47 g/ dL) (Figure-6). No significant difference was noticed between the two groups. A significant difference in globulin level was noticed between these two groups, but only at an age range of 26-35 years. Globulin level was significantly higher (p=0.023) in *Toxoplasma* negative subjects (3.58 g/dL) as compared to *Toxoplasma* positive subjects (2.88 g/dL) (Table- 6).

Albumin level was significantly lower (p= 0.007) in *Toxoplasma* positive subjects (4.17 mg/dL) versus a high level in *Toxoplasma* negative subjects (4.75 mg/dL). In addition, significant differences in albumin levels were noticed among these two groups at age ranges of 18-25 and 26-35 years (Table-7). On the other hand, no significant differences were noticed in total protein levels between *Toxoplasma* positive subjects (7.5 mg/dL) and *Toxoplasma* negative subjects (7.71 mg/d) (Figure-8).

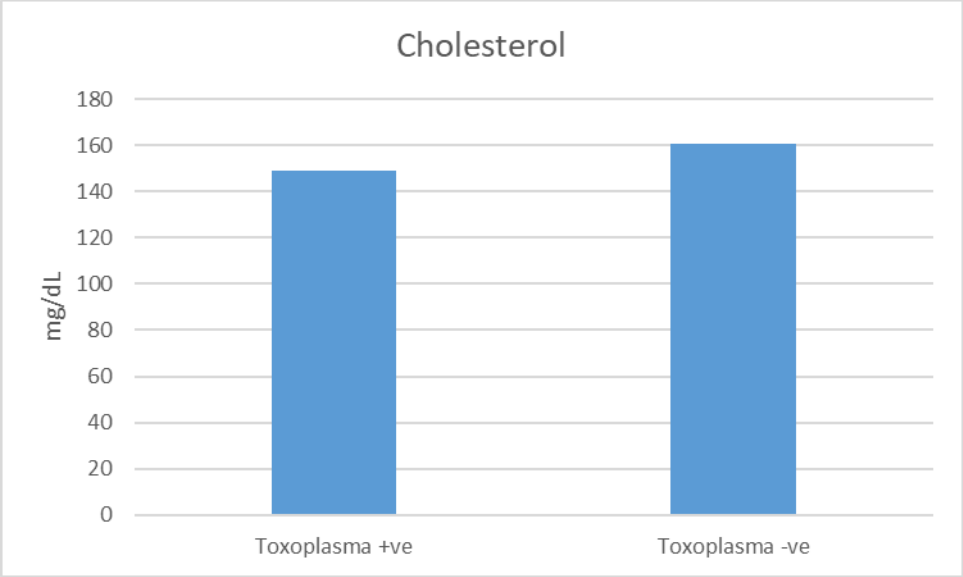


**Figure 1-**Cholesterol levels in *Toxoplasma* positive and *Toxoplasma* negative subjects.

**Table 1-** Cholesterol levels in *Toxoplasma* +ve and *Toxoplasma* –ve subjects in relation to age groups.

| Age/years | otal | *Toxoplasma* + *ve* | Mean of Cholesterol level(mg/dL) | *Toxoplasma* – *ve* | Mean of Cholesterol level (mg/dL) | p -value |
|---|---|---|---|---|---|---|
| 18-25 | 5 | 7 | 144 | 8 | 183 | 0.22 |
| 26-35 | 3 | 12 | 147 | 11 | 159 | 0.19 |
| 36+ | 1 | 2 | 161 | 9 | 130 | 0.91 |

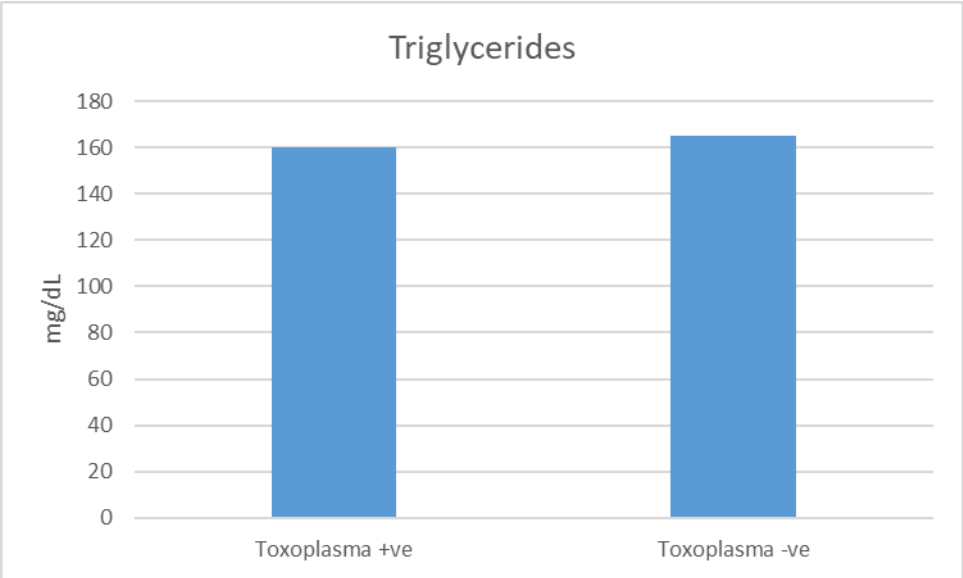


**Figure 2-**Triglyceride levels in *Toxoplasma* positive and *Toxoplasma* negative subjects.

**Table 2-** Triglycerides levels in *Toxoplasma* +ve and *Toxoplasma* –ve subjects in relation to age groups.

| Age/years | otal | *Toxoplasma + ve* | Mean triglycerides level(mg/dL) | *Toxoplasma – ve* | Mean triglycerides level (mg/dL) | p-value |
|---|---|---|---|---|---|---|
| 18-25 | 5 | 7 | 182 | 8 | 182 | 0.89 |
| 26-35 | 3 | 12 | 158 | 11 | 159 | 0.99 |
| 36+ | 1 | 2 | 105.5 | 9 | 160.8 | 0.82 |

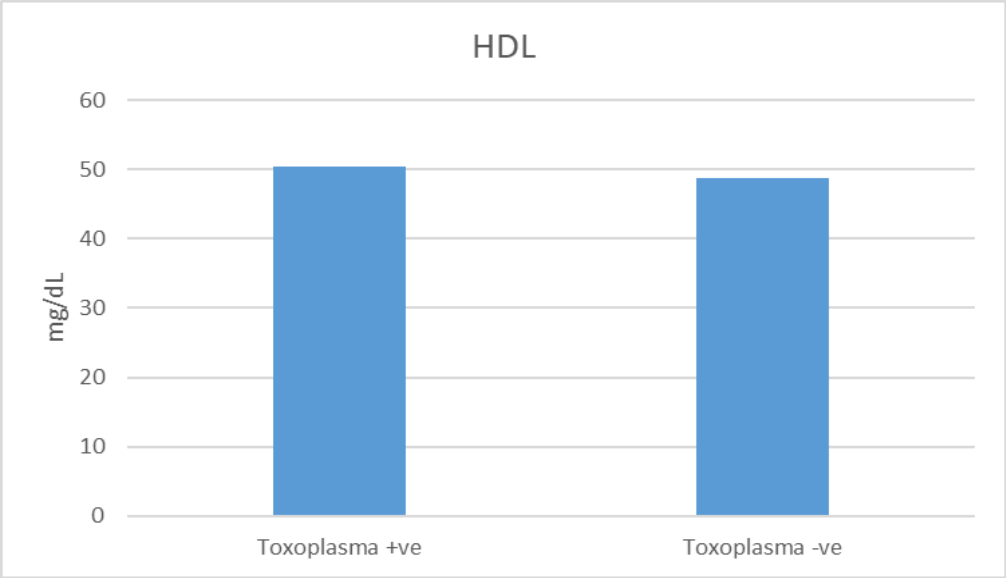


**Figure 3-**HDL levels in *Toxoplasma* positive and *Toxoplasma* negative subjects.

**Table 3-** HDL levels in *Toxoplasma* +ve and *Toxoplasma* –ve subjects in relation to age groups.

| Age/years | otal | *Toxoplasma + ve* | Mean HDL level(mg/dL) | *Toxoplasma – ve* | Mean HDL level (mg/dL) | p-value |
|---|---|---|---|---|---|---|
| 18-25 | 5 | 7 | 51.75 | 8 | 46.22 | 0.2 |
| 26-35 | 3 | 12 | 55.09 | 11 | 53.79 | 0.65 |
| 36+ | 1 | 2 | 49.9 | 9 | 51.9 | 0.72 |

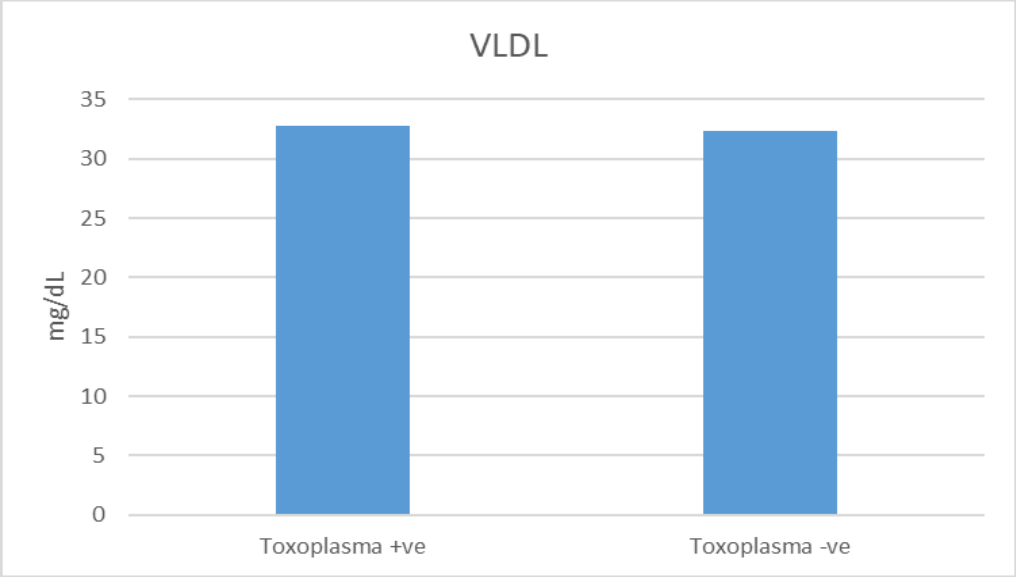


**Figure 4-**VLDL levels in *Toxoplasma* positive and *Toxoplasma* negative subjects.

**Table 4-** VLDL levels in *Toxoplasma* +ve and *Toxoplasma* –ve subjects in relation to age groups.

| Age /years | otal | *Toxoplasma + ve* | Mean VLDL level(mg/dL) | *Toxoplasma – ve* | Mean VLDL level (mg/dL) | p -value |
|---|---|---|---|---|---|---|
| 18-25 | 15 | 7 | 36.3 | 8 | 42.9 | 0.47 |
| 26-35 | 3 | 12 | 31.7 | 11 | 36.3 | 0.45 |
| 36+ | 1 | 2 | 30 | 9 | 30.3 | 0.96 |

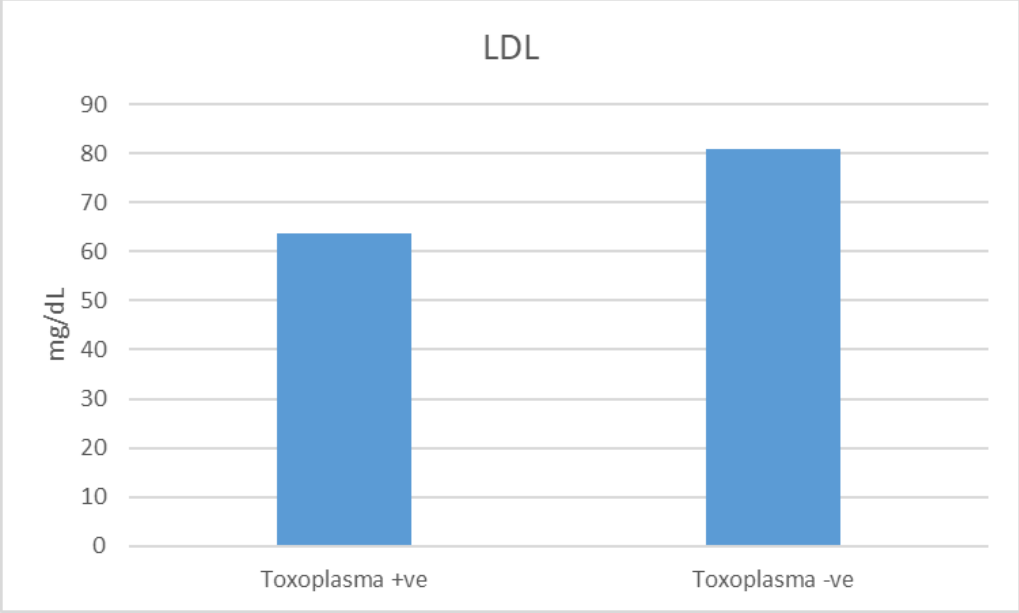


**Figure 5-**LDL levels in *Toxoplasma* positive and *Toxoplasma* negative subjects.

**Table 5-** LDL levels in *Toxoplasma* +ve and *Toxoplasma* –ve subjects in relation to age group

| Age /years | otal | *Toxoplasma + ve* | Mean LDL level(mg/dL) | *Toxoplasma – ve* | Mean LDL level (mg/dL) | p -value |
|---|---|---|---|---|---|---|
| 18-25 | 5 | 7 | 55.2 | 8 | 101 | 0.17 |
| 26-35 | 3 | 12 | 64.6 | 11 | 78.1 | 0.25 |
| 36+ | 1 | 2 | 47.4 | 9 | 62.8 | 0.42 |

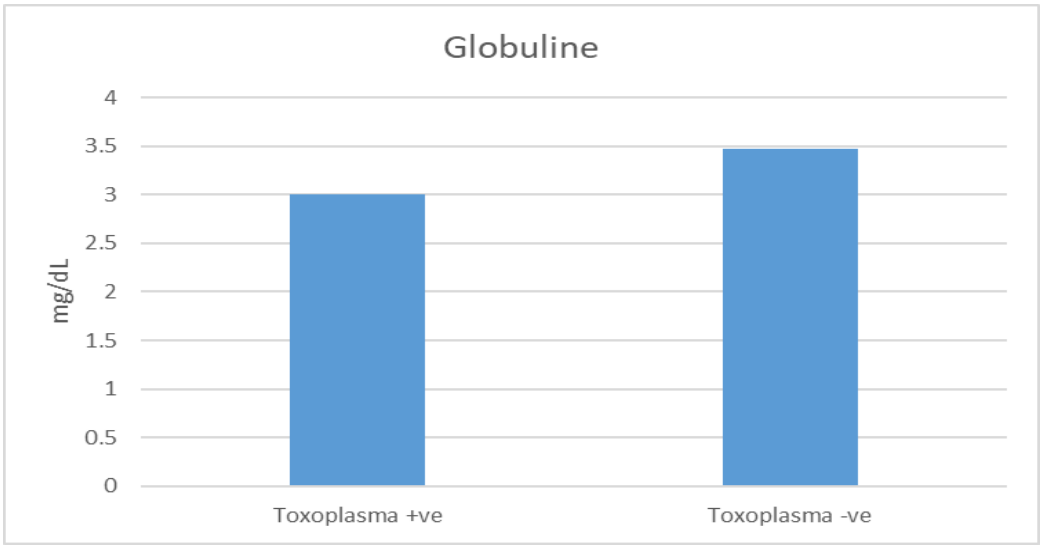


**Figure 6-**Globulin levels in *Toxoplasma* positive and *Toxoplasma* negative subjects.

**Table 6-** Globulin levels in *Toxoplasma* +ve and *Toxoplasma* –ve subjects in relation to age groups.

| Age /years | otal | *Toxoplasma + ve* | Mean globulin level(g/dL) | *Toxoplasma – ve* | Mean globulin level (g/dL) | p -value |
|---|---|---|---|---|---|---|
| 18-25 | 5 | 7 | 3.02 | 8 | 3.48 | 0.22 |
| 26-35 | 3 | 12 | 2.88 | 11 | 3.58 | 0.023 |
| 36+ | 1 | 2 | 3.17 | 9 | 3.20 | 0.95 |

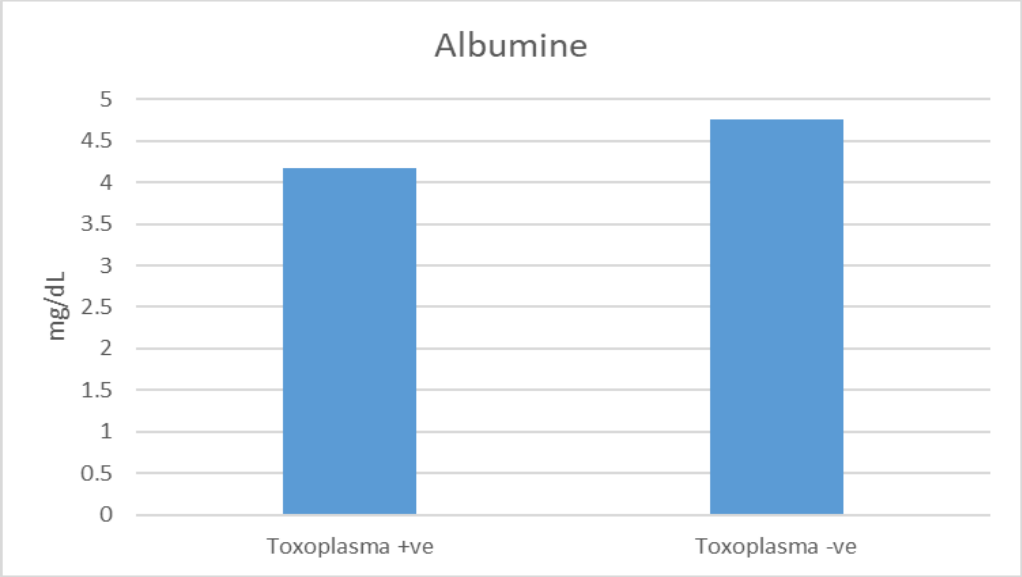


**Figure 7**-Albumin levels in *Toxoplasma* positive and *Toxoplasma* negative subjects.

**Table 7-** Albumin levels in *Toxoplasma* +ve and *Toxoplasma* –ve subjects in relation to age groups

| Age/years | otal | *Toxoplasma + ve* | Mean Albumin level(g/dL) | *Toxoplasma – ve* | Mean Albumin level(g/dL) | p -value |
|---|---|---|---|---|---|---|
| 18-25 | 5 | 7 | 4.88 | 8 | 4.1 | 0.026 |
| 26-35 | 3 | 12 | 4.76 | 11 | 4.01 | 0.0065 |
| 36+ | 1 | 2 | 4.40 | 9 | 4.1 | 0.49 |

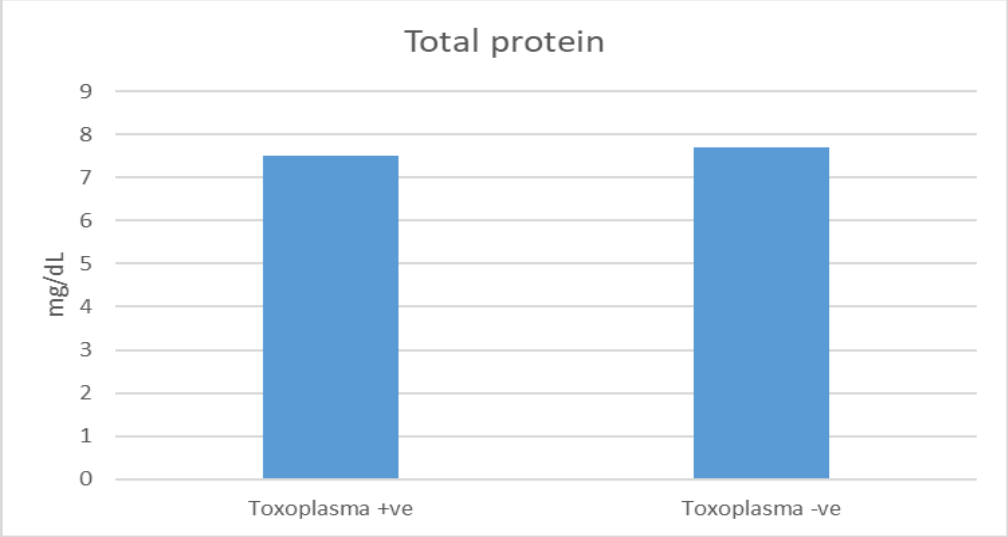


**Figure 8**-Total protein levels in *Toxoplasma* positive and *Toxoplasma* negative subjects.

**Table 8-** Total protein levels in *Toxoplasma* +ve and *Toxoplasma* –ve subjects in relation to age groups.

| Age /years | otal | *Toxoplasma + ve* | Mean total protein level(g/dL) | *Toxoplasma – ve* | Mean total protein level(g/dL) | *p -value* |
|---|---|---|---|---|---|---|
| 18-25 | 5 | 7 | 7.9 | 8 | 7.2 | .06 |
| 26-35 | 3 | 12 | 7.69 | 11 | 7.60 | 0.37 |
| 36+ | 1 | 2 | 7.56 | 9 | 7.61 | 0.47 |

**Discussion**

This investigation demonstrated interesting results approving that *T. gondii* alters some biochemical parameters. The results of lipid profile indicated a drop in cholesterol and triglycerides levels and the grade of lipid peroxidation, as characterized by a significant increase in HDL.

The association between the concentrations of cholesterol in people infected with some parasites has attracted the consideration of various researchers. *Toxoplasma* is incapable of synthesize cholesterol *de novo* and relies on recovering LDL-derived cholesterol from the host cell through LDL-mediated endocytosis or protein linked to the LDL receptor [13]. A mechanism has been proposed to control the entry of toxoplasma into cells by host and not parasite cholesterol [14]. These investigations revealed that cholesterol has a significant part in pathogenesis of *T. gondii* infection. However, facts on the lipid bases of *T. gondii* are very rare and the molecular tools by which *Toxoplasma* recovers lipids of the host cell are mostly unidentified [15]. Previous studies showed high concentrations of lipoproteins in people with protozoan infections [16]. Alterations in these lipoproteins were noticed in some other infections, as in HIV infected individuals who are under antiviral therapy [17]. Conflicting results were attained by other investigators, which might be attributed to the chronic nature of the disease, which is mostly the case in this study. However, in acute malaria infections, the levels of HDL and LDL were decreased, whereas that of triglycerides was increased temperately [18]. In another study, acute phase HDL was reported to be altered, with reduced HDL and antioxidant activity as well as other structural-compositional changes and inflammatory protein interactions [19]. A different study showed that the reason for the decline in HDL can be the hypertriglyceridemia-related acute phase response, which is an immediate response [20]. More investigations are needed to characterize the HDL particle during both the chronic and acute phases of toxoplasmosis. Finally, this study detected no significant differences in some parameters the lipid profile between the studied groups. This could be due to the limited number of cases.

These results are in disagreement with those of Yarim *et al*. [21], who observed hypoalbuminemia with a lower A/G ratio in infected dogs. Low concentration of albumin suggests damaged functions of the liver. Many factors can influence the fluctuation of serum proteins, including parasites and other infectious diseases [22]. Previous animal research reported a decrease in serum albumin levels in experimentally infected *Toxoplasma* mice due to liver injury [23]. However, in the present study, all *T. gondii* seropositive patients did not show any clinical features related to liver damage. Therefore, albumin level did not show the decrease reported by other studies.

Finally, this study revealed that *T. gondii* has a role in altering some biochemical factors in infected women, as characterized by the decline in the levels of cholesterol, triglycerides, and LDL, with the obvious increase in HDL concentration. Further investigations are required to investigate the effects of the phases of toxoplasmosis (chronic versus acute) on the alterations of all these parameters.

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