

Biochemical parameters in patients with *Trichomonas vaginalis* and *Toxoplasma gondii* in Erbil-Iraq

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Abstract

This study investigated the biochemical parameters of two parasites, *Trichomonas vaginalis* and *Toxoplasma gondii* among females attending the gynaecological out patients clinics in Maternity Rizgary Hospital, several health centers and some private clinics in Erbil-Iraq, from 16th July 2007 till 15th July 2008, by using different diagnostic methods. In addition, a comparative study to evaluate the biochemical changes due to trichomoniasis and toxoplasmosis. The serum samples were examined for some biochemical parameters like cholesterol, triglycerides, high density lipoproteins-cholesterol (HDL-C), low density lipoproteins-cholesterol (LDL-C), total proteins, albumin, globulin, with some elements such as calcium, sodium, potassium, chloride, iron, copper and zinc. Infection with *Trichomonas vaginalis* had no significant effects on the studied biochemical parameters among non-pregnant women. *Toxoplasma gondii* causes a significant decrease in total protein level among non-pregnant women, while in pregnant women, reduced calcium level was observed.

Key words: *T. vaginalis*, *T. gondii*, Erbil, Iraq.

Introduction

Trichomonas vaginalis a sexually transmitted parasite, adheres to the vaginal epithelium, causing vaginitis and other complications in women (1). It is an anaerobic protozoan flagellate, which lacks mitochondria and peroxisomes, but has a specialized double membrane-bounded organelle called the hydrogenosome, which is involved in metabolic process that extend glycolysis (2). *In vitro* study has shown that when serum was replaced by bovine serum albumin and cholesterol, it resulted in good growth (3).

T. gondii, an apicomplexan protozoan parasite, is an important pathogen of human and animals. It can cause serious infections in immunocompromised patients and in developing fetus (4). It has been shown that depleting host cell plasma membrane cholesterol blocks parasite internalization by reducing the release of rhoptry protein that is necessary for invasion (5).

Studies have shown elevated level of lipoproteins like high density lipoprotein (HDL), low density lipoprotein (LDL) and total cholesterol in patients suffering from parasitic infection (6).

Coopens et al. (7) demonstrated that the intracellular parasite, *T. gondii* acquires host cholesterol that is endocytosed by the LDL pathway, a process that is specifically increased in infected cells, interference with LDL endocytosis or cholesterol translocation reduced the intracellular survival of *T. gondii*.

Biochemical analysis of enzymes shown that the alanine transaminase (SGPT), aspartate transaminase (SGOT) and LDH level were decreased than normal range (8).

Several studies showed that the average zinc concentration in serum from toxoplasmosis seropositive women was lower than control group (8 & 9).

The present study was planned to determine the values of some biochemical tests (like cholesterol, triglycerides, high density lipoproteins-cholesterol (HDL-C), low density lipoproteins-cholesterol (LDL-C), total protein, albumin, globulin; some elements like calcium, sodium, potassium, chloride, and some of serum trace metals such as iron, copper and zinc in trichomoniasis and toxoplasmosis infected and non-infected women and between pregnant

and non-pregnant toxoplasmosis patients.

MATERIALS AND METHODS

The study was carried out on female aged between 15-45 years old, from 6th July 2007 and 15th July 2008, on patients attended to some private clinics, gynecological out patient's clinics in Maternity Rizgary Hospital and several health centers in Erbil Governorate for estimation of some biochemical parameters among those with *T. vaginalis* and *T. gondii* infections and control group.

Blood samples were withdrawn, from the patients and controls, centrifuged and sera were collected kept at 4-8°C for about 24-48 hours, if longer period was needed, then they were stored in a deep freezing, at -20°C for performing some serological and biochemical tests

T. vaginalis was diagnosed by in vaginal discharge using direct wet mount and culture method using Diamond Modified Broth (10).

Serological tests were used for detection of specific antibody to *T. gondii* by the use of Latex Agglutination Test (Latex Test Kits from Plasmatec Laboratory Products Ltd, U.K.) and IgM and IgG antibodies

using Enzyme-Linked Immunosorbent Assay (ELISA) technique (the Genesis Diagnostics, (UK) and BioCheck (CA) Toxoplasma IgM kits).

Biochemical tests: All the biochemical tests for sera of ninety-six patients were done in the Internal Laboratory of Erbil Teaching Hospital. Total cholesterol (CHOD-PAP method), Triglycerides (GPO Method), HDL-Cholesterol (PAP) precipitant, Total protein (Biuret Method), and Serum albumin (BCG Method) were measured using the Biolabo Kit-France.

LDL-Cholesterol: Calculation of LDL-Cholesterol was done according to Friedewald equation: $LDL-C = [Total\ cholesterol] - [HDL-C] - [Triglycerides/5]$.

Serum globulin was estimated by subtraction the value of serum albumin from total protein. The normal value of globulin is 2-3.6 g/dL (20-36 g/L).

Calcium (CPC method) was determined using Biolabo Kit-France. Quantitative determination of sodium, potassium and chloride in the serum sample was performed in the Internal Laboratory of Erbil Teaching Hospital by using an instrument called ELITE,

Electrolyte Analyzer System, from Electra Medical Corporation-USA.

Conditioning sodium solution was used for conditioning sodium electrode in ELITE analyzer and its content was 0.05 mol/L ammonium bifluoride (NH₄OHF₂).

Serum trace elements-Iron Fe, Copper Cu and Zinc Zn

Quantitative determination of serum trace elements (Fe, Cu and Zn) was carried out in the Quality Control Laboratory of Kurdistan Technology and Research Establishment in SulaimaniaCity by using atomic absorption spectrophotometer (AA200) PerkinElmer-USA

Statistical analysis: The statistical analysis was conducted using the software program Statistical Program Social System (SPSS version 13.0). Independent-Samples t-test was used for the biochemical tests for determination of the significant variations. The other remaining data were analyzed using Chi-square test (11).

RESULTS

A. *Trichomonasvaginalis*

The serum biochemical parameters were estimated in twenty three non-pregnant child-bearing age women (15-45years), 16 were *T. vaginalis* positive and 7 were negative.

It seems from the results of this study that *T. vaginalis* had no effect on cholesterol, triglycerides, HDL-C, LDL-C, total protein, albumin, globulin, calcium, sodium, potassium, chloride, iron, copper and zinc, as there was no significant difference in biochemical parameters between infected and control groups (Table 1).

Table 1: Levels of biochemical parameters in *T. vaginalis* seropositive and seronegative non-pregnant women.

Biochemical parameters	-ve cases	+ve cases	Statistical evaluation
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Cholesterol mg/dL	168.1±5.680	166.3±11.30	N.S
Triglyceride mg/dL	135.4±8.341	146.7±12.72	N.S
HDL-C mg/dL	39.57±1.510	39.75±2.126	N.S
LDL-C mg/dL	101.6±5.318	97.25±9.926	N.S
Total proteins g/dL	10.67±0.835	9.225±0.514	N.S
Albumin g/dL	5.029±0.440	5.069±0.162	N.S
Globulin g/dL	5.643±1.013	4.156±0.469	N.S
Calcium mg/dL	9.300±0.574	8.913±0.376	N.S
Sodium mmol/L	142.4±1.325	142.1±0.442	N.S
Potassium mmol/L	3.943±0.111	4.338±0.120	N.S
Chloride mmol/L	116.6±1.395	116.4±0.532	N.S
Iron mg/L	0.241±0.016	0.296±0.027	N.S
Copper mg/L	0.019±0.004	0.054±0.013	N.S
Zinc mg/L	0.330±0.023	0.419±0.029	N.S

B. *Toxoplasma gondii*

The same biochemical parameters were estimated in *T. gondii* infection, as mentioned in the *T. vaginalis* study. The study was conducted on 96 (76 non-pregnant and 20 pregnant) women using ELISA technique.

Out of 76 non-pregnant women, 51 were negative and 25 were positive for toxoplasmosis, and out of 20 pregnant women, who were enrolled in this study, 11 were negative and 9 were positive.

It is shown that *T. gondii* had no significant effect on cholesterol, triglycerides, HDL-C, LDL-C, albumin, globulin, sodium, potassium,

chloride, iron, copper and zinc in non-pregnant women except significantly decreased total proteins level (Table 2).

Table (3) indicates the levels of biochemical parameters in *Toxoplasma* seropositive and seronegative pregnant women. The current study revealed that there was no relation between seropositive and negative toxoplasmosis in the values of the biochemical parameters except calcium value, which showed a significant decrease among pregnant women. Although the total protein level decreased but statistically was not significant in seropositive pregnant women.

Table 2: Levels of biochemical parameters in *T. gondii* seropositive and seronegative non-pregnant women.

Biochemical parameters	-ve cases	+ve cases	Statistical evaluation
Cholesterol mg/dL	178.8±6.074	196.3±12.05	N.S
Triglyceride mg/dL	144.8±7.370	171.2±17.32	N.S
HDL-C mg/dL	39.41±1.397	35.48±1.602	N.S
LDL-C mg/dL	110.5±5.709	126.5±11.02	N.S
Total proteins g/dL	9.747±0.331	8.704±0.288	P<0.020
Albumin g/dL	4.851±0.140	4.728±0.214	N.S
Globulin g/dL	4.896±0.372	3.976±0.356	N.S
Calcium mg/dL	9.480±0.201	9.440±0.285	N.S
Sodium mmol/L	141.5±0.441	141.5±0.598	N.S
Potassium mmol/L	4.212±0.071	4.216±0.079	N.S
Chloride mmol/L	115.7±0.473	115.8±0.705	N.S
Iron mg/L	0.296±0.014	0.297±0.020	N.S
Copper mg/L	0.035±0.005	0.035±0.004	N.S
Zinc mg/L	0.368±0.015	0.322±0.031	N.S

Table 3: Levels of biochemical parameters in *T. gondii* seropositive and seronegative pregnant women.

Biochemical parameters	-ve cases	+ve cases	Statistical evaluation
Cholesterol mg/dL	201.5±15.30	177.8±10.40	N.S
Triglyceride mg/dL	144.1±25.29	154.3±20.63	N.S
HDL-C mg/dL	40.09±2.925	43.22±2.962	N.S
LDL-C mg/dL	132.7±12.04	103.4±12.09	N.S
Total proteins g/dL	9.109±0.758	8.322±0.514	N.S
Albumin g/dL	4.718±0.393	4.144±0.412	N.S
Globulin g/dL	4.391±0.903	4.178±0.753	N.S
Calcium mg/dL	10.18±0.509	8.500±0.386	P<0.021
Sodium mmol/L	142.3±2.098	138.8±0.521	N.S
Potassium mmol/L	4.009±0.113	4.100±0.087	N.S
Chloride mmol/L	118.2±3.547	114.2±0.795	N.S
Iron mg/L	0.241±0.039	0.218±0.029	N.S
Copper mg/L	0.019±0.005	0.038±0.009	N.S
Zinc mg/L	0.301±0.043	0.390±0.065	N.S

DISCUSSION

A-Trichomonas vaginalis:

T. vaginalis had no significant effect on biochemical parameters. This

might be due to small sample size of the studied group of patients or due to virulence and strain of the parasite. This finding is expected as *T.*

vaginalis an obligate extracellular, non invasive parasite (12) which is necessary to evoke a quantitative change in serum protein concentration because new protein produced as a result of tissue necrosis.

Al-Mudthafar and Al-Jeboori(13) showed that there is an intraspecific variation among the isolates of *T. vaginalis* which may suggest that there is more than one strain of *T. vaginalis* and as a result of a correlation between the biochemical data and the severity of trichomonal infection, there are severe types of infection and the mild-moderate ones. In addition, the host's immune response to the parasitic infection seems to be important determinants of virulence (14).

The results of this study are in accordance with the study of Fattah (15) that showed that the values of total protein, albumin and globulin were near normal range in both negative and positive cases of *T. vaginalis* infected patients.

B. *Toxoplasma gondii*

In general toxoplasmosis did not lead to severe changes in biochemical parameters. It is shown that *T. gondii* had no significant effect on cholesterol, triglycerides, HDL-C, LDL-C, albumin, globulin, sodium,

potassium, chloride, iron, copper and zinc. While in non-pregnant women decreased total proteins level, but in pregnant women reduced calcium level was observed.

Although the total protein level decreased but statistically was not significant in seropositive pregnant women. Kadiret *al.* (16) and Kadir and Khana (17) revealed that there was no relation between seropositive and negative toxoplasmosis in the level of serum protein and albumin in pregnant women while increase in globulin value was recorded. Significantly, higher serum total protein with slightly elevated albumin and globulin levels were recorded in toxoplasmic patients (18).

The reduced protein levels in the present study may be associated with the urinary tract infection, malnutrition and impaired intestinal absorption (19). All parasitic infections may cause loss of proteins via gastroenteritis, which leads to hypoproteinaemia (20).

The current study revealed that there was no relation between seropositive and seronegative toxoplasmosis in the values of the biochemical parameters except calcium level, which showed a significant decrease among pregnant women. This result was supported by Al-Zuhairy

(21) who demonstrated a significant reduction in calcium concentration in serum of ewes infected with acute and chronic toxoplasmosis in comparison with the controls, while no significant change in calcium was seen in experimentally infected rats. The reduced calcium level may be attributed to that the invasion of host cells by the obligate intracellular protozoan parasite *T. gondii* is calcium dependant and the intracellular concentration of free calcium in the host also varies in infected cells (22), in a concomitant study by Pingret *et al.* (23), revealed that 48 hours after infection, when the parasites had replicated and formed typical rosettes, cytoplasmic Ca^{2+} was significantly lower in infected cells than uninfected cells. It appears that once *T. gondii* has entered the cell, it is able to activate the pumping of Ca^{2+} from the cytoplasm of the host cell into the parasitophorous vacuole surrounding the tachyzoite parasite. The porous parasitophorous vacuole membrane would allow free diffusion of calcium across the membrane, and implicates the presence of products secreted into the vacuoles that are able to bind calcium. A candidate for this activity is the dense-granule protein, which is closely associated with the

tubulovesicular network of the parasitophorous vacuole and has a high affinity for calcium (5).

Calcium regulation in the host cell and the vacuolar compartment appears to be linked to both the growth and the release of the parasite from the host. An increase of Ca^{2+} in the parasitophorous vacuole may be the signal that triggers the egress of *T. gondii*, during which the parasite pushes the host plasma membrane out from the surface; the membrane is then ruptured as the parasite escapes, resulting in lysis of the host cell (23 & 24).

Another explanation for the reduced calcium may be due to that the animals with hypocalcaemia may be more susceptible to infection and invasion by the tachyzoites especially during pregnancy, which is considered, as a cause for calcium decrease, as a result of the high require of the calcium for the fetal skeletal mineralization during pregnancy which is a time of increased need for calcium (25), and there is an inverse relationship between calcium concentration in the cytoplasm of the host cell and the ability of the tachyzoites to invade the cells and increase of host cell cytosol Ca^{2+} ,

significantly decreased tachyzoite invasion (26).

It is recommended to carry on experimental infections in animals with hypocalcaemia and normal ones to investigate the ability of calcium to prevent or reduce the virulence of the disease.

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