Use of Sister Chromatid Exchange as an Indicator of Genetic Damage in Women and Ewes Infected with Toxoplasmosis in Baghdad City.

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Abstract

This study was conducted to determine the infection rate of *Toxoplasma gondii* in women and ewes during the period from November/ 2012 -March / 2013 at some area of Baghdad city .A total of 242 blood samples were collected from women and ewes: 100 blood samples collected from women who had abortion and pregnant women.92 blood samples collected from pregnant ewes, and 25 blood samples as control groups from each of women and ewes. Out of 100 women cases, the result showed that the total infection rate was 55%.While in ewes; the infection rate was 23.9%.Cytogenetic analysis was conducted in this study as a marker to reflect the genotoxic effect of *T.gondii* parasite on the lymphocytes. Sister Chromatid Exchange (SCEs) means showed a significant increase in the patients women and also in infected ewes when compared to the healthy controls with significant difference ($p \leq 0.01$). While the Mitotic Index and Replecative Index means in this study were found to be significantly lower in the patients than in healthy controls women and in infected and controls ewes. The means of SCEs was 8.27and 1.67 in patients and control women respectively; and 8.44and 1.49 in infected and control ewes respectively. The mean of Mitotic Index (M.I.) was 3.18 in patients and 18.08 in control women, and 2.48 in infected and 5.99 in control ewes; the mean R.I. was 1.19 in patients and 1.6 in control women; and 1.26 in infected and 1.61 in control ewes with significant differences ($p \leq 0.01$) .

Key Word: Sister Chromatid Exchange**,** Toxoplasmosis, Genetic Markers, Women and Ewes**.**

استخدام التبادل الكروماتيدي الشقيقي كمؤشر للضرر الحاصل بالمادة الوراثية للنساء والنعاج المصابة بداء المقوسات Toxoplasmosis في مدينة بغداد.

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الخلاصة

هدفت هذه الدراسة الى تحديد نسبة الاصابة بطفيلي *Toxoplasma gondii* في النساء والنعاج في مدينة بغداد للفترة من تشرين الثاني/2012 – آذار/2013 من خلال فحص242عينة دم 100عينة دم نساء مجهضات وحوامل و92 عينة دم نعاج حوامل ، فضلا عن25 عينة دم من كلا المجموعتين كمجموعتي سيطرة. بلغت نسبة الإصابة الكلية بالنساء 55% .اما في النعاج فكانت نسبة الاصابة الكلية 23.9 %باستخدام فحص الاليزا .استخدم الدم غير المتخثر للتحليل الوراثي – الخلوي للخلايا الليمفاوية والذي اظهر وجود تأثير معنوي $p \leq 0.01$ لمستضدات الطفيلي في تضاعف الخلايا الليمفاوية حيث كان معدل الانقسام الخلوي أقل معنويا لدى المرضى عند مقارنتهم بمجموعة السيطرة بالنسبة للنساء والنعاج، في حين كان متوسط تكرار التبادل الكروماتيدي الشقيقي اعلى من مجموعة السيطرة بالنسبة للنساء والنعاج .حيث كان متوسط تكرار التبادل الكروماتيدي الشقيقي 8.27 و 1.67 في النساء المصابات وغير المصابات على التوالي; 8.44 و 1.49 في مجموعة النعاج المصابة والسليمة على التوالي ; اما معدل الانقسام الخلوي فقد كان 3.18 في النساء المصابات و18.08 في النساء غير المصابات; و2.48 في النعاج المصابة و5.99 في النعاج السليمة. اما معامل التضاعف فقد كان 1.19 و 1.6عند النساءالمصابات وغير المصابات ; و1.26 في النعاج المصابة و1.61 في النعاج السليمة على التوالي .

الكلمات المفتاحية : التبادل الكروماتيدي الشقيقي، داء القطط ،النساء والنعاج والمادة الوراثية .

Introduction

Toxoplasma gondii is an intracellular zoonotic parasite and it causes the most common protozoal disease of animals and human (Tenter, *et al.,* 2000). The definitive host is the house cat and certain other Felidae (Ustun *et al*., 2004). The infection has a worldwide distribution. Approximately one –third of all humanity has been exposed to this parasite; this proves the importance of toxoplasmosis as a zoonotic disease (Jones and Dubey, 2010). In small ruminant (sheep and goats) , *T. gondii* infection has considerable economic importance as it causes abortion, stillbirth and neonatal loss, especially in sheep, or the birth of weak lambs, which may be accompanied by a mummified fetus (Dubey, 2008).

In humans investigated evidence of infection have been found in all population groups during the first few weeks post-exposure, the infection typically causes a mild flu-like illness or no illness. Thereafter, the parasite rarely causes any symptoms in otherwise healthy adults. However, those with a weakened immune system, such as AIDS patients, organ transplant , malignancy patients, and pregnant women, it can lead to severe or even lethal damage as an opportunistic parasite (Montoya and Liesenfeld, 2004; Weiss and Dubey,2009). Moreover, congenital toxoplasmosis is of great clinical importance. It occurs as acute maternal infection during pregnancy which affects the fetus, resulting in retinochoroiditis, intracranial calcifications, hydrocephalus, mental retardation and even spontaneous abortion and neonatal death (Petersen, 2007).Cytogenetic analysis is a widely employed indicator system for induced mutations. It allows objective evaluation of insults to the genetic material and is a method that permits visual analysis of chromosome damage (Tuker and Preston, 1996). Sister Chromatid Exchange (SCE) is the exchange of Genetic material between two identical sister chromatids of the same chromosomes. The (SCE) test has been used to detect genome stability in humans (Chaganti *et al.*, 1974) and the main livestock species (Ciotola *et al*., 2005), and to discover DNA damage caused by a variety of natural and artificial chemical compounds (Perucatti *et al*., 2006). A greatly increased incidence of SCEs has been demonstrated in cells from patients with schistosomiasis (Shubber *et al.,* 1991; Juma *et al*., 1999) and patient with toxoplasmosis (AL-khafajy, 2004) .

Aims of this study

- Investigate the cytogenetic changes in the lymphocyte of affected women and ewes by using blood samples.
- Estimating the infection rates of toxoplasmosis in aborted women and ewes regarding the age from different area.

Materials and Methods

The Cytogenetic analysis and scientific imaging of chromosome and cell was conducted in Ministry of

Science and Technology– Department of Environment and Water Pollution treatment center - Biological Research Department.

Collection of Blood Samples

A total of 242 blood samples were collected from aborted women and ewes which divided into: One hundred blood samples collected from aborted women they were referred to the AL-zahraa hospital and many private laboratories in Baghdad according to the physician's reports, indicating the possibility of being have toxoplasmosis. The woman ages ranged between 20-40 years old. Ninety two blood samples of ewes, collected from pregnant ewes from many

regions in Baghdad (College of Vet. Medicine 5 samples, Abu-Graib 20 samples, AL-Shulla 20 samples, Sabaa AL-Boor 16 samples and AL-Yousefia 31 samples). Five milliliter of blood samples were collected from median cubital vein of women and from jagular vein of ewes after antiseptic the site of collection by ethanol.The collected blood samples divided into two parts, 3ml in sterile tube for serological test, and 2 ml in heparinzed tube for cytogenetic test.

Control Groups

Twenty five samples (from each group) of healthy looking women, and ewes had been selected as control group to compare with the infected groups in the same parameters of this study. Serum and venous heprinized blood were collected from them and tested for anti-Toxoplasma gondii antibodies .Those that revealed any antibody titer against Toxoplasma gondii were excluded from this study Enzyme Linked Immune Sorbent Assay (ELISA) : The test was based on the reaction of *Toxoplasma gondii* .

Serological Tests

Proteins with monoclonal human's antibodies and sheep antibodies (IgG), and the diluted serum samples were added to the wells of the coated plate. After washing the bound, antibodies was detected by horse reddish peroxides (HRP) conjugated anti-humans conjugate and sheep peroxides conjugate. The color reaction in the wells is directly related to the concentration of anti- *Toxoplasma gondii* antibodies in the serum samples (The commercial kit produced under the authority of the Laboratories HUMAN® for human, and for sheep ID Screen® they were performed according to manufacturer's instructions). Cytogenetic analysis of human and animal blood lymphocyte were according to (shubber, 1987). All data were represented as means and Standard error and statically analyzed by using T-test and chi square by using spss soft ware. The level of statistical significant was set at ($P < 0.01$ and $P< 0.05$) (Snedecor and Cochran, 1989).

Results of ELISA Test

***Toxoplasma gondii* Infection Rate in Women** According to the result; out of 100 samples, 55 sera were positive by indirect ELISA with an infection rate 55%, while negative in control group (table 1).

Table (1) Infection Rate of Women Toxoplasmosis by Using Indirect ELISA Test

Test	No. of Samples	*Toxoplasma gondii* positive	Infection Rate %
ELISA IgG	**100**	**55**	**55**
Control	**25**	**0**	**0**

***Toxoplasma gondii* Infection Percentage in Ewes**

Out of 92 ewes samples, 22 sera were positive by indirect ELISA with an infection rate of 23.9%, while no infection recorded in the control group. (table 2) .

Table (2) Percentage of Ewes Toxoplasmosis by Using Indirect ELISA Test

Test	No. of samples	*Toxoplasma gondii* positive	Percentage %
ELISA IgG	92	22	23.9
Contorol	25	0	0

Results of Cytogenetic Analysis

In this study the mean of M.I. and R.I. for infected with Toxoplasmosis of both women and ewes were significantly ($p \leq 0.01$) lower than healthy controls, M.I. was 3.18± and 18.08± in patients and control women respectively, and 2.48, 5.99 in infected and control ewes respectively; R.I. was 1.19 and 1.6 in patients and control women respectively, and 1.26 and 1.61 in infected and control ewes , respectively with significant difference ($p \leq 0.01$) , while the mean of SCEs were significantly higher 8.27 and 1.67 in patients and control in women respectively,fig. (1, 2), and (8.44 and 1.49) in infected and control ewes respectively , with significant difference ($p \leq 0.01$). (tables 3 and 4).

Table (3) the Means and SD of Mitotic Index, Replecative Index and Sister Chromatid Exchanges in Women

Women	Mitotic index MI%	Cell Cycle Progression C.C.P			Replicative Index R.I%	Mean S.C.E/Cell
		M1%	M2%	M3%		
Toxoplasma gondii patients (55)	3.18 ± 0.13 B	81.6 ± 4.11 A	15.14 ± 0.55 B	2.69 ± 0.24 B	1.19 ± 0.01 B	8.27 ± 0.11 A
Control (25)	18.08 ± 0.47 A	56.92 ± 1.84 B	27.92 ± 1.36 A	15.56 ± 1.03 A	1.6 ± 0.03 A	1.67 ± 0.02 B

The different capital letters refer significant differences between different groups at ($P<0.01$).

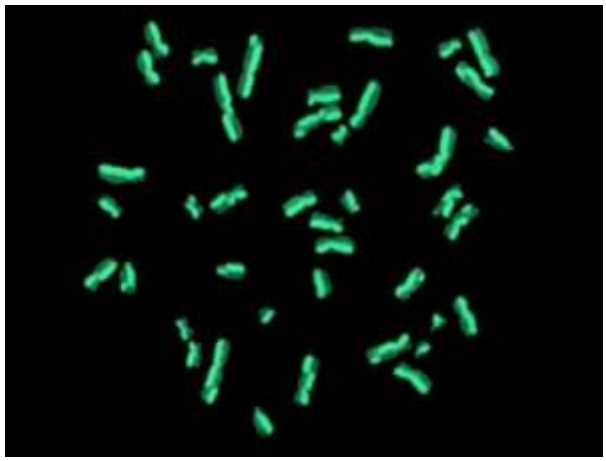

Figure (1): Sister Chromatid Exchanges in Women (100X).

Table (4) the Means and SD of Mitotic Index, Replecative Index and Sister Chromatid Exchanges Ewes

Ewes	Mitotic index MI%	Cell Cycle Progression C.C.P			Replicative Index R.I%	Mean S.C.E/Cell
		M1%	M2%	M3%		
Toxoplasma gondii Infection (22)	2.48 ± 0.18 B	78.13 ± 1.38 A	17.27 ± 1.03 B	4.54 ± 0.49 B	1.26 ± 0.01 B	8.44 ± 0.26 A
Control (25)	5.99 ± 0.2 A	56.12 ± 2.1 B	26.64 ± 1.25 A	17.2 ± 1.29 A	1.61 ± 0.03 A	1.49 ± 0.018 B

The different capital letters refer significant differences between different groups at ($P<0.01$).

The cytogenetic analysis was used to demonstrate the effects of parasite on the chromosome ,cell cycle kinetics, and analysis of the proliferation rate without use of radiolabeled thymidine (Shubber *et al*., 1985). Several studies were done on other parasites to estimate the immune state or to indicate the chromosomal damage. There was a significant decrease in the mean of M.I. in normal healthy controls (Baqir *et al*., 2001).

and R.I. of patients with *Schistosoma haematobium* (Shubber 1987; Juma and Shubber, 1999), this were agreed with our study. The low MI and RI manifest the decreasing of lymphocytes proliferation. This impairment extends to non-parasite antigen when lymphocytes responded to Phytoheamagglutinine (PHA) significantly lower than response

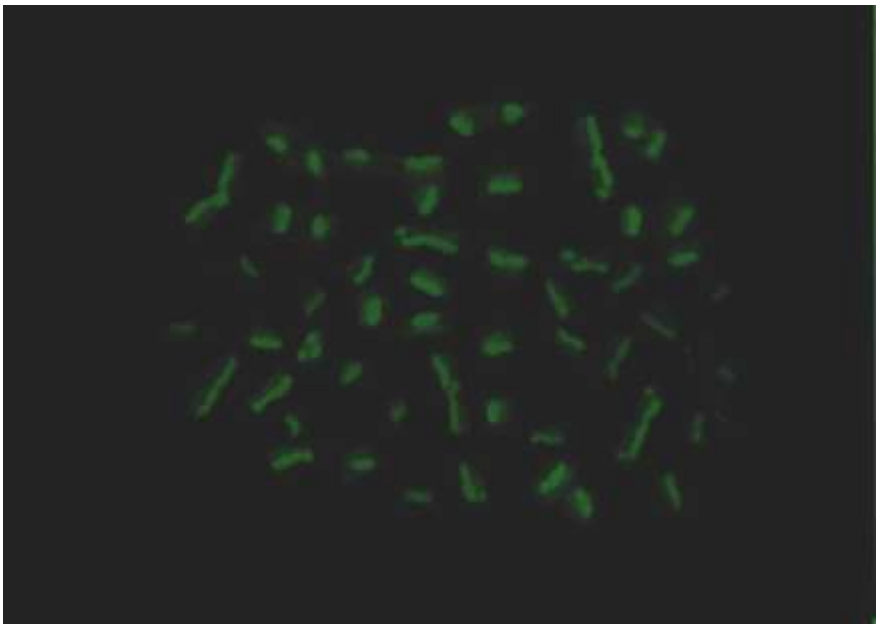

Figure (2): Sister Chromatid Exchange in Infected Ewes (100X).

Al-ubaidy (2002) found that the mean of M.I. and R.I. of patients with hydated disease in response to the PHA was significantly lower than those of healthy controls. This might indicate an immune unresponsiveness state against the parasite *Ecchinococcus granulosus.* AL-khafajy (2004) reported that there are significant decrease in M.I. and R.I., and significant increased of SCE in women infected with *T. gondii.*

The antigenic effect of *T. gondii* may interfere with the action of PHA on the lymphocyte proliferation in the women infected with Toxoplasmosis. This proliferation of lymphocyte did not indicate the immunopathological changes or immunoinflammation in those women because there were no signs and symptoms and the infection was only limited on fetus that lead to the abortion. Therefore, this result manifests the results of the previous studies that used the M.I and R.I. mean as parameters of the CMI of the patients, while the healthy controls usually show normal M.I and R.I. mean (AL-khafajy, 2004).The (SCE) test has been used to detect genome stability in humans (Chaganti *et al*., 1974), the main livestock species (Ciotola *et al*. 2005), and to discover DNA damage caused by a variety of natural and artificial chemical compounds (Iannuzzi *et al*., 2004; Perucatti *et al*., 2006).

Conclusions

The present study concluded the following:

- The incidence of the Toxoplasmosis among women was high (55%).
- The incidence of the Toxoplasmosis among ewes was (23.9%).
- The Toxoplasma has an effect on lymphocyte proliferation, and on the genetic material of the lymphocyte which caused a significant decrease in Mitotic Index and Replecative Index mean of the infected group when compared to healthy controls group.
- There was a significant increase in Sister Chromatid Exchange of infected group when compared to healthy controls group

Recommendations

The present study recommended the following:

- Further specific chromosomal studies to indicate the cytogenetic changes during the infection.
- Molecular analysis to determine Toxoplasma genotypes in Iraq.
- Further studies to develop a good vaccine to protect and control Toxoplasmosis in human and animals.
- Screening tests in pregnant women by using alternative sensitive diagnostic tests such as ELISA and PCR for diagnosis of Toxoplasma infection in human and animals.
- Good cooking of meat and meat products consumed by human in order to destroy the tissue cyst and follow hygienic precautions.
- Eradicate of stray cats to eliminate the life cycle of parasite.
- Wear gloves when gardening and during any contact with soil or sand because it might be contaminated with cat feces that contain Toxoplasma.

Wash hands with soap and warm water after gardening or contact with soil or sand.

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