

Cytogenetic Studies on Lymphocytes Isolated from Women with a History of Abortion Infected with *Toxoplasma gondii*

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Abstract

Sixty women who had abortion were selected for this study. ELISA test was used to detect anti-*Toxoplasma* specific antibodies. Venous heparinized blood and serum was collected from the women. Those women were divided into three groups according to the presence or absence of specific anti-*Toxoplasma* antibodies. These groups included women with IgG (18), women with IgM (14) and women with both IgG and IgM (12). The other 16 women had no antibodies against *Toxoplasma*. Twenty-four healthy-looking women had been selected as controls. Venous heparinized blood and serum was collected from those women. Serum was tested for anti-*Toxoplasma* antibodies. Those that revealed any antibody titer against *Toxoplasma* were excluded from the study. The M.I. and R.I. were found to be significantly lower in the patients when compared to the healthy controls, whereas S.C.E. was found to be significantly higher. This may indicate the effect of the parasite on the immune cells and their genetic material making it possible for the parasite to become established.

Keywords: Cytogenetic analysis, Toxoplasmosis, Abortion

Introduction

Toxoplasmosis, caused by the protozoan parasite *Toxoplasma gondii*, ranks third in the United States for deaths caused by food- born pathogens [1]. Toxoplasmosis can

be transmitted across the placenta in women who have the infection acquired either for the first time or recurring (especially in persons who are immunocompromised) during pregnancy [2]. Approximately one out of every 1000 pregnant women becomes infected, resulting in anywhere from 400 to 4000 cases of congenital toxoplasmosis each year in the United States [3]. *T. gondii* is an important water- or food-born pathogen that is capable of causing severe disease in infants from infected mothers and in the immunocompromised patients [4]. Following oral infection, *Toxoplasma* initially crosses the intestinal epithelium, disseminates into the deep tissues and traverses biological barriers such as the placenta and the blood brain barrier to reach immunologically privileged sites. It is here that the parasite causes the most severe pathology; disseminated congenital infections in the developing fetus [5], severe neurological complications in immunocompromised individuals [6] and ocular pathology in otherwise healthy individuals [7].

High *T. gondii* seroprevalence has been found in many countries such as France [8], tropical areas of Latin America or sub-Saharan Africa [9]. Studies carried out in Scandinavian countries [10] and in England [11] have shown low levels of seroprevalence. In other countries, toxoplasmosis had been investigated such as in Iran [12], Saudi Arabia [13] and in Egypt [14]. In Iraq, many studies were published about the seroprevalence of toxoplasmosis by using different techniques like IHA, IFAT and ELISA [15, 16].

Parasitic infections were found to cause chromosomal damage in the immune cells, leading to immune disturbances, which were shown to be associated with the degree of infection [17, 18]. A sensitive assay of biological and industrial mutagens in man and animals analyzes the sister chromatid exchanges, cellular proliferation and mitotic index using a thymidine analogue, mainly 5-bromodeoxyuridine [19]. These cytogenetic parameters are associated with mutation, carcinogenicity and teratogenic effects of several chemical, physical and biological agents [20, 21].

Materials and Methods

A. Subject collection

Sixty women who had abortion were selected for this study in a period between January 2003 and March 2003. They were referred to the Central Health Laboratory in Baghdad according to the physician's reports, indicating the possibility of them having toxoplasmosis and confirmed by using ELISA test to detect anti *Toxoplasma* specific antibodies. Venous heparinized blood and serum was collected from the women. Those women were divided into three groups according to the presence or absence of specific anti-*Toxoplasma* antibodies. These groups included women with IgG (18), women with

IgM (14) and women with both IgG and IgM (12). The other 16 women had no antibodies against *Toxoplasma*.

Twenty-four healthy-looking women had been selected as controls. Venous heparinized blood and serum was collected from those women. Serum was tested for anti-*Toxoplasma* antibodies. Those that revealed any antibody titer against *Toxoplasma* were excluded from the study.

B. Culturing and harvest of lymphocytes

Lymphocyte culture was performed according to Kelsey *et al.* [22] with the following modifications.

A tube of 4ml RPMI-1640 (Sigma) prepared medium containing 5-bromodeoxyuridine (15 µg/ml), brought to room temperature, was used for each individual. The following components were added:

Heparinized whole blood	0.5 ml
Human AB+ve-plasma	1.0 ml
Phytohemagglutinin (PHA)	0.2 ml

For mitotic index (M.I.) analysis, slides were stained with Giemsa stain for 2 minutes and examined with a light microscope under X20 objective lens. It was determined as a percent of the mitotic cells to interphase nuclei in 1000 cells. For sister chromatid exchanges (S.C.E.) (scored in 50 well spread second metaphases presented as mean S.C.E./cell ± standard error and cell cycle progression (C.C.P.) (analyzed in 100 consecutive metaphase cells presented as percent of M1, M2 and M3 cells), identical slides were stained with the fluorescent dye DAPI for 10 minutes. The dry slides were mounted with alkaline phosphate buffer and examined under X100 oil immersion objective lens using an Olympus-BH2 standard microscope equipped with a fluorescent epi-illumination system. The replicative index (R.I.) was calculated using the following equation:

$$R.I. = \frac{(1 \times M1) + (2 \times M2) + (3 \times M3)}{100} \quad [23]$$

C. Statistical analysis

The data were statistically analyzed using student's T-test according to Snedecor and Cochran [24].

Results

The Mitotic Index (M.I.): The mean M.I. of seropositive women was significantly lower ($p \leq 0.0001$) than that of healthy controls, and there was no significant differences ($P > 0.05$) among women who had IgG, IgM, or both IgG and IgM (table 1).

The Sister Chromatid Exchanges (S.C.E.): The mean S.C.E. of seropositive women was significantly higher ($P \leq 0.0001$) than that of healthy controls, and there was no significant difference ($P > 0.05$) among women with IgG, IgM, or both IgG and IgM (table 1).

The Cell Cycle Progression (C.C.P.): The mean replicative index (R.I.) of seropositive women was significantly lower ($P \leq 0.0001$) than that of healthy controls, and there was no significant difference ($P > 0.05$) among women with IgG, IgM, or both IgG and IgM (table 1).

Table (1): Cytogenetic parameters of blood lymphocytes from women with anti-*Toxoplasma* antibodies and healthy controls

Parameter	Control Group	women with specific anti- <i>Toxoplasma</i>			P
		IgG	IgM	IgG & IgM	
M.I.(%)	15.6 ± 4.4	2.26 ± 1.2	2.8 ± 1.3	2.68 ± 1.2	(P< 0.0001)
R.I.	1.53 ± 1	1.32 ± 0.05	1.2 ± 0.04	1.16 ± 0.05	(P< 0.0001)
S.C.E./cell	3.25 ± 1.4	7.6 ± 0.9	8.1 ± 1.5	7.6 ± 1	(P< 0.0001)
P: probability, M.I.: mitotic index, R.I.: replicative index, S.C.E.: sister chromatid exchange					

Discussion

The use of the cytogenetic analyses as the parameter of this study for the indication of the immune response in *T. gondii* infection has not been done before. Therefore, this study could be considered as the first in this aspect.

The mean M.I. and R.I. of patients with toxoplasmosis were significantly lower than the healthy controls. This does not indicate an immunosuppression state, because many studies indicated the strong and persistent cell mediated immunity (CMI) elicited by *T. gondii*, resulting in host protection against rapid tachyzoite growth and consequent pathologic changes. Also, recent studies on human peripheral blood T lymphocytes from previously unexposed donors indicate that *T.gondii* acts as a strong T cell mitogen, inducing high levels of proliferation and IFN- γ [25].

However, other studies were done on other parasites and on many types of carcinomas like leukemia and lymphoma to estimate either the immune state or to indicate the chromosomal damage. There was a significant decrease in the mean of M.I. and R.I. of patients with *Schistosoma haematobium* [26]. This may indicate an immunosuppression state due to the release of immunoregulatory substances from the parasite like these released from adult worms with inhibitory activities on lymphocyte proliferation [27]. Studies also indicate a significant decrease in the proliferation of lymphocytes from peripheral blood of active Kala-azar patients on stimulation with *Leishmania* antigen. The low M.I. and low R.I. manifest this decrease in lymphocyte proliferation. This impairment extends to non-parasite antigen when lymphocytes responded to PHA significantly lower than response in normal healthy controls [28]. The mean M.I. and R.I. of patients with hydatid disease in response to the nonspecific T cell mitogen, the PHA, was significantly lower than those of healthy controls. This might indicate an immune unresponsiveness state against the parasite *Echinococcus granulosus* [29].

The significant decrease of M.I. and R.I. mean in this study, although the noted increasing of lymphocyte proliferation, could indicate that proliferation was as a result of either *Toxoplasma*-antigen-pulsed or *Toxoplasma* infected antigen presenting cells (APC) effect on the lymphocytes [25]. In this study, the antigenic effect of the *T.gondii* may interfere with the action of PHA on the lymphocyte proliferation in the women with toxoplasmosis. This proliferation of lymphocytes did not indicate the immunopathological changes or immunoinflammation in those women because there were no signs and symptoms and the infection was only limited to the 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.

S.C.E. has been used frequently to indirectly assess the DNA damaging potential of various physical, biological and chemical treatments [30]. Moreover, females have been found to have higher S.C.E. frequencies than males depending on age; this is because they have more chromosomal material (3%) than males [30, 31]. This study shows there was a significant increase in S.C.E. mean of patients with toxoplasmosis

than of healthy controls. This result may be related to: (A) the type of treatment that is given to the pregnant women who have a history of abortion. Spiramycin is usually given from the first trimester, for the treatment of toxoplasmosis, prescribed along with pyrimethamine [33]. The latter drug is considered teratogenic in addition to causing bone marrow suppression and high levels of toxicity. (B) During the parasite metabolism, certain chemicals might be released that might have a direct effect on the chromosomes. Such results were shown in *S. haematobium* infection [34]. Therefore, the alteration of host metabolism for endogenous or exogenous mutagens and carcinogens may play an integral role in the induction of S.C.E. (C) The presence of *Toxoplasma* in the host cells may change the efficiency of cells for BrdUrd uptake and / or incorporation in cellular DNA. (D) The possible role of immune response in changing the type of sampled cells cannot be excluded, for there is a change in the type of immune cells during the infection [35]. (E) IgE has been shown to increase during *Toxoplasma* infection [36]. This increase was found to be directly proportional to the increase in S.C.E. [21].

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