The impact of toxoplasmosis on Seminal fluid analysis in association with infertility

Husam E. Ab-dulla*[1], Ahmed S. Abood[2], Haider S. Kadhim[3], Nada M. Al-bashier[3], Ula Al-kawaz[4]

Abstract— Toxoplasma gondii is a common protozoan parasite that infects warm-blooded animals throughout the world, including mice and humans. Mice and humans are both the intermediate hosts for Toxoplasma gondii. During infection, both, the parasite and the host, utilize various mechanisms to maximize their own reproductive success. As half of the human population is infected, developing a disease called toxoplasmosis, along with an ever-growing number of couples suffering with idiopathic infertility, it is therefore surprising that there is a lack of research on how Toxoplasma gondii can alter reproductive parameters. One of the common methods for determination of fertility status in males is seminal fluid analysis. This study was conducted on 83 males in which 64 infertile male patients, Include 25 males with primary infertility and also 39 males with secondary. Our aim was to determine the effect of toxoplasmosis infection on seminal fluid analysis parameters. ELISA was used to detect the chronic state of toxoplasmosis. The study showed there is no effect of toxoplasmosis on seminal fluid criteria in infertile males.

Index Terms— ELISA, IgG, Infertility, Seminal fluid analysis, Toxoplasmosis, Toxoplasma gondii.

1 INTRODUCTION

It has been 100 years since T. gondii was initially described in Tunis by Nicolle and Manceaux (1908) in the tissues of the gundi (Ctenodoactylus gundi) and in Brazil by Splendore (1908) in the tissues of a rabbit (1).

T. gondii is a ubiquitous, Apicomplexan parasite of warm blooded animals that can cause several clinical syndromes including encephalitis, chorioretinitis, congenital infection and neonatal mortality. Fifteen years after the description of T. gondii by Nicolle and Manceaux a fatal case of toxoplasmosis in a child was reported by Janků (2). In 1939 Wolf, Cowen and Paige were the first to conclusively identify T. gondii as a cause of human disease (3). The true importance of toxoplasmosis in humans remained unknown until the first reports of cases of congenital toxoplasmosis (4). Cysts are able to proliferate without passing through an intermediate tachyzoite stage, via both the migration of free bradyzoites and the fission of bradyzoite cysts, suggesting a mechanism for dissemination during chronic infection (5).

The first occurrence of toxoplasmosis was recorded in 1938 by Machattie, through histological isolation of the parasite from spleen and lungs of two stray dogs in Baghdad (6). There are several studies showing the seroprevalence of toxoplasmosis in Baghdad metropolis among them one investigates the seroprevalence in males in which detection had been recorded between 400 apparently healthy male blood donors by using (LAT) and ELISA (IgM, IgG), the results were (34 - 2.5 and 30.25) % respectively (7).

1 Iraqi Ministry of Agriculture, Veterinary Directorate.
2 College of Education, Al-Iraqia University.
3 College of Medicine- Al-Nahrain University.
4 High Institute of Infertility Diagnosis and Assisted Reproductive Technologies - Al-Nahrain University.
** Corresponding Author: husam.aljumaily@yahoo.com*

The principal modes of T. gondii transmission are ingestion of fecal oocysts or tissue cysts, and the transplacental transmission of tachyzoites from mother to unborn child. Infection with fecal oocysts may occur by accidentally ingesting contaminated soil (e.g. not washing hands after gardening or eating unwashed fresh produce), drinking untreated contaminated water, eating shellfish grown in contaminated water, or contact with cat feces (e.g. a cat litter box). Infection from tissue cysts may occur by consuming raw or undercooked meat, by accidentally consuming tissue cysts after handling raw meat and not washing hands thoroughly, or by cross-contamination of food prepared using unwashed utensils and chopping boards that have had contact with raw meat (8).

Venereal transmission of T. gondii in different studies were detected in semen and reproductive organs of experimentally infected male rat (9), rabbit (10), dog (11), goat (12), sheep (13), cattle (14) and pig (15).

There are some evidences propose that T. gondii can be transmitted with semen to female animals (10, 11, 16).

T. gondii detected in testicle, epididymis and seminal samples of experimentally infected male dogs. Moreover, the infected seminal samples were injected to toxoplasma-negative female dogs with artificial insemination. They observed all of the female dogs were infected. In two of the female dogs fetal reabsorption occurred at the beginning of gestation, likewise numerous Toxoplasmic cerebral cysts were isolated from four puppies of the dogs (11).

In rabbit, presence of T. gondii DNA in semen and blood of experimentally infected male has been observed at 7 to 88 days post infection (10). The infection in some Toxoplasma-negative female rabbits resulted from artificial insemination of infected semen (10).

A recent study showed that in sheep artificial insemination of semen experimentally contaminated with T. gondii tachyzoites was capable to infect sheep that suggested the possibility of venereal transmission of T. gondii in sheep (16). Furthermore, persistent anestrus, hydrometra, mucometra and follicular cysts along with histopathological lesions in placen-

tas were observed in female sheep that were infected with contaminated semen (13).

Infertility is defined as the inability of a sexually active non-contracepting couple to achieve pregnancy in one year (17). Most couples achieve conception within one year and approximately 15% of couples are unable to do so, approximately 20% of cases of infertility are caused entirely by male factor, with an additional (30-40) % of cases involving both male and female factors; therefore, a male factor is present in half of infertile couples (18).

A large proportion of infertile men fail to impregnate their female counterpart because of lack of sperm (azoospermia) or too little sperm (oligozoospermia); infertility may also be due to abnormal sperm morphology (tetratozoospermia) and insufficient sperm motility (athenozoospermia) (19).

Semen quality is taken as a surrogate measure of male fecundity in clinical andrology, male fertility, reproductive toxicology, epidemiology and pregnancy risk assessments. Reference intervals for values of semen parameters from a fertile population could provide data from which prognosis of fertility or diagnosis of infertility can be extrapolated (20).

1.1 Aims of the study:

To investigate the impact of toxoplasmosis positivity on the seminal fluid analysis parameters in males with and without fertility dysfunctions.

2 Materials & Methods

2.1 Subjects

Patients: Selection of sixty-four man who suffering from infertility for this study. Include twenty-five males with primary infertility and also thirty-nine males with secondary infertility.
Controls: Selection of nineteen healthy fertile males as control, to compare with our patients in the same parameters of this study, sera were collected from those fertile males. From the total number fourty four were referred to Al-nahrain University/ high institute of infertility diagnosis and assisted reproductive technologies according to the physician's report, and the rest from private laboratory in metropolis Baghdad from Al-taji city in a period between April 2104 and August 2014.
A questionnaire sheet, was filled out for each individual involved in the study. In addition, ethical approval was obtained.

2.2 Sample collection

From each male (patient and control) venous blood (5 ml) were collected in addition to seminal fluid. The blood was placed in a plain tube and left to stand for one hour at room temperature for clot formation. For serum collection, the tube was centrifuged for 10 minutes at 4°C at 450 XG. The serum was then aspirated using a Pasteur pipette and dispensed into sterile appendorf tubes (0.5 ml in each) and stored at -20 °C until used. The semen was collected in the laboratory by masturbation after 3-5 days of sexual abstinence. The ejaculate was deposited in a sterile wide mouth, screw capped, and plastic container.

2.3 Seminal Fluid Analysis

The seminal fluid was examined according to world health organization (WHO) 2010 criteria of seminal fluid analysis. Liquefaction time, volume, colour, viscosity and PH were assessed by slowly pouring the specimen from the collection bottle into a small glass- to slide measure graduate these parameters in the laboratory.

The following findings were assessed microscopically spermatozoa motility, the degree of motility, sperm concentration, the percentage and type of morphologically abnormal spermatozoa, agglutination and identification of other cell types within the ejaculate.

2.4 Quantitative ELISA

Kit for detection of IgG antibodies against T. gondii antigens in serum (HUMAN Gesellschaft for biomedical and diagnostical mbH) was used according to manufacturer`s instructions. The absorbance of the low positive control was measured, and its value represents the cut-off value of the assay. Then, the absorbance of the test sample was substrated by the cut-off value to decide if the sample is positive or negative. Such decision was made according to the following:

Positive sample: absorbance cut-off value ≥ 0.15
Negative sample: absorbance cut-off value < 0.15

2.5 Statistical analysis

The data were statistically analyzed depending on the nature of the character, according to Snedecor and Cochran (21) using computer software SPSS version 22.

3 Results

Distribution of studied individuals according to age groups frequencies, Toxoplasmosis positivity and anti-Toxoplasma IgG seroprevalence among study groups were published previously (22).
Statistical analysis to investigate the differences between the toxoplasmosis positive and negative groups in the parameters of seminal fluid analysis show no significant differences between the two groups in all of the parameters (See Table 1).

TABLE (1)
SFA PARAMETERS ASSOCIATION WITH TOXOPLASMOSIS POSITIVITY

SFA parameter	Toxoplasmosis		P value
	Negative	Positive	
Volume/(ml)	2.88±0.15	2.68±0.23	0.473
Liquefaction time/(sec)	38.98±1.68	42.00±2.31	0.307
pH	7.55±0.04	7.46±0.04	0.222
Concentration/(ml)	29.00±3.75	39.35±5.29	0.122
Progressive motility/ (%)	26.00±3.53	36.15±5.06	0.109
Non-progressive motility/ (%)	10.50±1.49	13.15±1.86	0.301
Immotile/ (%)	30.32±3.93	35.60±5.02	0.437
Total progressive/ (%)	1.72±1.36	3.90±2.62	0.450
Morphology/ (%)	40.53±4.96	51.00±5.95	0.218
Agglutination	1.32±1.21	.00±0.00	0.676

4 DISCUSSION

Male infertility is a multifactorial syndrome encompassing a wide variety of disorders, and in more than 50% of infertile males, the cause of their infertility is unknown (i.e. idiopathic) and can be congenital or acquired; however, several factors are involved, and they include genetics, immunological and environmental factors, in addition to hormonal imbalance (23). Immunological factors have been suggested to be involved in the etiology of male infertility, and much concern has been focused on anti-sperm antibodies (ASAs) and cytokines, and their effects on semen quality have been questioned (24). Immune privilege in the testis is essential to maintain immunological tolerance to male germ cells during their development into spermatozoa, but immunity to sperm through the production of ASAs is thought to contribute to infertility, with 9-36% of infertility in couples being attributed to an immunological mechanism (25). In the current study the comparison between the basic seminal fluid parameters with the positivity of toxoplasmosis table (1), there were difficult to get significant differences at (p value ≤0.05) between parameters according to the positive and negative of toxoplasma infection, that might due to the number of selected patients, strain of T. gondii, age of patients, the nutritional state of patient even the genetic factor of the patients, and duration of toxoplasmosis infection.

We observed the M±SE of most seminal fluid parameters in positive toxoplasmosis higher than from these SFA parameters from negative toxoplasmosis Even if were within maximum normal value (38.98±0.15, 29.00±3.75, 26.00±3.53, 10.50±1.49, 30.32±3.93, 1.72±1.36, and 40.53±4.96) were values for negative toxoplasmosis to SFA parameters (Liquefaction Time, Concentration, Progressive Motility, Non-Progressive Motility, Immotile, Total Progressive, and Morphology, respectively) and the values of these SFA parameters in positive toxoplasmosis were (42.00±2.31, 39.35±5.29, 36.15±5.06, 13.15±1.86, 35.60±5.02, 3.90±2.62, and 51.00±5.95, respectively).

Also, attention toward others SFA parameters (volume and agglutination) when compared between negative toxoplasmosis and positive were shows mean± SE for negative (2.88±0.15, 1.32±1.21 respectively) higher than positive toxoplasmosis (2.68±0.23, 0.00±0.00 respectively). There are no adequate research about the effect of toxoplasma infection on SFA parameters, while (Lopez, Z.) (26) explained that the role of semen as a potential source of Toxoplasma infection has not yet been investigated. Previous experimental study on fertility and toxoplasmosis agreed with the study done by (Costa) whom inoculated boars with T. gondii tachyzoites and subsequently evaluated sperm motility, sperm concentration and sperm morphology, but they did not observe any changes to those characteristics due to toxoplasmosis (27).

5 CONCLUSIONS

The presented results confirming previously published studies, those investigate the role of toxoplasma gondii infection in alteration of seminal fluid characteristics, in which there are no impact of infection.

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