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Study the Role of Trichomoniasis in In Vitro Fertilization (IVF) Failure

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Abstract

Trichomoniasis is a sexual transmitted disease that affects human fertility. In women, trichomoniasis has been related to infertility due to pelvic inflammatory disease that compromises tubal patency, while in men, trichomoniasis may contributed to infertility by deficit of sperm cell quality and function due to physical damage. In this article, asymptomatic fifty couples with unexplained infertility, undergoing In Vitro Fertilization (IVF) were examined for Trichomonas vaginalis (T.V.) infection. In 70% of couples, embryo implantation failed and pregnancy was not achieved, probably as a consequence of trichomoniasis. The result indicates that T. vaginalis pathogenicity, adverse reproductive health outcomes, in time diagnosis, and treatment may improve implantation rate in patients with unexplained infertility undergoing Assisted reproductive technology (ART).

Key word : *Trichomonas vaginalis* , (IVF) Failure**,** infertility; sexual transmitted disease

Introduction

Trichomonas vaginalis is a globally occurring anaerobic/ microaerophilic protist parasite which colonizes the epithelium of the human urogenital tract [(1)]. Aside from human papillomavirus, trichomoniasis is the most common sexually transmitted infection worldwide, based on estimates of the World Health Organization (WHO) from 2016 [(2)], *T. vaginalis* infection , affect approximately 156 million people every year. Medical opinion has traditionally held that it plays little role in health complications in women, and it is rarely seen in men, the Centers for Disease Control and Prevention (CDC), reports that only 30 percent of people with trichomoniasis report any symptoms at all [(3)]. However, evidence has recently accumulated implicating *T. vaginalis* as a contributor to a variety of adverse outcomes among both sexes. Among both women and men, the association of *T vaginalis* with human immunodeficiency acquisition and transmission has been shown in multiple studies [(4, 5)]. Among women, trichomoniasis may play a role in development of cervical neoplasia, postoperative infections, and adverse pregnancy outcomes and as a factor in atypical pelvic inflammatory disease and infertility [(6, 7)]. Among men, trichomoniasis has emerged as a cause of nongonococcal urethritis and contributes to male factor infertility [(8)]. Published reports clearly show that the rate of trichomonas infections among infertile couples is higher than that among fertile ones [(9)].

The interaction of vaginal pathogens with epithelium and mucosa of the reproductive tract affects the immunological harmony needed for the success of embryo implantation. Nowadays, *T. vaginalis* is a latent pathogen in the reproductive tract, in this way is responsible of the adverse reproductive health outcomes in humans. Trichomoniasis proves the way for several bacterial intruders of the inflammation processes, thereby increasing the risk of failure in reproductive capacity and increasing the risk by 1.5-3 times of HIV and VPH acquisition [(10,11)]. *T. vaginalis* evades host immunity by the presence of adhesion proteins, cysteine proteases, and lipophosphoglycan molecules, all of which increase the pathogenicity of this intruder. The parasite adheres to the vaginal and cervical epithelial cells and triggers an immunosuppressive response from monocytes, macrophages, and dendritic cells. Also, *T. vaginalis* carries viruses and other parasites, such as mycoplasma

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and gardenella, causing chronic mucosal damage and an inflammatory reaction which gives rise to severe consequences in reproductive outcomes [12-15]. Goodman *et al*. [15] reported the presence of a Totiviridae viral family, which is a virus with a doubled stranded RNA that concurrently, is infecting the *T. vaginalis* parasite [15-17]. Infection of *T. vaginalis* by such a virus increases severely the immunological genesis of trichomona virulent factors by changes in its genome organization, protein coding, and replication signals. Trichomona virus increases the recurrence of the parasite infection and resistance to the metronidazole treatment [15,18,19-21].

Materials and Method

Ethics Statement: All human participants were enrolled in conformity with informed consent, privacy and confidentiality of patients who were sampled and analyzed anonymously during study (No. 53.2017).

Sampling:

From August 2017 through to August 2019, a prospective and case-control study at Dr. Khawer center for infertility and In vitro fertilization (IVF) in Erbil province of Kurdistan region of Iraq, were conducted. For research purpose only, fifty women under the age of 35 years, had normal body index, and nonsmokers and their spouses (normal seminal fluid analysis according to the world health organization parameters (WHO) at 2010), [22] with unexplained infertility, were enrolled in this study. High vaginal swab and semen sample had been collected from the participate couples.

DNA extraction and PCR:

The genomic DNA was extracted by the extraction kit gSYNC^{TM} (Geneaid England) directly from high vaginal swab, following the manufacturer's instructions.

PCR steps

The primer set targeting beta tubulin gene 9/2 which is a conserved region found in all of *T. vaginalis* (gene bank accession No. L05470) were used to amplify a 112 bp piece of the gene, the sequence of the primers.

Forward primer sequences (5'–3'): CAT TGA TAA CGA AGC TCT TTA CGA T

Reverse primer sequences (5'–3'): GCA TGT TGT GCC GGA CAT AAC CAT

PCR protocol:

The PCR master mixture prepared at final volume of 20 µl in 0.2 mL PCR tubes. Component and their volume are explained in Table (1).

Table (1): the PCR construct master mixture for amplification of (BTUB) gene:

Reagents	Volume for 1X
Master mix(Prime taq Primemix)	10.0 µl
Forward primer	1.0 µl
Reverse primer	1.0 µl
Nuclease free water	4.0 µl
DNA	4.0 µl
Total	20 µl

The PCR amplifications were performed on a program according to the cycle

The construct reaction conditions for (BTUB) as shown in table (2):

Table (2): the construct reaction conditions for (BTUB):

Stage	Temperature	Time	Cycles
Initial Denaturation	95	5 minutes	1 cycle
Subsequent denaturation	95	30 second	10 cycles
Annealing	(64-55)	30 second	
Extension	72	1.0 minute	
Subsequent denaturation	95	30 second	40 cycles
Annealing	55	30 second	
Extension	72	1.0 minute	
Final Extension	72	10 minutes	1 cycle
	4	To time end	

A 3 µl volume of each reaction was subjected to electrophoresis on 3.0 % agarose gel in Tris-acetate-EDTA buffer pH 8.5 (45 min at 10 V/cm) and staining

with safe dye. The DNA bands were visualized under UV light (transillumenator).The size of the amplified products was assessed by comparison with a commercial weight marker, Smart Ladder (50 bp) GeneDiex|Korea and photographed by digital camera.

PCR products sequencing:

To reconfirm the PCR results, 10 amplified PCR products were directly sequenced by targeting Beta tubulin gene in both directions using, the Big Dye Terminator method. Sequencing was performed bi-directionally using the same primers used in PCR amplification of the beta tubulin genes (Macrogen /South Korea). The obtained sequences were aligned (by using Bio edit software) with normal sequence from GenBank.

Results and Discussion

Unfortunately, all the examined women for trichomoniasis were positive by detection of BTUB gene and 40% of their spouses also. Only 30% of these women successfully achieve pregnancy, as shown in table 3.

Table (3): the percentage of trichomoniasis in males and females and the successful percentage of IVF.

Gender	T.V. infection percentage	IVF percentage	IVF success percentage	IVF failure percentage
Female	100%	100%	30%	70%
Male	40%			

Although, couples that enrolled in this study were chosen very carefully, to overcome the IVF failure causes and to insure getting good eggs quality (eggs without chromosomal changes) and quantity, not defective embryo (that can implanted successfully in the uterus), yet it has long been regarded as a sexually transmitted infection of minor importance, trichomoniasis is not routinely screened in asymptomatic patients and the infection can persist for 3-12 months in the genital tract. [23-26]. Patients with trichomomiasis are asymptomatic in 70-100% of male cases *versus* 35-85% of female cases [23], however, the asymptomatic women may also experience, at least part of pathological signs, could be due to the genetic diversity of the parasite strains to express virulence factors, which increase cytoadherence, cytolysis and cell detachment [27].

Additionally, previous studies have reported that trichomoniasis has high rates of reinfection due to resistance of the parasite to metronidazole treatment [18, 26] making this microorganism a serious reproductive tract pathogen.

The *T. vaginalis* protozoan attaches to vaginal epithelial cells through its barbed tail, membrane expression of surface protein p270, secretion of proteases and a cell-detaching factor, leading to an intense host inflammatory response, inducing local cytotoxic effects, genital tract damage, and reproductive effects [28,29]. Because, trichomonas has been been isolated from fallopian tubes, peritoneal fluid, and the pouch Douglas, scientists suggesting that motile trichomonas may be able to invade the whole genital tract [30,31]. There are several studies that conclude that *T. vaginalis* causes urogenital damage to different types of cells and tissues, such as connective and muscular tissues, due to an excessive cytotoxic local effect suggesting high risk of reproductive failure [31-37]. Trichomonas bind to the cells inducing membrane retraction, cell blebbing, and apoptosis. These changes of cell architecture can be evidenced under microscopy and characterized by condensed chromatin and intense cytoplasm vacuolization [37]. In this study, the female partners did not report any infectious symptom and, therefore, they were not tested for *T. vaginalis*, but they might have been affected with latent trichomoniasis. They are an examples of asymptomatic patient who obtained a negative outcome of implantation failure after ART. More than 20% of women with trichomoniasis have a chronic inflammatory process in the reproductive tract and that may explain the embryo implantation failure after ART procedure. Consequently, the clinical implication of embryo implantation failure depends on both the quality of the embryo and the receptivity of endometrium mainly marked by the correct and exact immune environment needed for a successful pregnancy.

On the other hand, men infected with *T. vaginalis* display abnormal motility of the spermatozoa and high semen agglutination. A relevant tropism from *T. vaginalis* microorganism to the head or flagella of the human spermatozoa has been reported. The adhesion of *T vaginalis* and sperm affects sperm motility followed by phagocytosis, lysis, and digestion of sperm cell [38, 39]. In 2008, Benchimol *et al*.

[40] report that, after one hour of interaction between *T. vaginalis* and sperm cells in an *in-vitro* environment, 75% of the sperm cells were immotile or dead. Additionally, cyto-adhesion and phagocytic activity of trichomonas to ingest and digest spermatozoa in an *in-vitro* environment suggest a similar behavior in an *in-vivo* environment as a cause for decreasing numbers of motile sperm directly affecting reproductive success. Latent trichomoniasis infection could be the cause of the unexplained infertility in men, since sperm damage evidenced by severe asthenozoospermia.

This search showed that, Couples with unexplained infertility must be widely screened and tested for sexually transmitted pathogens to ensure adequate conditions of the female reproductive tract needed to achieve pregnancy. Further studies are required to accurately fill the gap of knowledge between trichomoniasis, unexplained infertility, and implantation failure.

In conclusion: The result indicates that T. vaginalis pathogenicity, adverse reproductive health outcomes, in time diagnosis, and treatment may improve implantation rate in patients with unexplained infertility undergoing Assisted reproductive technology (ART).

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

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