



Serum Levels of Prolactin and Complement Components (C3 and C4) in Women Infected with *Trichomonas vaginalis*

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Abstract: *Trichomonas vaginalis* (TV) is a sexually transmitted anaerobic protozoan parasite. In women, the infection frequently associated with infertility. However, the exact effect of the parasite that leads to infertility is a debate issue. This study aimed to determine the impact of TV infection on serum levels of prolactin (PRL), C3 and C4 and the correlation of these parameters with the infertility. A total of 142 women were investigated for TV infection and infertility. Serum levels of PRL, and complement (C3 and C4) were estimated using mini Vidas and single radial immune diffusion assay respectively. Infertile women positive for TV had significantly higher serum levels of PRL than infertile women negative for TV, while serum levels of C3 decreased significantly in both fertile and infertile TV-infected women compared with their counterparts of non-infected women. Thus, it can be concluded that increased serum levels of PRL could be incriminated as a cause of infertility in TV-infected women.

Keywords: *Trichomonas vaginalis*, prolactin, complement, women infertility.

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Introduction

Trichomonas vaginalis (TV) is one of the most common pathogenic protozoan of human with annually estimated 180 new infections worldwide. Furthermore, trichomoniasis (the disease caused by the protozoan) accounts for almost half of all curable sexually transmitted diseases (STDs) in the world (1). This ranks the disease first among STDs in countries with low prevalence of acquired immunodeficiency syndrome (AIDS).

Trichomoniasis affects both men and women; however, it causes symp-

tomatic disease almost exclusively in women (2). Adaptive immune response to TV seems to be only partially effective since the reinfection rate could reach 36% on follow up studies (3). On the other hand, inflammatory response predominated by neutrophils and mast cells could be the most reliable mechanism of host defense against this infection (4). The exact mechanism of how these cells accumulate and mediate TV killing after acute infection is not fully understood. Complement components are known as

chemoattractant and activators of neutrophils in such circumstances (5), with the alternative pathway is almost the predominant pathway (6).

Prolactin (PRL) is a 23 kDa polypeptide hormone secreted by the lactotroph cells of the anterior pituitary gland (7). It is a potent immune-modulator that exerts stimulatory effects on immune cells. Protective effect of PRL has been documented in vivo in many protozoal infections. Dzitko *et al.*, (8) found that women with hyperprolactinemia had low sero-prevalence of anti-toxoplasma antibodies than those with normal PRL. Among the potential sequelae of TV infection are infertility (9, 10), and cytological abnormalities of the cervix (11). The exact mechanism by which TV affect women fertility is not well-understood, and previous studies mainly focused on the direct effect of TV by products on the sperm rather than the effect of the parasite on infected female. We hypothesized that TV induces a hormonal as well as immunological changes in the infected female. Accordingly, we carried out this study which aimed to investigate the impact of TV infection on serum level of PRL, C3, and C4 and association of these parameters with infertility of infected female.

Materials and Methods

A nested case-control study was performed which involved a total of 142 non-pregnant women (age range 15-47 years, mean=31 years) who were attending Gynecology department /Al-Imamain Al-Kahadhumian Medical City and The High Institute of Infertility Diagnosis and Assisted Productive Technologies/ Baghdad during the period from February, 2013 to April,

2014. Cases included in this study were assured not to have taken oral or topical metronidazole during 4 weeks prior to specimens' collection. This study was approved from the Ethic Committee/ College of Medicine / Al-Nahrain University. The status of infertility was determined according to the World Health Organization (WHO) which is failure of women in a reproductive age to achieve clinical pregnancy after 12 months or more of regular un protected intercourse (12).

Samples

1- Vaginal swabs: Vaginal swabs were collected from women who were placed in a lithotomy position and the sterile metal speculum was inserted into vagina without any lubricant or solution. Swabs were taken from the posterior fornix. The swab was pressed between the In-Pouch TV System media (Biomed Diagnostics, USA) walls. Then the swab discarded and the top edge of In-Pouch media chamber was folded down and rolled three times. A wire tape was folded at the end tabs behind the pouch, and the In-Pouch medium was incubated at 37 °C for three days. The culture was examined every day for three successive days before being considered negative (13). White sediment along the side and bottom of the chamber followed by darkening of the media was considered positive result.

2- Blood samples: Five ml of venous blood was obtained from each participant. Serum was separated from these samples and kept at -20C until be used.

Serum Levels of Prolactin

A ready kit (BioMerieux/ France) was used for determination of serum levels of PRL. Mini vidas technique was used according to the manufacturer's manual. Briefly, 200 μ l from each serum sample was transferred into the well containing alkaline – phosphatase labeled antiprolactin (conjugate) of the strip. The Solid Phase Receptacle (SPR) was placed in the mini Vidas.

The sample-conjugate mixture was then automatically cycled in and out of the SPR several times. Prolactin in the serum sample binds to both the antibodies on the SPR and to the conjugate forming a sandwich. In the final detection step, the substrate (4-methyl-umbelliferyl phosphate) was cycled in and out the SPR where the conjugate enzyme catalyzed this substrate into a fluorescent product (4-methyl-umbelliferone). The intensity of fluorescence was measured at 450 nm. This intensity is proportional to the concentration of PRL in the sample. Results were automatically calculated by the instrument in relation to the calibration curve.

Serum Levels of C3 and C4

Single radial immune-diffusion assay (LTA,15LF-Italy) was used to estimate serum levels of C3 and C4 with normal reference values for C3 (91-156 mg/dl) and for C4 (20-50 mg/dl). Briefly, wells of the plate were filled with 5 μ l of samples. The plate was then closed and placed in a moist chamber. After 72 hours of incubation the precipitating was measured to the closest 0.1mm. These scores were converted into concentration depending on the conversion table supplied with the kit.

Statistical Analysis

Values are expressed as mean \pm standard deviation (SD). The Statistical Package for the Social sciences (SPSS, version 14.0) was used for statistical analysis. T-test for independent samples was used to compare the means of different parameters between each two groups. The acceptable level of significant was p -value < 0.05 .

Results

Out of 142 women, 47 (33.09%) were found to be positive for TV infection. Accordingly, the study population was divided into the following groups:

Group A: Women who were fertile and positive for TV infection (23 women).

Group B: Women with primary or secondary infertility and positive for TV infection (24 women).

Group C: Women who were fertile and negative for TV infection (13 women randomly selected from 49 women).

Group D: Women with primary or secondary infertility and negative for TV infection (15 women randomly selected from 46 women).

Serum Levels of Prolactin

Generally women infected with TV (both fertile and infertile) had higher serum levels of PRL than non-infected counterpart women; however the differences were not always significant. Mean serum level of PRL among fertile women positive for TV infection was 23.19 ± 17.53 ng/ml which did not differ significantly from that of non-infected fertile women (17.18 ± 9.2 ng/ml) ($t=1.145$, $P=0.26$). On the other hand, infertile women positive for trichomoniasis showed significantly

higher mean of PRL serum level (28.19±17.53 ng/ml) than that of non-infected infertile women (12.83±6.917ng/ml) ($t=2.063$, $P=0.049$) (Table 1).

Table 1: serum levels of prolactin, C3 and C4 in different groups

Groups	Prolactin (mean±SD)	C3 (mean±SD)	C4 (mean±SD)
Fertile women positive for TV	23.19±17.53 ng/ml	110.12±39.25 mg/dl	21.65±9.03 mg/dl
Infertile women positive for TV	28.19±17.53 ng/ml	97.25±43.33 mg/dl	21.63±8.95 mg/dl
Fertile women negative for TV	17.18±9.2 ng/ml	147.51±49.01 mg/dl	147.51±49.01 mg/dl
Infertile women negative for TV	12.83±6.917ng/ml	137.65±42.53 mg/dl	24.39±9.0 mg/dl

TV: *Trichomonas vaginalis*

SD: standard deviation

Serum Levels of C3

Table 1 shows means of C3 serum levels in different groups. Infected women (either fertile or infertile) showed lower serum levels of C3 than their counterparts non-infected women. Mean serum level of C3 in fertile infected women was 110.12 ±39.25 mg/dl which was significantly lower than that of non-infected fertile women (147.51±49.01 mg/dl) ($t=2.357$, $P=0.028$). Similarly, mean serum level of C3 for infertile infected women and non-infected women were 97.25±43.33 mg/dl and 137.65±43.33 mg/dl respectively with significant difference ($t=2.865$, $P=0.008$).

Serum Levels of C4

Although non-infected groups showed higher serum levels of C4 than infected ones, the differences were not significant. These levels were 21.65±9.03 mg/dl and 24.12±9.2 mg/dl

in fertile and non-fertile women respectively ($t=0.779$, $P=0.443$), and 21.63±8.95 mg/dl and 24.39±9.0 mg/dl in infertile infected and non-infected women respectively ($t=0.933$, $P=0.358$) (table1).

Discussion

Growing body of evidences support the role of TV infection in women infertility (10). The current study aimed to investigate the possible mechanisms employed by this parasite which lead to infertility. Different mechanisms have been suggested such as sperm killing by the parasite's byproducts (14), and decrease and increase in sperm motility and deformity respectively (15,16). However, the implication of this infection with hormonal disturbance received less attention. Our study focused on the association of TV infection with women infertility from hormonal and immunological aspects.

Interestingly, the results partially proved such hypothesis, and infertile TV-infected had significantly higher serum level of PRL than infertile non-infected women.

The PRL is one of the most effective hormones in women fertility and conception. Hyperprlactinemia in women, which is an elevation of PRL serum levels beyond 25ng/ml (17), is associated with women infertility. Hyperprlactinemia in TV-infected women can be explained by the indirect effect of the infection on dopamine. This hormone mediates inhibition of PRL secretion through binding to D2 receptors on lactotroph cells membrane, and inducing several signal transductions resulting in inhibition of *PRL* gene transcription (7). Thus, inhibition of dopamine is the main physiologic control of PRL secretion. Histamine is considered as one of the most effective compounds that inhibits dopamine secretion (18). Many reports have pointed out the accumulation and increased activity of mast cells in TV-infections (4,19).

Consequently, the possible mechanism of hyperprlactinemia in TV infection is the increased releasing of histamine (and may be other compounds) from mast cells which inhibits dopamine, and thus release the secretion of PRL for pituitary gland.

The present study revealed a reduction in the two main complement components in TV-infected women compared with two non-infected. This reduction was significant for C3 and non-significant for C4. These results can be explained from two aspects. Firstly, the impact of TV infection on serum levels of complement as whole, and secondly why there was significant reduction in C3 but not C4. Regarding

the first aspect, women enrolled in this study are non-pregnant, and the menstrual blood of infected women supplies adequate amount of iron for the parasite which is known for its relatively high requirement for iron (20). This iron facilitates the growth and multiplication of TV, and also it regulates trichomonad adhesion and cysteine proteases (21). These two factors cause degradation of complement which binds to the surface of the parasite (6), and eventually reduction in serum level of complement. Because of their pivotal role in the complement cascade, C3 and C4 are consumed by activation of both classical and alternative pathway. However, low levels of C3 accompanied by normal levels of C4 suggest the activation of alternative pathway (22). In fact, it is well documented that the alternative pathway of complement activation is almost the most common pathway for fighting TV (6). This pathway increases the consumption of C3 but not C4 (23). This explains the significant reduction in C3 not C4 in TV-infected women compared with non-infected.

As both fertile and infertile TV-infected women differed from their counterparts non-infected women regarding C3 and C4, it is nonsense to assume that decreased levels of either C3 or C4 have an association with fertility status of infected women.

One limitation of this study is the relatively small number of TV-infected women which does not allow drawing a solid conclusion about the role of PRL as a proposed cause for infertility in such infection. Another limitation is the probability of coinfection of TV with other sexually transmitted infections (STIs) which was not ruled out because

some technical limitations. Thus a study using larger number of subject with ruling out other STIs is required for more confident conclusion.

However, it is plausible to conclude that TV infection causes increase in serum level of PRL which involves in infertility of infected women.

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