

Full Length Research Paper

Levels of tumor necrotic factor alpha and interleukin-10 in vaginal discharge of women with infertility disorders and infection

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The mucosal immune system of the female reproductive tract is uniquely adapted to facilitate specialized physiological functions whilst eliminating threatening sexually transmitted and environmental pathogens. In the present study, tumor necrotic factor alpha (TNF- α) and interleukin (IL-10) were evaluated in vaginal discharge of infertile women infected with *Chlamydia trachomatis* and *Candida albicans*. A total of 70 women were used in this study when they came for medical consultation at the infertility clinic of Kammal Al-Sammaree Hospital and also at private gynaecology clinics in Baghdad city. For 26 women with infertility disorders, six of them were found infected with *C. albicans* and 20 had *C. trachomatis* infection. Another 24 women were fertile including 17 infected with *C. albicans* and 7 had *C. trachomatis* infection. In addition, 20 healthy women were selected as control group. Vaginal samples were collected and assessed for TNF- α and IL-10. High vaginal swaps were taken for wet amount, Gram stain and culture. Elevated levels of TNF- α and IL-10 were detected in the vaginal discharge of women with infertility disorders and infected with *C. albicans* as well as *C. trachomatis*, and fertile infected women as compared to the control group ($p= 0.0005$).

Key words: *C. albicans*, *C. trachomatis*, IL10, TNF α , female infertility.

INTRODUCTION

Infectious agents could impair various important human functions, including reproduction. Bacteria, fungi, viruses and parasites are able to interfere with the reproductive function in both sexes (Pellateia et al., 2008). The lower female genital tract provides a barrier against infectious microorganisms by a selected population of immune cells which are capable of producing a large array of cytokines

depending on the nature of the stimuli as these cytokines were thought to have an important role in intracellular communication underlying immune defense and reproductive processes (Anderson, 1996; Fichorova and Anderson, 2000).

Candida albicans is the most common form of mucosal fungal infection. The consequence of fungal infection, such as colitis and endometritis might cause infertility (Kranjcic-Zec et al., 2004). Local immunity is important in host defense against this infection accordingly cytokines are produced by vaginal epithelial cells at various levels quantitatively with considerable levels of TNF- α , and IL-

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10 (Steele and Fidel, 2002).

Chlamydia trachomatis is a major cause of sexually transmitted diseases in developing countries (Schachter, 1999). It is an obligate intracellular gram-negative bacteria which selectively colonized epithelial cells in the human host. Infection is insidious and, though often asymptomatic, could have serious consequences particularly for women. In some cases of cervical infection, the organism ascended into the upper genital tract, causing pelvic inflammatory disease with a sequel that included infertility (Paavonen and Eggert-Kruse, 1999).

C. trachomatis infection was thought to be immunologically mediated, resulting in local recruitment of lymphocyte subsets and inducing the production of cytokines (Reddy et al., 2004), leading to induction of an inflammatory response that was crucial in resolving acute infection but might also played a key role in the pathogenesis of *C. trachomatis* associated infertility (Öhman et al., 2006). The study was aimed at isolating and identifying *C. albicans* and *C. trachomatis* which are associated with vaginal infection in women patients, and evaluating the levels of TNF- α and IL-10 cytokines in vaginal discharge of women positively infected with *C. albicans* and *C. trachomatis*.

MATERIALS AND METHODS

Patients and methods

Patients group consisted of 50 females who attended the Infertility clinic of Kammal Al- Sammaree Hospital and also the private gynaecology clinics in Baghdad city from September 2009 to October 2010. Their ages ranged from 18 to 48 years (median 31 years). A detailed clinical questionnaire was done to each patient for collecting information on reason for referral, gynaecology history including menstruation, symptoms of genital and urinary tract infection, obstetrics and medical histories including recent history of treated sexually transmitted disease (STD) infection. The exclusion criteria were included the recent antibiotic therapy, infertility cases related to a male factor and a history of recent treated STD infections. Vaginal swabs were taken by a gynaecologist from the posterior fornices of vagina with use of sterile speculum of all participants for diagnosis of *C. albicans* and *C. trachomatis*. Two cotton swabs of vaginal discharge was drawn into sterile tubes with 2 ml of sterile phosphate buffered saline (PBS) pH 7.2 and kept at -20°C until used for cytokine assayed (Mittal et al., 1995). Swabs were stained by Gram's method and examined microscopically for yeast cells and hyphae of *Candida*. In addition, vaginal material was inoculated onto Sabouraud's medium for culturing *Candida*, followed by conformation test using API Ca system. *C. trachomatis* was diagnosed according to clinical practice guidelines for the detection of *C. trachomatis* suggestively, presumptively, followed by serological test (Black, 1997).

The patients were classified into the following groups depending on clinical examination and laboratory diagnosis: Group A: Patients were positively infected with *C. albicans* (n=23). This group was further subdivided into two subgroups according to fertility status; infertile (n=6) and fertile women (n=17). According to the definition of WHO, infertility is the inability to conceive a child. A couple may be considered infertile if, after two years of regular sexual

intercourse, without contraception, the woman has not become pregnant (and there is no other reason, such as breastfeeding or postpartum amenorrhoea). Primary infertility is infertility in a couple who have never had a child. Secondary infertility is failure to conceive following a previous pregnancy. Infertility may be caused by infection in the man or woman, but often there is no obvious underlying cause (WHO, 2010). Group B: Patients positively infected with *C. Trachomatis* (n=27), who were also subdivided into two subgroups, infertile (n=20) and fertile patients (n=7); Group C: Control group which consisted of 20 non- pregnant fertile multipara healthy women matched for age.

Cytokines were detected in vaginal secretion by sandwich ELISA using (BIOTECH, USA) kits for TNF- α and IL-10 according to the manufacturer's instructions.

Our study was carried out according to the ethical standards for human experimentation. The permission of medical ethics committee was obtained for all groups (infertile, fertile, and healthy controls). The study was approved by the Al- Kindy College of Medicine and informed consent was obtained from the patients and controls.

Statistical analysis

To determine the statistical significance differences of variables, SSPS system was used with ANOVA test followed by LSD value. P values less than 0.05 were considered as statistically significant while P-value more than 0.05 was considered as statistically not significant.

RESULTS

Cytokine levels in vaginal secretion

This study was designed to evaluate cytokine levels in vaginal secretion of women with infertility disorders and infection. TNF- α and IL-10 were measured locally in vaginal secretion using ELISA technique.

There was a significant elevation of mean vaginal levels of TNF- α in *C. albicans* positive infertile women as compared to its level in *C. albicans* positive fertile women and normal control group (p= 0.005) (Table 1 and Figure 1). In addition, higher levels of vaginal TNF- α was detected in *C. trichomatis* positive infertile women as compared with the corresponding levels in *C. trichomatis* positive fertile women and normal group. These results scored significant at p= 0.0005 level as demonstrated in Table 1 and Figure 2.

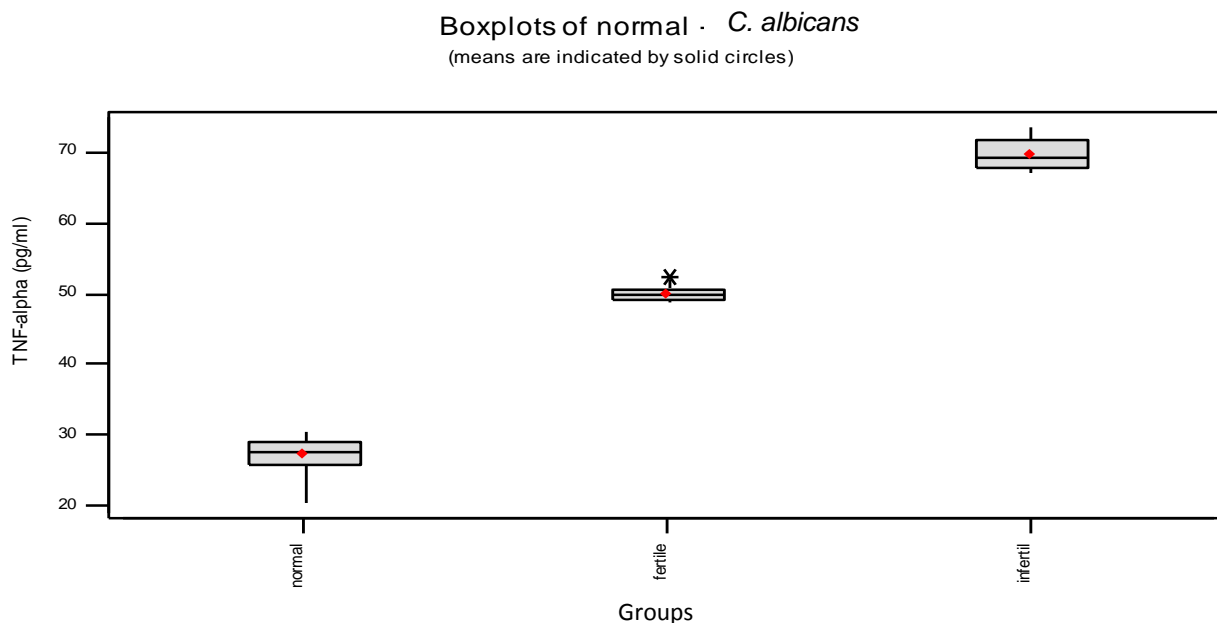
The vaginal discharge of *C. albicans* positive infertile women showed significant increased levels of IL-10 as compared with the corresponding levels in *C. albicans* positive fertile women and normal women (p= 0.0005) as shown in Table 2 and Figure 3, but this result failed to reach the significant level between fertile infected group and normal group (p= 0.172).

On the other hand, there was a significant difference between three groups in the case of vaginal IL-10 levels in *C. trichomatis* positive infertile women and *C. trichomatis* positive fertile women as compared to control group (p= 0.0005) (Table 2 and Figure 4).

Table 1. Vaginal levels of TNF- α in women positively infected with *C. albicans* and *C. trichomatis* and normal groups.

| Group | Sub group | Number | TNF- α (pg/ml) | |
|-----------------------|-----------|--------|-----------------------|--------|
| | | | Mean | SE |
| <i>C. albicans</i> | Infertile | 6 | 69.66 | 0.83* |
| | Fertile | 17 | 50.03 | 0.26 |
| <i>C. trichomatis</i> | Infertile | 20 | 100.21 | 0.7* |
| | Fertile | 7 | 67.76 | 1.48 |
| Healthy controls | | 20 | 27.34 | 0.54§† |
| Total | | 70 | | |

SE, Standard error; *P= 0.0005, *C. albicans* positive infertile patients vs *C. albicans* positive fertile patients; †P= 0.0005, *C. trichomatis* positive infertile patients vs *C. trichomatis* positive fertile patients; §P= 0.0005, controls vs *C. albicans* positive infertile patients and *C. albicans* positive fertile patients; †P= 0.0005, controls vs *C. trichomatis* positive infertile patients and *C. trichomatis* positive fertile patients.

**Figure 1.** TNF- α level (pg/ml) in controls, *C. albicans* positively fertile women and in *C. albicans* positive infertile women.

Relation of TNF- α and IL-10 levels with the infected microorganisms and fertility status

To find out any relation between the vaginal levels of studied interleukins and the image of fertility status of those women infected with *C. albicans* and *C. trichomatis*, and those who were normal, ANOVA test with LSD was applied.

Concerning the TNF- α level in vaginal secretion, our results represented in Figure 5 show a significant increase of TNF- α levels in women infected by *C. trichomatis* compared with the patients infected by *C.*

albicans; both had infertility disorders (100.2 ± 0.7 vs. 69.66 ± 0.83 pg/ml respectively). Both results scored as significant levels of $p = 0.0005$ when compared to the control group (27.34 ± 0.54 pg/ml). Additionally, there was a significant difference in relation between fertile groups infected by *C. trichomatis* (67.76 ± 1.48 pg/ml) and *C. albicans* (50.03 ± 0.26 pg/ml) as compared with the control group ($p = 0.0005$) as shown in Figure 5. There was a significant difference between *C. trichomatis* infected women and *C. albicans* infected group; both had infertility disorders as compared to control group regarding IL-10 level (128.14 ± 0.83 , 49.32 ± 1.96 , and

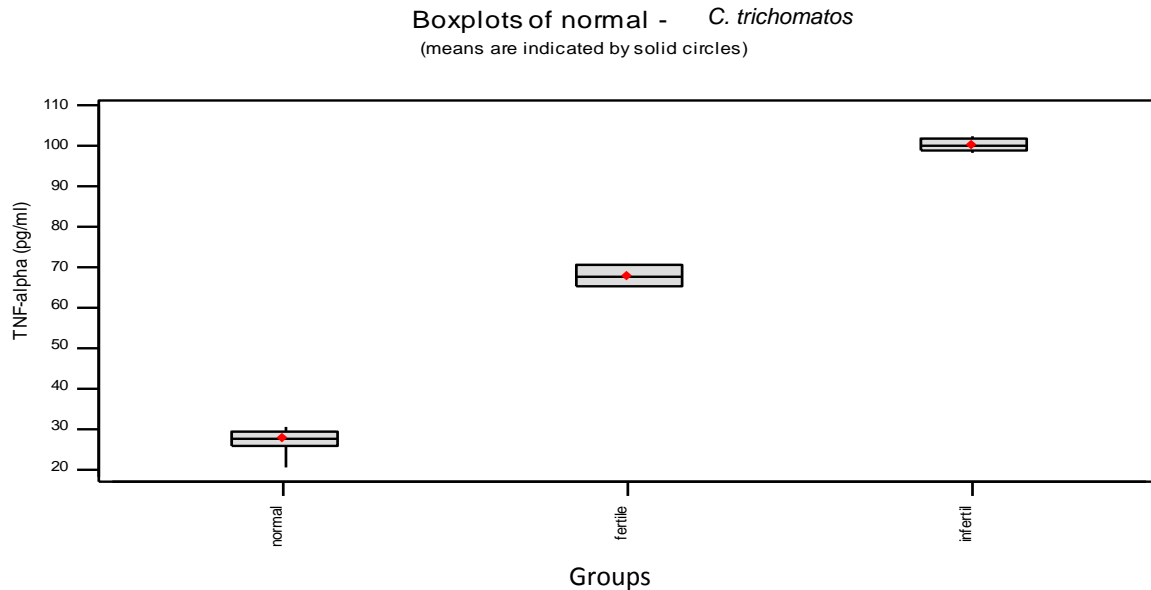


Figure 2. TNF- α level (pg/ml) in controls, *C. trichomatis* positive fertile women and in *C. trichomatis* positive infertile women.

Table 2. Vaginal levels of IL-10 in women positively infected with *C. albicans* and *C. trichomatis* and normal groups.

| Group | Sub group | Number | IL-10 (pg/ml) | |
|-----------------------|-----------|--------|---------------|--------|
| | | | Mean | SE |
| <i>C. albicans</i> | Infertile | 6 | 49.32 | 1.96* |
| | Fertile | 17 | 12.08 | 0.31 |
| <i>C. trichomatis</i> | Infertile | 20 | 128.14 | 0.83* |
| | Fertile | 7 | 63.18 | 1.27 |
| Healthy controls | | 20 | 11.05 | 0.29§† |
| Total | | 70 | | |

SE, Standard error; *P= 0.0005, *C. albicans* positive infertile patients vs *C. albicans* positive fertile patients; *P= 0.0005, *C. trichomatis* positive infertile patients vs *C. trichomatis* positive fertile patients; §P= 0.0005, controls vs *C. albicans* positive infertile patients †P= 0.0005, controls vs *C. trichomatis* positive infertile patients and *C. trichomatis* positive fertile patients.

11.05 \pm 0.29 pg/ml respectively, p=0.0005) as shown in Figure 6. The same hold true, a significant difference (p= 0.0005) appeared in relation between fertile group infected by *C. trichomatis* (63.18 \pm 1.27 pg/ml) and *C. albicans* (12.08 \pm 0.31 pg/ml) after comparison with the control group (11.05 \pm 0.29 pg/ml) as shown in Figure 6.

DISCUSSION

Little is known about genital mucosal immune responses

to infection in women with or without sequel. This study was designed to assess the potential to stimulate putative vaginal associated immunity in women with vaginal infection mainly by *C. albicans* and *C. trichomatis* and evaluated the levels of cytokines in vaginal secretion of women who had infertility disorders.

In the present study, higher levels of TNF- α and IL-10 were detected in the vaginal secretion of *C. albicans* and *C. trichomatis* positive infertile women than positively infected fertile women. TNF- α which displayed anti-Chlamydia properties was also known to play an

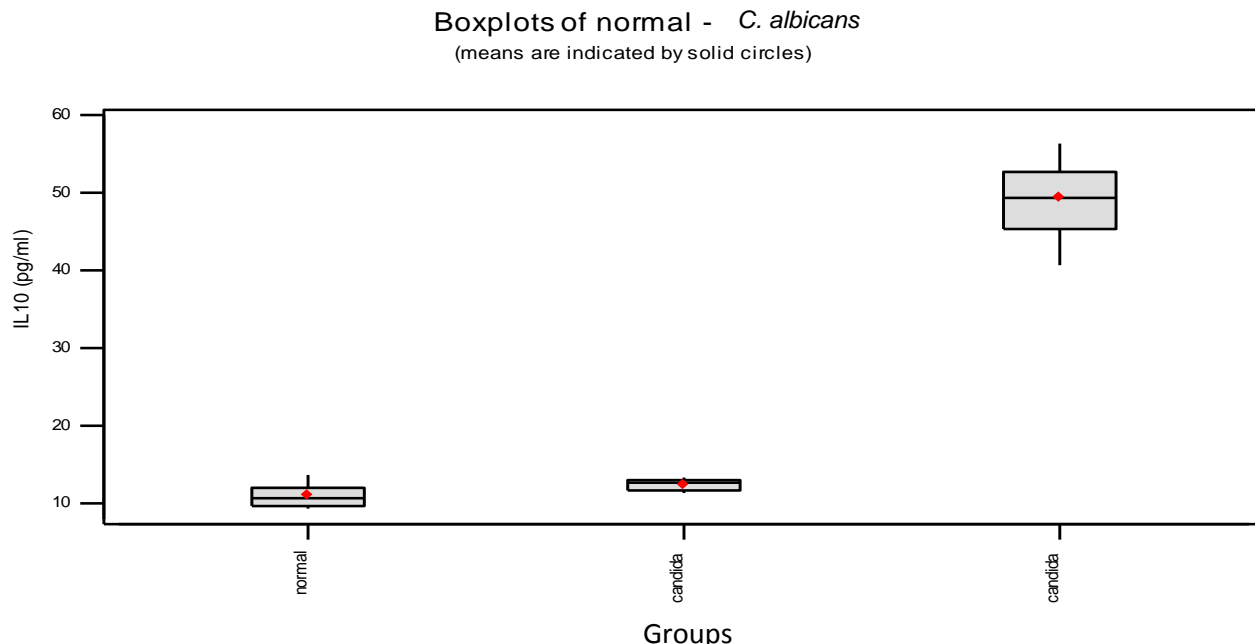


Figure 3. IL-10 level (pg/ml) in controls, *C. albicans* positive fertile, women and in *C. albicans* positive infertile women.

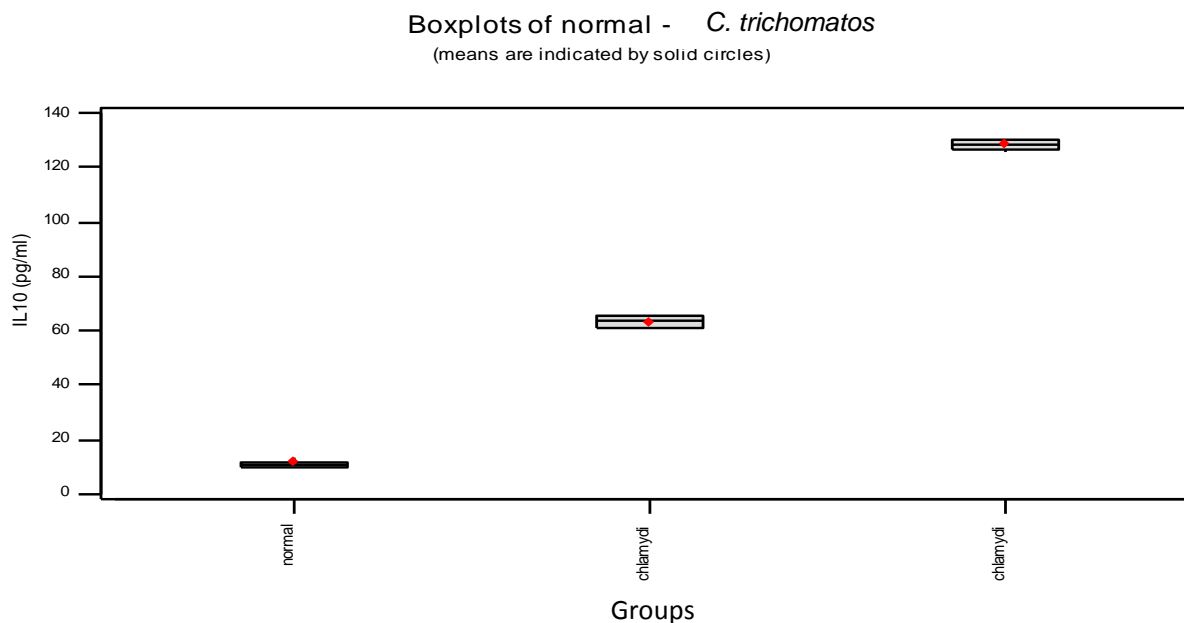


Figure 4. IL-10 level (pg/ml) in controls, *C. trichomatis* positive fertile women and in *C. trichomatis* positive infertile women.

important role in the initiation of inflammatory responses (Shemer-Avni et al., 1988). In the mouse genital tract, infertility associated with endometriosis had been shown to be related to the production of TNF- α (Darville et al., 2000). Proinflammatory cytokines were also known to

derive the lipid peroxidation of the spermatozoa plasma membrane to the levels that could affect the sperm fertility capacity (Martínez et al., 2007). Estrada et al. (1997) found that both TNF- α and IFN- γ had effects on sperm motility, viability, membrane integrity and lateral

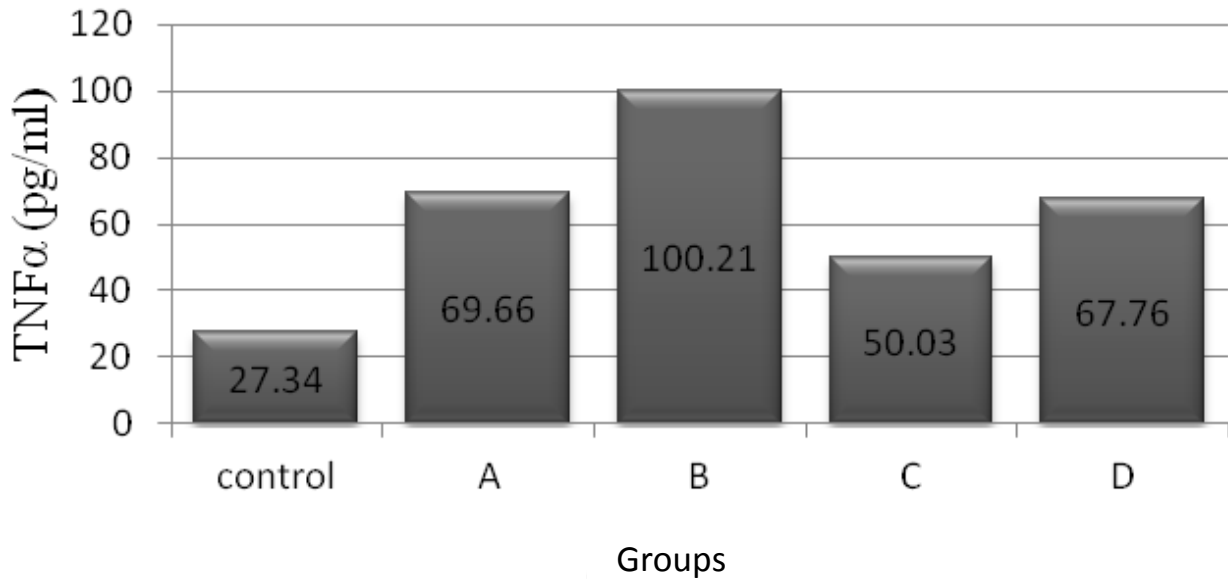


Figure 5. Mean vaginal secretion level of TNF- α . A, infertile women positively infected with *C. albicans*; B, infertile women positively with *C. trichomatis*; C, fertile women positively infected with *C. albicans*; D, fertile women positively with *C. trichomatis*.

head displacement, suggesting poor fertilizing potential of human spermatozoa during inflammatory conditions.

TNF-alpha had anti reproductive effects (Arck et al., 1997) exerting an autocrine activation (Fukui et al., 2008). Thum et al. (2007) had found that elevated level of TNF-alpha and altered Th1/Th2 cytokines expression are associated with high levels of activated NK cells and this may subsequently exert a negative impact on reproduction.

Our data are consistent with the previous studies in which higher serum levels of IL-10 were detected in women positively infected with *C. trichomatis* (Cohen et al., 1999) and in the serum of women positively infected with *C. albicans* (Hashim, 2010). IL-10 is known to selectively suppress Th1-mediated cellular immunity by inhibiting the production of inflammatory cytokines such as IFN- γ , TNF- α and IL-1 (Mosmann and Moore, 1991) and there was a decreased production of IL-10 by decidual T cells of women with impaired infertility when compared with decidual cells of fertile woman (Piccini et al., 1998). IL-10 is an anti-Th1 cytokine produced during genital Chlamydial and Candida infection. It had been previously suggested that the existence of differences in the vaginal milieu between women with recurrent vulvovaginitis and the control group was associated with the expression of IL-10 (Giraldo et al., 1999). Several cytokine genes were known to be linked with a protective Th1 response and chemotaxis and activation of macrophages, neutrophils, and lymphocytes *in vivo* were up-regulated during experimental infection of mucosal surfaces by *C. albicans*. It was found that the expression

of IL-1, IL-6, IL-8, IL-10, GM-CSF, IFN- γ , and TNF- α , were all up-regulated, suggesting that vaginal mucosa was an important source of proinflammatory cytokines in response to *C. albicans* (Taylor et al., 2000; Schaller et al., 2002; Steele and Fidel, 2002).

Yang et al. (1996) found that increasing susceptibility to infection by Chlamydia was associated with shifting away from the production of IFN- γ towards the production of IL-10, as it was conceivable that the temporary or inadequate immunity commonly induced against Chlamydia might be due to the negative immunoregulation of IL-10 (Stephens et al., 1999), causing the production of relatively low frequency of chlamydial-specific Th1 cells. The enhanced levels of IL-10 might not be acting as anti-inflammatory mediator and might be involved in prolonging the infection by exerting immunostimulatory effects (Conti et al., 2003). IL-10 might have detrimental effects on human resistance to infection and might act as a risk factor for infection (Cohen et al., 2005; Srivastava et al., 2008).

Conclusion

The intent of the study provides some perspective on the ways in which elevated levels of pro-inflammatory TNF- α as well as anti-inflammatory IL-10 cytokines in women with infection might contribute to infertility. TNF- α initiated the inflammatory processes and might have affected the sperm fertility capacity. IL-10 might not be acting as anti-inflammatory mediator and might be involved in prolonging

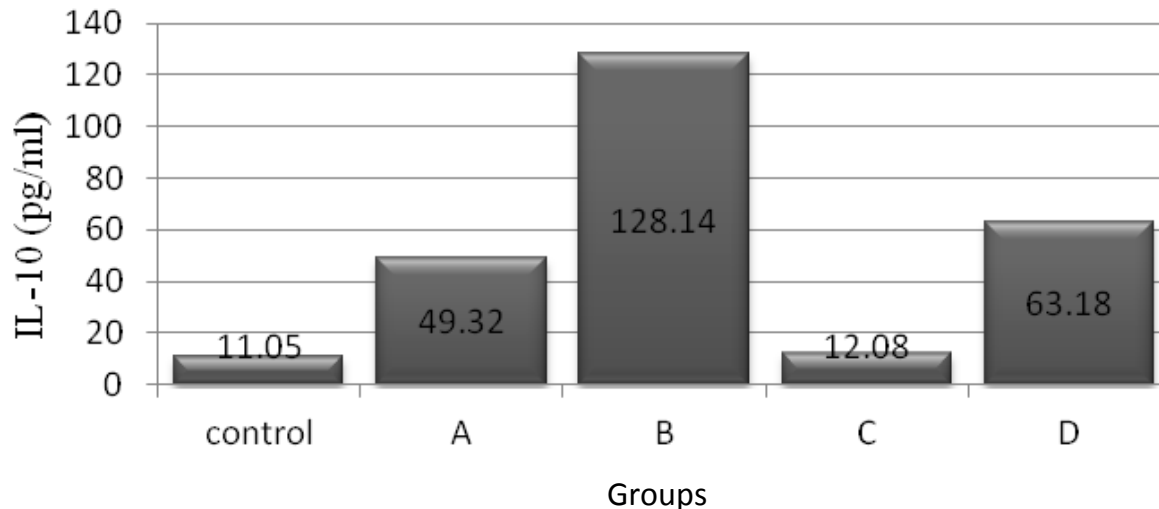


Figure 6. Mean vaginal secretion level of IL-10. A, infertile women positively with *C. albicans*; B, infertile women positive for *C. trichomatis*; C, fertile women positively infected with *C. albicans*; D, fertile women positive for *C. trichomatis*.

the infection.

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