

## Study level of Interleukin-1 $\beta$ and Interleukin-2 in women infected with *Trichomonas vaginalis* parasite

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### الخلاصة

صممت هذه الدراسة لتحديد الاصابة بالمشعرات المهبليّة لدى النساء المشكوك باصابتها سريريّاً في مدينة النجف الاشراف وذلك باستخدام طريقة الفحص المجهرى المباشر حيث اشتملت الدراسة الحالية على 450 حالة مشكوك بها سريريّاً بالأصابة بطفيلي المشعرات المهبليّة و 30 حالة من نساء غير مصابات ارتادوا وحدة العقم في مدينة الصدر الطبيّة ومستشفى الزهراء في محافظة النجف الاشراف للمدة من كانون الثاني حتى اب 2012 . اظهرت الدراسة الحالية ان نسبة وعدد المصابات بطفيلي المشعرات المهبليّة كانت 10.88% و 49 على التوالي . كما تم عزل اعلى عدد و نسبة للطفيلي من الافرازات المهبليّة ذات اللون الابيض المغبر ذو الفقاعات 40 (68.96%) على التوالي وذو الرائحة الكريهة 55 (94.82%) كما كشفت النتائج وجود زيادة معنوية في مستوى الساييتوكينات(انترلوكين- بيتا1 وانترلوكين-2 في النساء المصابات مقارنة بمجموعة السيطرة.

### Abstract

The study was conducted on 450 out patients and 30 healthy women, whom have visited the department of infertility at Al-Sadder medical city, Al-Zahra Hospital and in Najaf Province during the period from January till August, 2012. The infection with *T.vaginalis* in clinical suspected women determine by using the wet amount microscope, the infection women numbers and percent by wet mount microscope gave 49 (10.88%). The results revealed the frothy white discharge 40 (68.96%) while the malodor discharges gave 55 (94.82%) respectively. The results showed significant increased ( $P<0.01$ ) in concentration of interleukin-1 $\beta$  and interleukin-2 in serum of *T.vaginalis* infection patients in compared to healthy group.

### Introduction

*Trichomonas vaginalis* is one etiological agent of vaginitis; more than half of end cervical infections do not cause sufficient inflammation to result in clinical signs and symptoms. However, symptoms can include dysuria, vaginal discharge, dyspareunia, perineal itching and pelvic discomfort or pain, erythema multiform and swelling of the vulva or labia suggest trichomonal infection (1). *T.vaginalis* infection with *N.gonorrhoeae* induced an immune response (IL-1 and IL-6) that was the same when *T.vaginalis* was the causative agent of infection alone (2). Interleukin - 1 $\beta$  (IL-1 $\beta$ ) is a member of the IL-1 super family containing IL-K, IL-1 $\beta$ , and IL-Ira receptor antagonist. IL-1 $\beta$  is known as catabolic (IL-IF2). IL-1 $\beta$  is

corresponding to two different isoelectric forms, acidic PI 5 and neutral PI7, respectively, (3). They are produced mainly by macrophages and monocytes, processed and released during apoptosis, and bind with high affinity to specific receptors on target cells, while only the mature form of IL-1 $\beta$  has biological activity, both the pro and mature forms of IL-K are active (4).

*T.vaginalis* lacks mitochondria and some necessary enzymes cytochromes; instead uses, it use the hydrogenosome to accomplish fermentative carbohydrate metabolism. The hydrogenosome appears to have common ancestry with mitochondria based on similarities in protein import. Nutrient is taken up by transport through the cell membrane and

by phagocytosis. The organism is able to maintain energy requirements by the use of a small amount of enzymes to provide energy via glycolysis of glucose to glycerol and succinate in the cytoplasm (5). The parasite infects the epithelium of the genital tract. Trichomonal cytoadherence to epithelial cell is a highly specific critical step during the initiation phase of the infection and subsequent pathogenesis. The mechanism of cytoadherence has been studied, relying on genetic and biochemical approaches. (6). Adhesion of *T.vaginalis* to vaginal epithelial cells (VECs) plays an important role in pathogenesis of trichomoniasis, four different parasite surface proteins mediate adherence, and adhesions are up regulated during attachment to VECs (7). *In vitro*, studies have indicated time dependent significant

difference in the percentage of vaginal epithelial cell (VEC) attached by *Trichomonads* as well as number of parasites attached per VEC in *T.vaginalis* isolates from symptomatic women. Significant differences in attachment of VECs was observed only during first 15 minutes while maximum number of VECs attached by parasite was significantly different at initial 20 to 25 minutes following incubation of VECs and parasites (8). *T.vaginalis* factors that mediate adhesion to host cells have focused on the abundant surface lipophosphoglycan (LPG) for which there is sound evidence for a role in attachment to host cell (9,10). The aim of this study to estimate the level of interleukin-1 $\beta$  and interleukin-2 in serum of *T.vaginalis* infection patients in compared to healthy control group.

## Materials and Methods

### The subjects

The study was conducted on 450 women with pelvic inflammatory disease and 30 of healthy women as control groups, when all these cases were examined and defined as suspected with *T.vaginalis* by obstetrician when attended to AL-Zahra, maternity and pediatric, AL-Sadder teaching hospital in AL-Najaf province from January to August 2012.

### Sample collection

From suspected women vaginal discharge was carefully collected from the posterior vaginal fornix after putting the patient at a lithotomic position and taking swab after opening the vagina by a sterile speculum, the swab are immersed in a tube with ml of a sterile normal saline (11). The swab was examined in wet mount preparation and two ml of blood was collected from each clinical suspected woman with *T.vaginalis* infection and non-suspected women ( as control group) by disposable syringe , blood samples was drawn in sterile plain tubes and remains for 30 minutes at room

temperature. After that the samples were centrifugation at 3000 rpm for 5 minutes (Back man/counter, Germany) to separate the serum and collected in another sterile tubes, each sample of serum was divided into 2 parts.

### Wet mount Examination

Immediately, 1 drop from one of the tubes was applied to a glass slide, covered with a cover slip, and examined under the microscope by using the high power objective (X40) for the presence of *T.vaginalis*. The wet mounts were examined for at least 10 minutes (12). Positive results were defined as the presence of one or more Trichomonads with characteristic motility (jerky movement) and morphology (Demoeet *al.*, 1996). The Trichomonads may be inactive and non-motile as in chronic or asymptomatic condition (13). The wet amount is also used to demonstrate the presence of clue cells in vaginal secretions, these cells were epithelial cells covered by masses of bacteria of varying morphology (14).

**Interleukin- 1Beta (IL-1 $\beta$ )**

The Assay Max Human Interleukin -1 Beta (IL- 1 $\beta$ ) ELISA kit was achieved according to the manufacturing company (Assay pro, U.S.A.).

**Interleukin-2 (IL-2)**

The Assay Max Human Interleukin -2 ELISA kit was conducted according to the manufacturing company (Assay pro, U.S.A.).

**Results****Vaginal discharge color**

The result of the percentage study revealed the effect of vaginal discharge color in relation with diagnosis of infection with *T.vaginalis*, the results showing that the highest infection found with frothy discharge which the number

and percentage of infection was 40 and 68.96 % respectively followed by the women with yellow to green discharge 14 (24.13%), followed by the women with bloody discharge 3 (5.17%), followed by the clear discharge 1 (1.72%), as seen in Table (1).

**Vaginal discharge odor**

The number and percent of the women with malodor vaginal discharge 55 and 94.82% respectively, while the women with odorless vaginal discharge had the number and percentage 3 and 5.17% respectively, this results as shown in Table(2).

**Interleukin – 1 beta (IL – 1 $\beta$ ) and interleukin-2 (IL-2)**

The current study revealed that the concentration of (IL-1 $\beta$ ) and (IL-2) in women infection with *T.vaginalis* were of highly significant increase ( $P < 0.01$ ) in compared to healthy control group, as seen in Table (3).

Table (1):The Vaginal Discharge Color and Infection with *Trichomonas vaginalis*

| Discharge color  | Positive cases |       |
|------------------|----------------|-------|
|                  | No.            | %     |
| Frothy discharge | 40*            | 68.96 |
| Yellow to green  | 14             | 24.13 |
| bloody discharge | 3              | 5.17  |
| Clear discharge  | 1              | 1.72  |
| Total            | 58             | 100   |

The highest infection with *T.vaginalis*.\*

Table (2): The Vaginal Discharge Odor and Infection with *Trichomonas vaginalis*.

| Discharge odor | Positive cases |       |
|----------------|----------------|-------|
|                | No.            | %     |
| Malodor        | 55*            | 94.82 |
| Odorless       | 3              | 5.17  |
| Total          | 58             | 100   |

The highest infection with *T.vaginalis*.

Table (3): The Mean of Cytokines Concentration (Pg / ml) in the Group of Patients Suffering from *T. vaginalis* Infections.

| Cytokine (Pg / ml) | Healthy control (n = 30) | <i>T. vaginalis</i> patients (n = 60) |
|--------------------|--------------------------|---------------------------------------|
| IL - 1 $\beta$     | 3.723 $\pm$ 0.362        | 12.132 $\pm$ 0.582*                   |
| IL - 2             | 14.952 $\pm$ 2.185       | 101.894 $\pm$ 2.185*                  |

\* Significant difference  $P \leq 0.01$  between control group and patients.

## Discussion

The percentage of two vaginal discharge frothy discharge and yellow to green discharge were (68.96%) , (24.13%) had higher infected cases with *T.vaginalis* parasite ,while the bloody was 3(5.17%), followed by clear discharge was 1 (1.72%) ,the result of this study agree with study of (15) who recorded that the frothy discharge was in (50%) of the infected cases, followed by yellowish to greenish in (40.47%), and the discharge tinged with blood was found in (7.14%), clear discharge was found in (2.38%) of the cases, and differ from the study was done by (16) who recorded that the frothy discharge was (48.58%) of infected cases, follow by yellowish to greenish in (40%) of the cases, while the bloody and clear discharge was found in equal percentage rate (5.71%). A study done by (17) who reported the frothy discharge in only (12%) of the positive cases, un frothy in (69%) of the infected cases, menses or no discharge was reported in (19%) of the infected cases. This does not agree with current study, maybe because the real color varies from examiner to another, and maybe to new *T.vaginalis* strain produce different toxic materials and lead to differ in discharge color.The higher percentage of infected with *T. vaginalis* parasite was found in malodorvaginal discharge which was noted (94.82%) of infected women with *T. vaginalis* and the lowest rate of infection was found in the cases, which have no odor in their discharge (5.17%) and this similar to a study done by (16) who found that the positive case was (65.71%) in malodor vaginal discharge and (11.43%) in odorless discharge. The reason of the malodor maybe because of the metabolic by products of anaerobic *T.vaginalis* and other anaerobes that increased the

concentration during Trichomoniasis which may contribute to the malodor discharge. The fishy discharge odor maybe caused by volatilization of amine, most commonly putrescence and cadaverine, which are produced by bacterial metabolism (Coexistence between *Gardenrella vaginalis*- with *T.vaginalis*), the amine odor often increases with menses and after intercourse when the amine is volatilized by alkalization (18).

The result of the present study showed a highly significant increase in the concentration of (IL-1 $\beta$  and IL-2) cytokines in serum of patient infected with *T.vaginalis* compared to healthy control group. Increasing the (IL-1 $\beta$  and IL-2) level maybe due to increasing the monocyte or macrophages which stimulated by *T.vaginalis* infection caused vaginitis lead to stimulated host immune response cellular and humeral ( 19) ; this result agrees with study of (20, 21,22) and 23). The study by20),proved that *T.vaginalis* infection induced an immune response (IL-1 $\beta$  and IL-2) that was causative agent infection .The study by (21) showed that the IFN- $\gamma$ , IL-1 $\beta$  were significant higher in mice infected with asymptomatic *T.vaginalis* isolates by using *T.vaginalis* crude antigen extract and nonspecific mitogen (Con A) as stimulants .(22) revealed that the IL-1 $\beta$  , IL-2 , IL-6 and IL-8 present in higher level which correlated with infection agent example *T.vaginalis* , but low or undetectable levels of other cytokines . The result of the (23) showed that the human neutrophils and macrophages stimulated by *T.vaginalis* which produced the (IL-8) and proinflammatory cytokines tumor necrosis factor - $\alpha$ , IL-6 and IL-1 $\beta$ .

## Conclusions

1-The higher isolation rate of *T.vaginalis* was found in frothy malodor discharge.

5-The infection with *T.vaginalis* causes increase in concentration level of IL-1 $\beta$  and IL-2.

## Recommendation

1-Many studies may be carried on the virulence strains of *T.vaginalis* and determine the strain which more prevalence in Iraqi.

2-Deeply experiment study about the influence of *T.vaginalis* on the all type of interleukins level should be recommended.

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