Molecular and immunological study for detection of IL-6 in men infected with *Trichomonas vaginalis* parasite in Al-Najaf province; Iraq

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الخلاصة

الهدف من هذه الدراسة هو تشخيص طفيلي *Trichomonas vaginalis* بأستخدام تقنية الPCR وقياس الـ IL-6 في الرجال المصابين بطفيلي المشعرات المهبلية *Trichomonas vaginalis* . جمعت عينات الإدرار وعينات الدم من 85 رجل من المختبرات الخارجية في محافظة النجف الذين يعانون من التهاب العضو التناسلي والتهاب البروستات للفتره من شهر حزيران إلى شهر تشرين الاول 2012 . وكذلك جمعت عينات من 20 رجل سليم كسيطرة . جمعت عينات الدم في أنابيب خاصة لعزل المصل . أستخدم المصل لقياس الـ IL-6 باستخدام تقنية ELISA.عزل الطفيلي من 15 شخص وكانت نسبة الإصابة 17.64% باستخدام تقنية الـPCR بينما أظهرت الدراسة زيادة معنوية ($P<0.05$) في مستوى الـ IL-6 في الرجال المصابين بطفيلي إلـ *Trichomonas vaginalis*.كما اظهرت الدراسة ان تقنية الـ PCRهي افضل طريقة للتشخيص طفيلي الـ *Trichomonas vaginalis*.

Abstract

The aim of this research was to diagnosis of *Trichomonas vaginalis* by use PCR technique and evaluate interleukin-6 (IL-6) in men infected with *Trichomonas vaginalis*; urine and blood specimens were collected from 85 men whom have visited the department of infertility at Al-Sadder medical city, Al-Zahra Hospital in Najaf Province during the period from June till October, 2012. Twenty healthy looking age matched men taken to serum tubes and serum was separated. Serum was used for evolution of the IL-6.The IL-6 was evaluated in serum using ELISA technique .*Trichomonas vaginalis* was isolated from 15 men with a prevalence rate 17.64% by using PCR technique. The results revealed a significant increase in IL-6 in men infected with *Trichomonas vaginalis* in compared to control group and the PCR is accurate method used in diagnosis of *Trichomonas vaginalis* parasite.

Introduction

Sexually transmitted infectious are caused by microorganisms that are fastidious in nature and therefore need intimate contact between individuals for transmission. Since these infections have only one host, i.e. man, they are in principle, ideal candidates for elimination (1). The conventional methods for diagnosis involve the direct microscopic examination of wet mount or culture-based systems for vaginal smears (2) .The factors associated with high prevalence are the same as those of sexually transmitted disease, poor personal hygiene, multiple sexual partners, and low socio/ economic status (3). Trichomonal infection in women ranges from an asymptomatic carrier state to profound, acute, inflammatory disease. The parasite principally infects the squamous epithelium of genital tract but can be recovered from the urethra and has been found in the fallopian tubes and the pelvis. In males; *Trichomonas vaginalis* causes urethritis and prostatitis. Respiratory infections are acquired perennially from infected mothers. (4). The molecular biology based diagnostic methods such as hybridization assay and PCR have been employed in the

diagnosis of *Trichomonas vaginalis* in different sittings (5). Approximately 180 million women worldwide may be infected with *Trichomonas vaginalis.* Prevalence estimates vary between populations studied, but range from 4-5% in women and 5-29% in men (6). Immune responses to infection with *Trichomonas vaginalis have* been described, including specific secretary antibodies in vaginal secretions and (IgM and IgG) antibodies in serum (7).

Immune responses including humoral and cell- mediated immunity and evokes lymphocyte function including cytokine production. Cytotoxic effects and antibodies produced after presentation by antigen manufacturing cells (8). Relative risk of developing invasive cervical cancer (9) and six fold higher probability of infection by human immunodeficiency virus (HIV) are linked to this disease (10).

T-cell subsets and cytokines serve a central function as key factors in the regulation of mucosal responses in various parasitic infections (11). Nucleic acid amplification techniques have been developed and applied for the detection of sexually transmitted pathogens in clinical specimens .Nucleic acid amplification tests have advantages with respect to sensitivity, specificity and rapidity. For detection of sexually transmitted pathogens compared with culture techniques (12). The aim of this study to estimate the concentration of cytokine IL-6 in serum of men infected with *Trichomonas vaginalis* by ELISA technique and the infection which diagnosis by PCR technique in urine specimens.

Materials and methods

The study was conducted on 85 men with urethritis and prostatic disease and 20 of healthy men 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 men urine was carefully collected and two ml of blood was collected from each clinical suspected men with *T.vaginalis* infection and non-suspected men (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 tube.

DNA Extraction

1-Cell pellets were collected from urine sample by centrifugation at 16000 rpm for 10 minutes

2-The cell pellets were resuspended in 500 µl of 0.5% Tween 20.

2-The suspension was boiled for 20 min in water bath at 100Cº.

3- Centrifugation at 12,000 rpm for 5min.

4- The supernatant was discarded, and the pellet was emulsified by vigorous flicking in 25 µl of chloroform phase.

5- The aqueous phase containing water-soluble components including nucleic acids was harvested by centrifugation at 12,000 rpm for 2min.

6- The tube which contains DNA was stored at - 20Cº till used for amplification. 13), PCR Identification of *Trichomonas vaginalis* according procedure by as (14) with some modifications and products analysis according procedure used by (13) as seen in figure (1).

Results

The results revealed that the number and the percentage of men infection with *Trichomonas vaginalis* were 15 (17.64%). The present study on trichomoniasis in men's revealed a significant increase ($p<0.05$) in IL-6 concentration of patients serum (4.21 ±0.16 pg /ml) in compared to the control group (0.0765 ± 0.0045 pg /ml) ,(figure 2) .

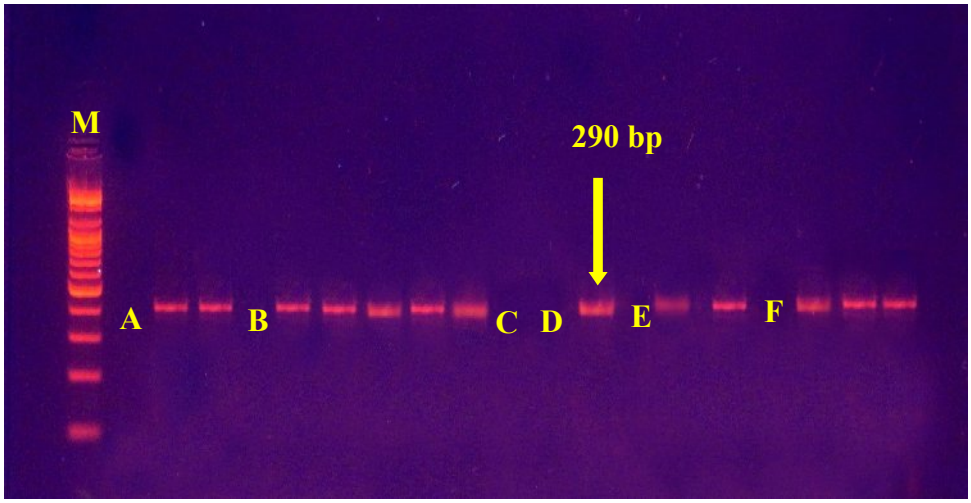

Figure: (1). PCR product using primer OP1 □ 2 to amplify specific target sequence from genomic DNA of *T.vaginalis* .M-DNA ladder 100bp.E-negative control. Other lane represents the PCR product (290 bp) A, B, C, D and F represent negative sample.

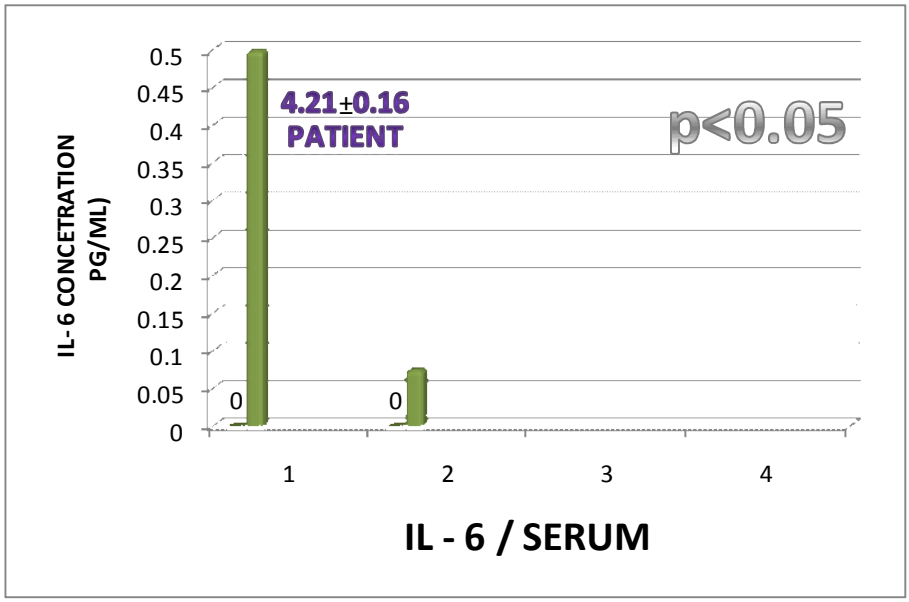

Figure (2): Comparison the mean of concentration of IL-6 in serum between patients with *Trichomonas vaginalis* and control groups.

Discussion

Data from the present study showed a significant increase in the concentration of IL-6 in comparison with control group maybe due to increase in number of monocyte as response to inflammation caused by *Trichomonas vaginalis* . This result agrees with the experimental trichomoniasis study conducted by (11).This result also corresponding to the result of Young *et al*. (2012). Who confirmed that *Trichomonas vaginalis* causes significant increase in IL-6 concentration of patients' serum comparison to the control group. Another study showed that human neutrophils or macrophages stimulated with live *T.vaginalis* produce the chemokine interleukin (IL-8) and the proinflammatory cytokines tumor necrosis factor-x, IL-6 and IL-1β (15,16) this study agrees with the current study. The results of present study agrees with study of (17), which proved that PCR technique more sensitivity than the other method which used for diagnosis of *T.vaginalis*.

Although cut off value was calculated for each parameter included in the present study to eliminate the effect of concomitant infection on the results, studies have indicated that the systemic immune response (serum) is not altered by the concomitant infections. From this present study concluded that there is a significant increase in IL-6 in peripheral blood in men infected with *Trichomonas vaginalis* when compared to the control group. And the PCR technique was accurate method for diagnosis of *Trichomonas vaginalis* parasite.

Conclusions

1-PCR the accurate method used in identification of the *T.vaginalis* parasite.

2-The infection with *T.vaginalis* causes increase in concentration level of IL-6.

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.

3-Deeply molecular studies for diagnosis all type strains found in Iraq and determine the more virulence strain.

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