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USE PCR TECHNIQUE TO DETECT *TRICHOMONAS VAGINALIS* AMONG MEN IN BASRAH PROVINCE

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ABSTRACT

The current study is aim Trichomoniasis among men in Basrah province using polymerase chain reaction (PCR) for the first time in Iraq compared with classically microscopic examination method and explain the association between infection and Urethritis and infertility as well as age ,marital status and location of them . Study is show the rate of infection 2.3% by microscopic examination and 16 % by PCR also , PCR show high sensitive and specific method to detect Trichomonas vaginalis , many infected men with Trichomoniasis are asymptomatic concluding that men only a vector , transmitting organism among females .

INTRODUCTION

Trichomonas vaginalis **is a parasitic protozoan that is cause of Trichomoniasis , a sexually transmitted disease (STD) of worldwide importance (1) , found in reproductive tract of both men and women . It live in the vagina and urethra of women and in the men prostate , seminal vesicles and transmitted primarily by sexual intercourse (2). Symptoms of disease in men are involved urethral discharge (ranging from scant to purulent) , dysuria and urethral pruritus . Trichomoniasis in men associated with urethritis , prostatitis , balanoposthitis , epididymitis and infertility (3). The prevalence of infection in men is less describe because many men are asymptomatic and may not seek evaluation , Furthermore, the diagnosis is less commonly sought in men (4). Routine clinical diagnosis usually depend on microscopic observation of motile parasite in wet – mount preparation . Recently , several assays for the diagnosis of Trichomoniasis based on polymerase chain reaction (PCR) have been developed and evaluated (5) , the most common of which use DNA repetitive sequences as the target . (6) . The current study is use wet – mount preparation (direct microscopy) and PCR method to detect *Trichomonas vaginalis* among men in Basrah province depend on urine sample .**

MATERIALS & METHODS

Samples collection:**Ten milliliters of urine sample were collected by sterilized test tube from 472 men who entered infertility center in maternity and pediatric hospital, private sexually transmitted diseases (STD) clinics and laboratories in Basrah province. Personal information were collected from symptoms and asymptom man was include: age, location, marital status, using of condom and symptoms. Urine sample was centrifuged at 3,000 xg for 5 min and sediment were divided in two group : one of them directly examined at 40X for diagnose the *T. vaginalis* , the sediment of another group were washed twice and stored in 500 µl of Tris – EDTA (pH:8) -20 for PCR (7), (6) .**

DNA extracted and PCR for T. vaginalis: **DNA were extracted from *T. vaginalis* using urine samples depend on Proteinase K \ SDS method (8) and**

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electrophoresed by UV light transilluminator for viewing DNA . Aset of primer (TVK3 and TVK7) targeting a conserved region of *T. vaginalis* DNA was used to amplify 300 bp piece of genome by PCR procedure. the sequences were as follows: for TVK3 (5'ATTGTCGAACATTGGTCTTACC CTC'3) and for TVK7 (5'TCTGTGCCGTCTTCAAGTATGC '3). A total volume of 25 µl of PCR reaction was performed in 0.2 µl microtube which consist of : 1 µl of each primer set , 5 µl of T. vaginalis DNA , 12 µl of Go Taq green master mix and 5.5 µl of distilled water and mixed well , finally about 25 µl of mineral oil were add to reaction . PCR protocol was include 5 min of denaturation at 94 C° , followed by 35 cycles of each consisting of 5 min of denaturation at 90 C° , 30s of annealing at 60 C° and 2min of extension at 72 C° . A final extension step at 72 C° for 7 min was included (9). Seventy five from each positive and negative urine sample were tested . A positive result showed about 300 bp piece of genome compared with 1500 DNA ladder .

RESULTS

The current study show that the rate of infection with *Trichomonas vaginalis* is 2.3 % (11\ 472) using microscopic examination compared with 16 % (12\ 75) that show by PCR method . Asymptomatic men show a high rate of infection 58.3 % whereas urethral itching show a low rate (8.3%) of infection with *T. vaginalis* among symptomatic men . Table (1).

A high rate of infection were recorded at age group (30 – 40 years) which is 50 % and there is no infection at < 20 years when study the association between infection with *T. vaginalis* and age of men . Table (2) Examination of married men shows that all of them found be infected (100%) with *T. vaginalis* in current study , which explain the closely association between the infection and marital status .Table (3) Men whom are suffering from infertility consist 1.6 % (3\12) of infection with *T. vaginalis* after examined 178 infertile men from 472 and there is no infection among men whom use condom during sexual activity. The current study explain a rate (58.3 %) of infection with *T. vaginalis* among men whom live in rural city compared with men whom are live in urban city . Table (4)

Wet – mount preparation (direct microscopy) is use together with PCR method to detect T. vaginalis from urine sample , results show that PCR diagnose 12 sample from 75 men compared with direct microscopy which diagnosed only 11 sample from 472 men . PCR appear high sensitive and specific method to detect *T. vaginalis* than direct microscopy when determination of sensitivity and specificity of each method during diagnosis of two sample by PCR which are negative by direct microscopy. Table (5) , (6) , Figure (1) .

DISCUSSION

Data on prevalence of sexually transmitted infection (STI) such as T. vaginalis among men in Iraq are very limited . Current study describe the prevalence of T. vaginalis among men with and without symptoms in Basrah \ Iraq for the first time by PCR technique compared with direct microscopic examination . In the present study the prevalence of T. vaginalis among men are low (2.3 %) and this are not accepted with data cited in other studies of the world such as 25 % in Guinea (10) , 18 % in Canada (11) , and 71.7 % in United States of America (12). Several factors are lead to infection in that countries such as illegal sexual relationship which in uncommon in Iraq because of Iraq is one of the Islamic countries , for instance , Strategies to prevent sexually transmitted infection in Islamic countries have to abide by the Islamic rules and values such as " Safe sex " which concepts in Islam is a monogamous sexual relationship through legal marriage (13) ,

Furthermore health education , sanitation and good using of water cycles are very importance factors to prevent infection with T. vaginalis (14) .

Result are exclusive among married men when show the association between the infection with T. vaginalis and marital status of men and the reason responsible of that is the infection happen directly by sexual contact with infected women or by use of the contaminated towels (15) . T. vaginalis is prefer to infect the vagina of women which is provide the important source for organism alive such as iron and lipids (16) therefore many of men may infect from there wife or from another infected women . furthermore , women may harbor T. vaginalis for 3 – 5 years (17) .

Some of men that infected with T. vaginalis suffering from symptoms such as Urethritis , dysuria and urethral itching (18) . a high rate (58.3 %) of infected men in current study are asymptomatic and this are not accepted with (19) who found a high rate of infection among symptomatic men in Basrah but accepted with (12) whom found 71.7 % of infected men in united states of America are asymptomatic , therefore symptoms in another men may return to other GTI , UTI infection and this accepted with (20) whom reported the failure to detect T. vaginalis in 100 Japanese men with or without Urethritis . Men with age ranging from 30 – 40 years consist high rate of infection with T. vaginalis which is 50 % this is accepted with study presented by (12) because of more of them are married and may have infection from their wife or another infected women during direct intercourse or from contaminated towels (3) , therefore there is no infection report in men whom age are less than 20 year because many of them are unmarried , also this period is consider as adolescence and Islamic rules are permitting adolescents to get married with no age limit for marriage to prevent no – marital sex (13). Some studies were suggested an association between infection with T. vaginalis and infertility (21) and explained that T. vaginalis may inhibit the movement of sperm (22) such these suggest is accepted with current study because the infertile men consist 1.9 % of infection in Basrah province. The majority of men in Basrah province use of condom to prevent microorganism from reaching to reproductive tract (23) , therefore , current study show that men whom use condom are not infect with T. vaginalis . Men whom are live in rural city in Basrah consist high rate of infection with T. vaginalis than others whom lives in urban city , this is accepted with results presented by (10) and the reason of this may related to the low economic status , low education , low use of condom and ignorance . Reported prevalence rates of infection with Trichomonas vaginalis in male have varied depending on the population studied and the diagnostic techniques used . classically , diagnosis is dependent on the demonstration of organism by direct microscopy of any discharge or urine (24). The current study use PCR for diagnosis T. vaginalis in men for the first time in Iraq and there are only two study have been previously reported the infection in United states of America (25),(26) therefore PCR show as a high sensitive and specific (100% and 90.9%) method to detect T. vaginalis than direct microscopy which is able to number of sample that fail to detect by direct microscopic examination of urine and the reason of this result related to that the PCR has the advantage of requiring only DNA , from either viable or non – viable organisms , and in concentration as low as one organism per PCR reaction (19) so this result is accepted with each study presented (25) and (26). Only one sample has a positive result when examined directly by microscope but negative with PCR and that may return to laboratory problems and transport of sample also urine may contain some chemicals which inhibit PCR amplifying (9).

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TABLES:

Table (1) show the association between the infection with *T. vaginalis* and symptoms

Status	No. positive sample (%)
Asymptomatic	7 (58.3)
Urethritis	3 (25)
Dysuria	2 (16.6)
Dyspareunia	3 (25)
Urethral itching	1 (8.3)

Table (2) Distribution the infection rate with T. vaginalis at age group

Age of men(years)	No. positive sample (%)
< 20	0
20 – 29	2 (16.6)
30 – 40	6 (50)
> 40	4 (33.3)
Total	12

Table (3) show the association between infection with T. vaginalis and marital status of men

Marital status	No. positive sample (%)
Married	12 (100)
Single	0
Total	12

Table (4) show the association between infection of T. vaginalis and location of men

Location of men	No. positive sample (%)
Urban	5 (41.6)
Rural	7 (58.3)
Total	12

Table (5) show the comparison between PCR method and direct microscopy to detect T. vaginalis

Method	No. positive sample (%)	Sensitivity (%)	Specificity(%)
PCR	12 (16)	100	90.9
Direct microscopy	11 (2.3)	84.8	83.3

Table (6) show association positive sample between PCR and direct microscopy

No. positive sample	Results by direct microscopy	Results by PCR
10	+	+
2	-	+
1	+	-
Total	11	12

Figure

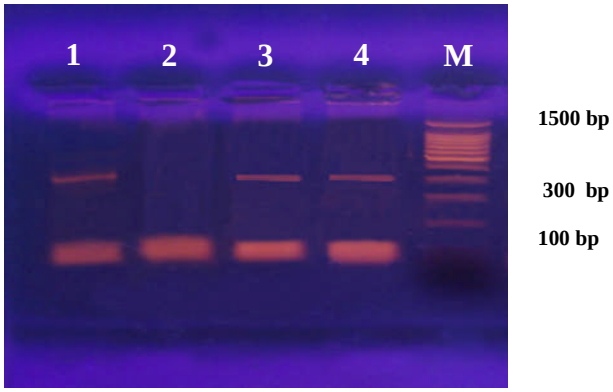

Figure (1) picture show DNA amplification of T. Vaginalis after electrophoresis in 0.2 % of agarose gel , the sample 1,3,4 show positive results , 2 show negative result , M is DNA Ladder to compare results

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استخدام تقنية تفاعل البلمرة التسلسلي PCR في الكشف عن طفيلي المشعرة المهبلية *Trichomonas vaginalis* بين الرجال في محافظة البصرة

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الخلاصة :

استهدفت الدراسة الحالية داء المشعرات المهبلية بين الرجال في محافظة البصرة باستخدام تقنية تفاعل البلمرة التسلسلي لأول مرة في العراق ومقارنتها بطريقة الفحص ألمجهري التقليدية ومن ثم توضيح العلاقة بين الاصابة بالداء والتهاب الاحليل والعقم عند الرجال اضافة الى العمر والحالة الاجتماعية والواقع السكني لهم . تبين من الدراسة ان نسبة الاصابة بالداء كانت ٢,٣ % باستخدام طريقة الفحص المجهري و ١٦% باستخدام تقنية تفاعل البلمرة التسلسلي كما تبين ان تقنية تفاعل البلمرة التسلسلي اعلى حساسية وخصوصية من طريقة الفحص المجهري في تشخيص طفيلي المشعرة المهبلية ، معظم الرجال المصابين بالداء لم تظهر عليهم الاعراض مما يدل على ان الرجل مستودع للمرض فقط ناقلا الاصابة بين الاناث .

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