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Comparison of Real time PCR with traditional tools for diagnosis of Trichomonas vaginalis in patient with vaginal discharge at Kut city, Iraq

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Abstract :

Trichomoniasis is a sexually transmitted disease (STD) and most common form of STD which is caused by *Trichomonas vaginalis*. This is a descriptive analytical study. Vaginal swabs were collected from sixty female patients with suspected *Trichomonas vaginalis* infection aged 14-53 years old during September 2013 to March 2014 who attended the Gynecology Clinic of Kut city, Iraq. Two swabs were collected from each woman; one for wet mount microscopic examination and the other for Real Time – PCR technique. Wet-mount microscopy were positive for *T. vaginalis* in 5/60 cases (8.33%) and positive Real-time PCR 13/60 (21.67%). The present study revealed that the highest incidence of *T.vaginalis* infection occurs in age group(24-33) years with the percentage of (46.1%). The sensitivity of Real-time PCR was 100% compared with 38.5% for wet-mount microscopy. The aim of the study was to compare a Real-time PCR assay with wet-mount examination for the detection of *T. vaginalis*.

Keywords: Real Time – PCR; *T.vaginalis;* Vaginal discharge; Human.

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Introduction :

Trichomoniasis caused by *Trichomonas vaginalis* with annual incidence of 170 million is the most common curable form of STD in all over the world. Trichomoniasis mostly is asymptomatic, clinical presentation of the disease is vaginitis, cervicitis and urethritis, and moreover increases the risk of other STD such as HIV infection. [(1)].

Trichomoniasis accounts for 4–35% of the vaginitis diagnosed in symptomatic women presenting in primary care settings. Clinical manifestations of the infection in women are generally non-specific, but may include vaginal discharge, vaginitis and irritation [(2)]. Although generally considered a disease of women, *T. vaginalis* also infects men, but in majority of the cases is asymptomatic [(3)].

Wet-mount microscopy is the most commonly cost effective method for diagnosis [(4)]. Although the test is rapid and easy but it has a limited sensitivity of 20–60% [(5,6)]. Culture is the golden standard diagnosis test, but is not routinely used [(7)]. PCR method using various regions of *T. vaginalis* genome have been employed and showed 89–98% sensitivity. The present study aimed to determine the occurrence and prevalence of *T.vaginalis* and to compare between two diagnostic methods.

Materials and Methods:

Study population and samples collection:

Vaginal swabs were collected from sixty female patients (14-53 years old) with suspected Trichomonas vaginalis infection during September 2013 to March 2014 who attended the Gynecology Clinic of Kut and Al-Zahra'a Hospital at Wasit Province, Iraq. Two swabs were collected from each patient; one for wet mount microscopic examination and the other for Real Time PCR.

Microscopic examination :

All vaginal samples were observed microscopically at 400x power for the presence of motile, oval flagellated protozoan and when motile trophozoite of T.vaginalis was observed the specimen considered as positive.

Real Time PCR :

Primers

The Real-Time PCR primers which were used in this study were design based on complete sequence of repeated DNA target for Trichomonas vaginalis genome (GenBank: L23861.1) using NCBI Gene-Bank data base and Primer 3 plus online and were provided by (Bioneer company, Korea) as following table:

Primer	Sequence		Amplicon
repeated DNA	F	CATTGACCACACGGACAAAAAG	67bp
	R	CGAAGTGCTCGAATGCGA	

Results and Discussion:

Comparison between Real-time PCR and wet mount:

Out of 60 vaginal discharge samples which were tested; 13 samples were positive for T. vaginalis using Real-Time PCR (rate of21.67%) and 5 (8.33%) of the above 13 samples were positive using wet-mount examination. The highest number of positive samples was in age group of 24-33 years old, the data are presented in table 1.

Table (1) The rate and age distribution of *T. vaginalis* positives based on Real-Time PCR and wet-mount test.

Age Group	Real -Time PCR	(%)	Wet mount	(%)	Total
14-23	4	30.8	1	20	13
24-33	6	46.1	2	40	22
34-43	2	15.4	2	40	15
44-53	1	7.7	0	0	8
54-63	0	0	0	0	2
Total	13/60	100	5/60	100	60

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Sensitivity and accurate rate:

The sensitivity and accurate rate of two T. vaginalis diagnosis method were done according to (Levi et al.,1997; Shimano et al., 2004). The calculation of both parameters was shown in tables(2,3) . The sensitivity of Wet mount was lower (38.5%) compared to, Real Time-PCR , while the accurate rate was (86.7%) for Wet mount and Real Time-PCR (100%) respectively.

Table (2) Comparison the sensitivity and accurate rate between two detection methods

Detection methods	Positive case		Sensitivity %	Accurate rate %
	No.	%		
Wet mount	5	8.33	38.5%	86.7%
Real -Time PCR	13	21.67	100%	100%

Table (3) The sensitivity and accurate rate of wet mount

+ Ve	True+Ve	False+Ve	Total	Sensitivity=$\frac{5}{13} \times 100 = 38.5\%$
	5	0	5	
- Ve	False-Ve	True -Ve	Total	
	7	47	54	Accurate rate= $\frac{5+47}{60} \times 100 = 86.7\%$

Real-Time PCR :

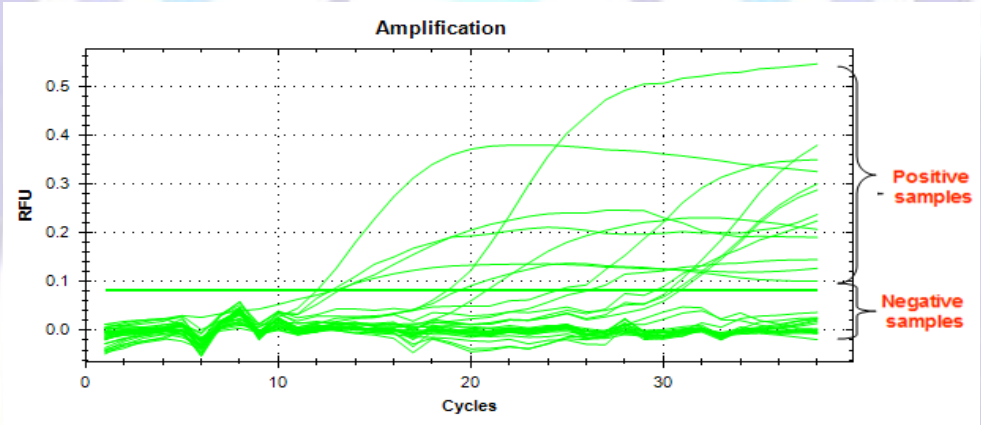

Figure (1) Real-Time PCR amplification plot of repeat DNA genome of *T. vaginalis* in positive and negative samples

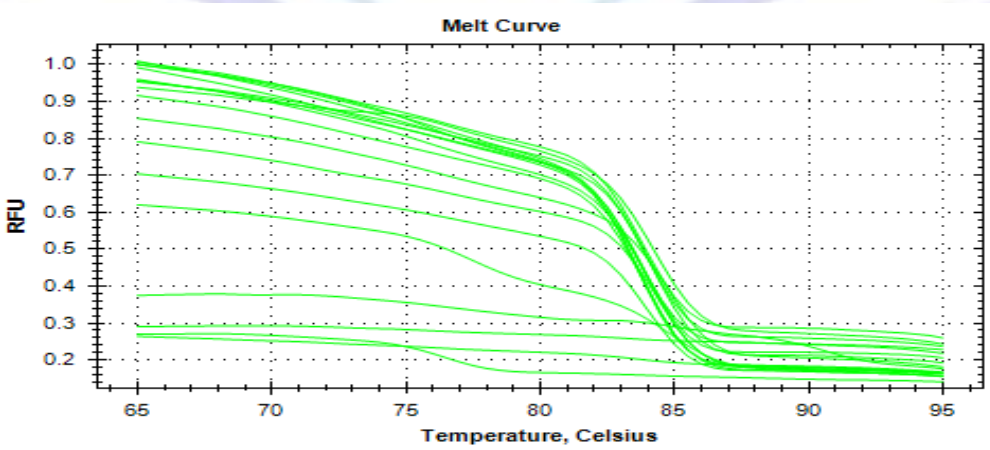

Figure (2) Real-Time PCR melt curve of repeat DNA genome of *T. vaginalis* in positive and negative samples

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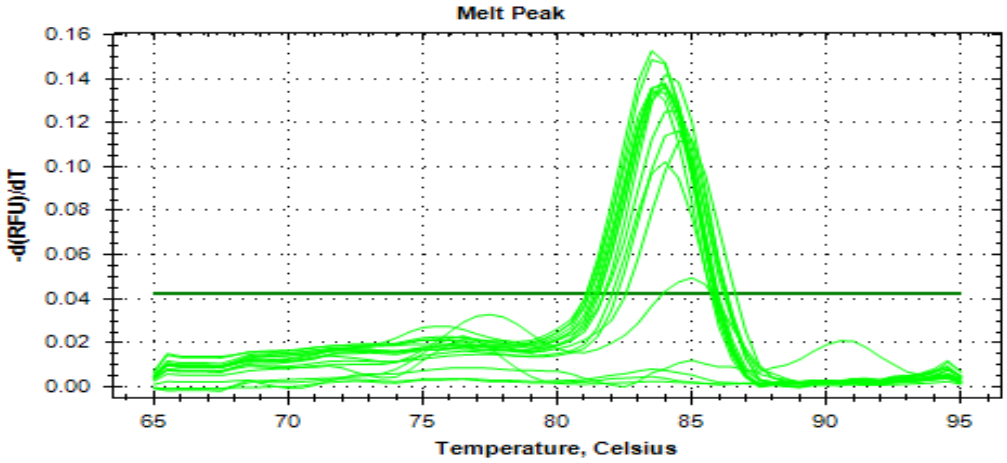

Figure (3) Real-Time PCR melt peak of repeat DNA genome of *T. vaginalis* in positive and negative samples

Discussion :

A molecular method such as PCR allows to monitor the amount of DNA while it is amplified. DNA was extracted from each specimen and amplified in Real Time PCR and the *T.vaginalis* DNA was detected using fluorescent reporter dye probes specific for *T.vaginalis* DNA using internal control. Internal control (IC) serves as an amplification control for each individually processed specimen and to identify possible reaction inhibition. IC is detected in a channel other than *T.vaginalis* DNA [(9)].

The higher frequency rate of *T.vaginalis* obtained in this study is consistent with those found by other researchers. In two studies completed in Iraq the incidence rate of trichomoniasis was shown to be 19.16% and 19.13%)[(10, 11)], and two other studies the incidence rate was 18.2% and 15.6%)[(12,13)]. Similarly incidence of trichomoniasis in Baghdad was reported 21% in 2012 21% 2003 in Baghdad, Iraq[(14,15)].

Much lower incidence of trichomoniasis was reported in Iraq by Saleem (2013) [(16)];Saba (2011) [(17)]; Al-Saeed (2011) [(18)]; Khalil *et al*., (2012) [(19)] and Amal (2010)[(20)] who recorded (12.88%),(12%), (5.4%),(7.2%) (4.5%) respectively, and in Italy by Mengoli *et al*., (2009) [(21)], who found (3.8%). The prevalence of *T.vaginalis* was 18% in a study using Real Time PCR in South Australia by Simpson *et al*., (2007) [(22)] and 68.9% in study completed by Pillay *et al*., (2007) in South Africa [(23)] and 89% in Sudan by Nazik (2011) [(24)]. In a study performed in 504 women in Egypt in 2003 it was shown that 11.9%, 23% and 66% of the samples were positive for *T.vaginalis* using wet-mount microscopy, culture and Real-time PCR, respectively [(25)].

The results of current study have shown that the highest incidence of T.vaginalis infection occurs in age group of 24-33 years old with the percentage of 46.1%. The current results correspond with the data generated with other (26,27), that showed a higher prevalence in 40-49 years age group. This phenomena might occur due to the ability of the parasite to alternate the vaginal environment for its survival and higher sexual activity. A study done by Constance et al., in 2013 showed that the highest infection rate of 80% was seen in women in reproductive age of 25-45 years, and 9% of the samples were positive in females with less than 25 years old and 30% in women more than 45 years old (28).

The present study showed that the wet mount test has the least sensitivity (38.5%) and the highest sensitivity (100%) was shown with Real Time-PCR , while the accurate rate was, (86.7%) for wet mount and Real Time-PCR (100%) respectively. This result was agreement with many previous studies that revealed differences in the sensitivity and the accurate rate of many different identification methods used in the diagnosis of T. vaginalis (21,29).

Conclusions:

1. The real-time PCR technique was more sensitive and accurate in diagnosis of T. vaginalis than the wet-mount.
2. Women aged (24-33) years had significantly higher prevalence of trichomoniasis than other age groups.
3. The prevalence of T.vaginalis at Kut city was high 21.67%.

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