Detection of Endometrial TB in Patients with AUB using PCR Method of Assessment of Menstrual Blood Flow of Iraqi Females

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Abstract

Background: Iraq still considered one of the countries in the region with high prevalence of TB, there are about 20000 patients, accounts for 3% of the total number of cases, and the estimated death from TB exceeds 4000 per year. TB in Iraq until nowadays considered a health, public and social problem, and because of the wars and political problems, many programs, campaigns, and researches were hundred by this situation

Objective: To detect endometrial Tuberculosis in women complaining from AUB (abnormal uterine bleeding) using real time PCR method of assessment via taking an endocervical swab.

Patients and methods: Prospective cross-sectional study carried at Department of obstetrics and gynecology in Baghdad teaching hospital /medical city over a period of eight months starting from October2019, to Augest2020. A total of 60 women complaining from abnormal uterine bleeding(AUB) who were scheduled to have endometrial biopsy for histopathology by dilatation and curettage(D&C), endocervical swab assessed for female genital tuberculosis(FGTB) by real time PCR.

Results: Out of 60 samples taken 6 were positive by real time PCR 10%, while there was only one case positive by histopathology 1.66% which was also positive by PCR the same patient gave history of pulmonary TB and received a nine month course of treatment, according to these findings the sensitivity of PCR found to be 100% while the specificity92% and an accuracy of 92%.

Conclusion: Real time PCR considered one of the valuable diagnostic tools for mycobacterium tuberculosis, and because Iraq still showing high incidence of the disease, one should suspect mycobacterium tuberculosis in any women complaining from abnormal uterine bleeding and offer PCR in addition to other diagnostic modalities, luckily it will allow treatment at an early stage of the disease.

Keyword: *Endometrium, TB, AUB, PCR*

Introduction

Iraq still considered one of the countries in the region with high prevalence of TB, there are about 20000 patients, accounts for 3% of the total number of cases, the estimated death from TB exceeds 4000 per year [(1).]

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TB in Iraq until nowadays considered a health, public and social problem, and because of the wars and political problems, many programs and campaigns and researches were hindered by this situation [(2).]

Tuberculous PID is largely limited to patients from developing countries. Pelvic infection usually occurs secondary to hematogenous spread from an extragenital source. Nevertheless, occasionally *Mycobacterium tuberculosis* can be transmitted sexually [(3).]

Genital TB occupy about 9%of extrapulmonary TB [4], TB endometritis occupy about 50-60% of genital TB and tends to be sporadic lesions whereas ulceration, caseous changes and bleeding seen later on to ends up by adhesion and amenorrhea [5]. Diagnosis of genital TB suspected depending on patient's symptoms with infertility being at the top of the list, then AUB whereas dyspareunia and dysmenohrroea at the bottom of the list [6].

One of the conventional methods used for diagnosis of tuberculosis is acid –fast bacilli staining which mainly relied on identification of *Mycobacterium tuberculosis* by microscopy or its growth on culture medium [7]. Culture still considered a gold standard method in the diagnosis of genital TB with a low detection rate contributes to the paucity of mycobacteria in the genital tract and delay in the diagnosis due to slowly growing pathogen which requires about 3-6 weeks [8]. histopathology required the presence of epithelioid granuloma in the examined tissue and because of the shedding of the endometrium there might be no time for the granuloma to be formed so the endometrial aspirate and endometrial sample will be negative and many cases will be missed for this reason [9].

PCR method are widely used nowadays, a rapid procedure requires few hours for the results to be obtained and can identify the nucleic acid sequence specific to mycobacterium tuberculosis and other mycobacteria in female genital TB, with a high detection rate <10 bacilli/ml including dead bacilli [10]. Compared to acid –fast bacilli staining by Zeihl-Neelsen stain which needs 104-106bacilli/ml on the other hand culture requires 10-100bacilli/ml of tissue /fluid sample **for** the diagnosis [5]. It is possible now to catch hidden genital tuberculosis by PCR, real time PCR noticeably decreases the false positive results because the amplification and detection takes place in the same reaction tube with a sensitivity reaches about 90-94% and specificity of 70-78% [11].

Material and Methods

Our prospective present study carried out in the department of obstetrics and gynaecology/ Baghdad teaching hospital, from October 2019 till August 2020, a total of 60 patients aged between 30-68 years attends the gynecology outpatient clinic complaining from abnormal uterine bleeding all were prepared for diagnostic dilatation and curettage(D&C) and endometrial sampling, after full history taking including any positive history for TB, examination, and full investigation done as part of preparation for D&C including chest X-ray, only one patient gave positive history of pulmonary TB and received a 9-month course of anti-TB drugs. after patient consent endocervical swab was taken before commencing D&C, the swab then kept in 1 ml of transport medium then diagnostic curettage was done and endometrial sample sent for histopathology after being fixed with formalin.

Swabs then sent for laboratory for processing by real time PCR using AmpliSens MTC-FRT PCR kit, mycobacterium tuberculosis detection by the PCR is based on the amplification of pathogen genome specific region using specific mycobacteria tuberculosis primers. AmpliSens MTC-FRT PCR kit is a qualitative test that contains the internal control (internal control STI-87) [12].

Results

Table 1 show that age group between (40-49) years was the main group (61.6%) with the mean age (47.3±5.6) years, blood group A+ represented (41.7%), parity between (4-6) was the main group, while Menorrhagia was the most common symptom (85.0%) and the (means ±SD) of endometrial thickness was (9.27±4.5) mm.

Table 1: Socio demographic characteristics of the studied group

Variable		N	%
Age	30-39	1	1.7
	40-49	37	61.6
	50-59	19	31.7
	≥60	3	5.0
Total		60	100.0
Age (Mean±SD) years		47.3±5.6	
Blood group	A-	1	1.7
	A+	25	41.7
	AB+	4	6.7
	B-	1	1.7
	B+	7	11.6
	O+	20	33.3
	O-	2	3.3
Total		60	100.0
Parity	Null	1	1.7
	1-3	10	16.7
	4-6	29	48.3
	≥7	20	33.3
Total		60	100.0
Symptoms	Menorrhagia	51	85.0
	PMB	7	11.7
	Metromenorrhagia	2	3.3
Total		60	100.0
Endometrial thickness (means±SD) mm of the studied group/mm		9.27±4.5	

Figure 1 show that TB were positive in 6/60 (10.0%) of the patients and the rest 54/60 (90.0%) were negative by PCR test, while histopathological finding show that all patients 60 (100.0%) were TB negative.

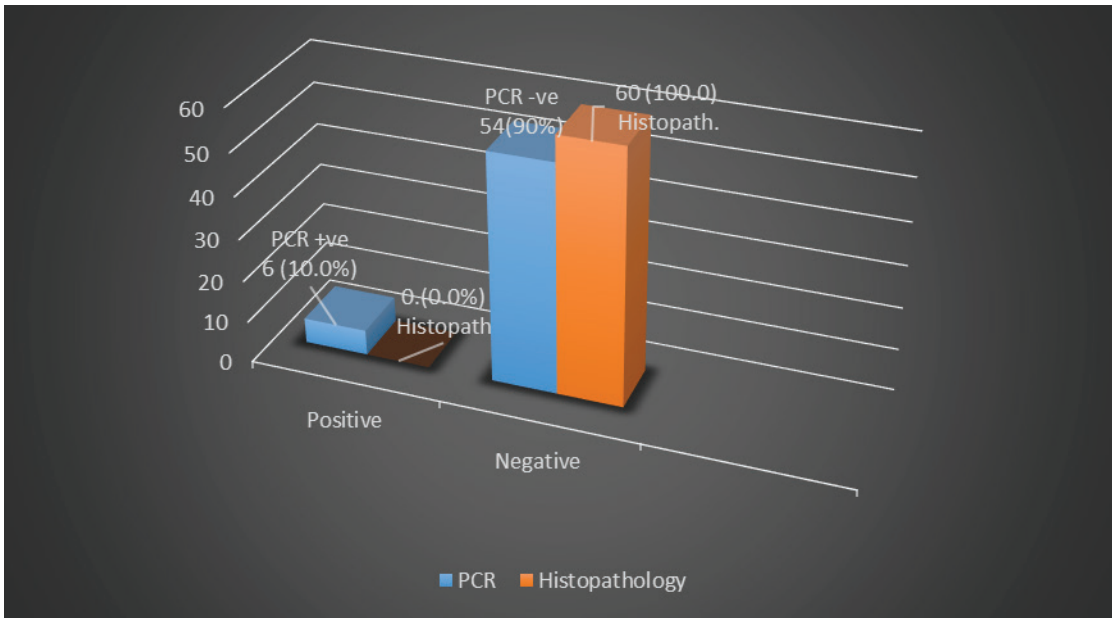

Figure 2: Distribution of the studied group according to PCR test and histopathological finding

As shown in table 2 all positive PCR were suffering from menorrhagia while no cases were found regarding other two symptoms (PMB and Metromenorrhagia). No significant difference was found between PCR outcome and symptoms of the disease (P≥0.05).

Table 2: Relation between PCR and symptoms

		Menorrhagia	PMB	Metromenorrahgia	P value
TB	PCR +ve (n=6)	6	0	0	0.5 Ns
	PCR -ve n=54	45	7	2	

Ns= Not significant

Table 3 show that there is no significant association between endometrial thickness, parity and blood group with TB patients regarding PCR outcome. Moreover, no significant association between means of endometrial thickness and age with TB patients diagnosed by PCR (P≥0.05) (table 4).

Table 3: Relation between different parameters and PCR outcome

		TB (PCR+) n=6	Normal (PCR-ve) n=54	P value
Endometrial thickness	≤5mm	2	8	0.2 Ns
	>5mm	4	46	

Cont... **Table 3: Relation between different parameters and PCR outcome**

Parity	Null	0	1	0.6 Ns
	1-3	0	10	
	4-6	4	25	
	≥7	2	18	
Blood group	A-	0	1	0.7 Ns
	A+	2	23	
	AB+	1	3	
	B-	0	1	
	B+	0	7	
	O-	0	2	
	O+	3	17	

Table 4: Relation between means of endometrial thickness and age with PCR outcome

	TB (PCR+) n=6	**Normal (PCR-ve) n=54**	**P value**
Endometrial thickness	11.8±4.67	8.98±4.5	0.1 Ns
Age	48.1±5.87	48.4±5.6	0.9 Ns

The validity results of histopathological findings regarding PCR test were sensitivity (0.0%), specificity (100%), +ve predictive value (0.0%), -ve predictive value (90.0%) and accuracy (90.0%). All these findings were shown in table 5.

Table 5: Validity test

Validity test		**PCR**		
		Abnormal (+ve)	**Normal (-ve)**	**Total**
		No. (%)	**No. (%)**	**No. (%)**
Histopathology	Abnormal	0 (0.0)	0 (0.0)	0 (0.0)
	Normal	6 (10.0)	54 (90.0)	60 (100.0)
	Total	6 (10.0)	54 (90.0)	60 (100.0)
Sensitivity		0.0%		
Specificity		100%		
+ve predictive value		0.0%		
-ve predictive value		90.0%		
Accuracy		90.0%		

Fisher exact test

Discussion

The incidence of FGTB differs worldwide; this is due to differences in studied groups, social stigma, the type of tests used to diagnose TB and its sensitivity and specificity, and the time at which the sample was taken with respect to the menstrual cycle. Although genital TB occurs secondary to pulmonary TB through hematogenous and lymphatic routes, primary genital TB can occur rarely when the male partner has genital TB such as TB epididymitis by transmission of infected semen.

Diagnosis of TB endometritis needs high index of suspicion because the disease is a symptomatic in majority of cases in addition to the paucity of the bacteria that's why the diagnosis require a test with high sensitivity and specificity to detect the disease in early stages. [13, 14]

Depending on the fact that real time PCR has a high sensitivity and specificity in detecting mycobacterium tuberculosis and histopathology usefulness in detecting endometrial pathologies we conducted this study. In Histopathology the diagnosis of tuberculous endometritis based on the presence of epitheloid cell granulomas with or without Langerhans giant cells, and caseation necrosis which is seen on late disease (1).

In our study, out of 60 patients complaining from abnormal uterine bleeding in form of menorrhagia, metro menorrhagia and polymenorrhoea, endocervical swabs revealed that 6(10%) of those women were found to be positive for mycobacterium tuberculosis by real time PCR while histopathology detect only one case(1.7%) which is also positive by real time PCR and have positive history of pulmonary TB, the low detection rate of histopathology, in the current study, can be explained either to an early stage of the disease or the continuous shedding of the endometrium specially in our study when all the PCR positive women complain from menorrhagia, or due to presence of focal lesions which may be missed by curette. meaning that histopathology is false negative in 5 patients and thus give a sensitivity and specificity for real time PCR of 100% and 92% respectively.

These results when compared to study conducted by Malhorta et al [8], who reported an overall positivity by real time PCR alone 23.78% which is higher than in our study (10%) and a sensitivity of 94.28% which is approximate to current study.

Whereas Thangappah et al [9] study showed tuberculosis were positive in 36.7% by PCR and 6.9% for histopathology giving a lower sensitivity 57.1% for PCR and higher sensitivity for histopathology10.7% as compared to our study which was explained by Thangappah as cyclical shedding of the endometrium resulting in to poor formation of the granuloma and thus the endometrium will not show evidence of tuberculosis in all cycles so multiple specimens from different locations is advisable.

Our efforts in this study was to decrease the false positive cases, all the patients complain from abnormal uterine bleeding, have no clinical sign or symptoms of TB except one known case of pulmonary TB.

The other five out of six positive cases detected by PCR could be explained by early stages of the disease and the bacteria known to be indolent in genital tract or latent disease detected by PCR and the patient still without symptoms.

Other sources of false positivity could be due and the presence of dead bacillus or previous infection which was excluded by past medical history other causes could be the contamination which was overcome in this study by the type of PCR kit which includes the enzyme uracil-DNA glycosylase to reduce the risk of contamination, also the kit contains internal control (STI-87) used in the extraction procedure in order to control the extraction process of each sample individually and thus recognition of any reaction inhibition, furthermore the kit uses (hot-start) which greatly reduces the frequency of nonspecifically primed reaction [9] [12].

Conclusion

Real time PCR considered one of the valuable diagnostic tools for mycobacterium tuberculosis, and because Iraq still showing high incidence of the disease, one should suspect mycobacterium tuberculosis in any women complaining from abnormal uterine bleeding and offer PCR in addition to other diagnostic modalities, luckily it will allow treatment at an early stage of the disease.

No conflicts of Interest

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