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The Link between Chlamydial Trachomatis and Peri-menopausal Bleeding: A Cross Sectional Study

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ABSTRACT

Objective: This cross sectional clinical trial aimed at exploring the association between chlamydia trachomatis infections and peri-menopausal bleeding presenting at Al-Azhar University Maternity Hospital outpatient clinics.

Patients and methods: This was a cross-sectional study involving 150 women divided into two equal groups of 75 women in each group. Women were allocated from the out-patient clinic of Al-Azhar Maternity University Hospital and they were counseled and arranged for Pipelle endometrial samples. Women of group I suffered from peri-menopausal bleeding while group II were presented at the hospital due to any cause other than vaginal bleeding. Pipelle endometrial biopsy was taken and sent for detection of Chlamydia trachomatis by real time PCR.

Results: In group-I, 44 (58.7%) samples were positive for Chlamydial infection while the other 31 (41.3%) samples were negative for Chlamydia. In group II 17 (22.7%) samples were PCR positive. There was an apparent significant difference between the two groups regarding the prevalence of C. trachomatis among symptomatic peri-menopausal women suffering from uterine bleeding.

Conclusion: It is advisable to screen for Chlamydia trachomatis in women with peri-menopausal bleeding.

Keywords: Chlamydia endometritis; Peri-menopausal bleeding.

INTRODUCTION

Worldwide, C. trachomatis is considered the most prevalent sexually transmitted bacterial disease. C. trachomatis is responsible for a diverse spectrum of genital infections as cervicitis, endometritis and salpingitis. Pelvic inflammatory disease (PID) is a major problem affecting the female genital system, C. trachomatis is the main organism causing it. Infertility in women and men, ectopic pregnancy and chronic pelvic pain, epididymitis and proctitis and arthritis in both men and women are spectra and common sequelae of this infection [1].

Pregnancy complications and implantation failure after in vitro fertilization also are reported as common sequels of these bacteria [2–5]. Young adolescent females having, multiple sexual partners with unprotected intercourse, are at highest risk to contract C. trachomatis. In about 70% of the cases, the progression of infection from the lower into the upper genital tract occurs without symptoms (subclinical PID) [1]. Reproduction is often delayed until a woman reaches her twenties, thus, early detection of C. trachomatis infection to prevent permanent tissue damage to the upper genital tract is critical. Histopathological and microbiological assessments of the endometrium have been useful in identifying cases of subclinical PID. Quantitative changes in endometrial histopathology and microbiology were observed as the disease progressed from the lower to the upper genital tract in these women [6–8].

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The diagnosis of endometritis based on the number of neutrophils in the glandular epithelium and plasma cells in the endometrial stroma, however, continues to be problematic [9]. This is likely due to different levels of experience of the observers in identification of plasma cells and neutrophils.

The presence of chlamydial antigens in the endometrium has also been linked to abnormal uterine bleeding (AUB) in women on birth control pills [10]. Because so many young women present with this condition, AUB is the most common clinical diagnosis for patients of reproductive age who undergo minor gynecologic procedures. The condition is initiated by either an infectious process (endometritis) or by structural alterations of micro vessels [11]. Whether AUB is a risk factor for subclinical PID remains unknown. In a proof of the concept study AUB improved after antibiotic treatment, lending further support to an infectious etiology of this condition.12 In spite of the extensive research of the endometrium in subclinical PID and the suggestion for an association between AUB, plasma cell endometritis and C. trachomatis, there remains an absence of markers for identification of C. trachomatis infection of the uterine lining.

This cross-sectional study utilizing endometrial samples aimed at confirmation of the possible relation between the clinical diagnosis of AUB and C. trachomatis infection of the endometrium.

PATIENTS AND METHODS

This was a cross-sectional study performed in Al-Azhar Maternity University Hospital involving 150 women; group I: 75 cases with peri-menopausal bleeding and a control group II: 75 peri-menopausal women with normal menstruation attending gynecologic outpatient clinic, for any reason other than bleeding. Pipelle endometrial biopsies were collected and sent for detection of *Chlamydia trachomatis* by PCR. This study was carried out in the period from January 2014 to December 2015. Explanation of the procedure and verbal consent was taken from every patient.

Inclusion criteria:

1. Peri-menopausal females; age 40 – 50 years.
2. Complain of dysfunctional uterine bleeding in group I
3. No gross uterine lesions were detected by vaginal US.

Exclusion criteria:

1. Patients who are immediately post-partum or post abortion or known cases of sexually transmitted diseases
2. Patients with any uterine abnormality detected by transvaginal sonar or hysteroscopy.
3. Patients with suspicion of pregnancy or malignancy.

All patients were subjected to:

1) Full history taking and physical examination.

2) Counseling and verbal consent was taken from every patient.

3) Pipelle endometrial biopsies were taken and sent to confirm *Chlamydia trachomatis* endometritis by real time PCR. The endometrial biopsy was placed into a transport medium composed of 2-sucrose phosphate buffer (PH 7.0) supplemented with 5% fetal bovine serum, 50 ug of streptomycin / ml, 100 ug of vancomycin per ml and 12.5 ug of amphotericin B (Fungizone) per ml (Phosphate Buffer Saline).

Detection of the *C. trachomatis* DNA in the collected specimens

A- Extraction of DNA: This was performed using the QI Amp DNA mini kit (QIAGEN GmbH, Hilden, Germany Cat. No.51304) as described by the manufacturer.

B- Real-time PCR assay:

Each run included the testing of the positive control, Tris-EDTA buffer in four reactions (no-template controls), and diethyl-pyrocarbonate-treated water (QIAGEN Germany) in duplicate reactions (negative reagent controls). The no-template controls and negative reagent controls were used to detect any nonspecific fluorescent signal or carry-over contamination. Run acceptability required obtaining the expected results from each control. Samples were considered positive if the amplification plots (i.e., change in normalized reporter signal versus PCR cycle number) showed definite exponential increase in fluorescent signal. If the fluorescent signal did not increase within 45 cycles, the sample was considered negative.

Statistical methodology: Retrieved data were recorded on an investigative report form. The data were analyzed with SPSS® for Windows®, version 15.0 (SPSS, Inc, USA). Description of quantitative (numerical) variables was performed in form of mean, standard deviation (SD) and range. Description of qualitative (categorical) data was performed in the form of numbers and percent. Analysis of numerical variables was performed by using student's unpaired t-test (for two groups) or ANOVA (for more than two groups). Analysis of categorical data was performed by using Fischer's exact test and Chi-squared test. Significance level was set at 0.05.

RESULTS

This cross-sectional study involved 150 women consented to participate in this study; group I (test group): 75 cases with peri-menopausal bleeding and group II (control group): 75 peri-menopausal women with normal menstruation recruited from outpatient clinic, and complaining from any reason other than bleeding. Pipelle endometrial biopsies were collected and sent for detection of *C. trachomatis* by real time PCR. Both groups were comparable in terms of age, body mass index, gravidity, parity, duration of marriage, frequency of coitus per week, mode of delivery (vaginal or cesarean), level of education, occupation (house wife or employed/business woman) and previous use of IUCD or hormonal methods as shown in **Table 1**.

Table 1. Clinic-demographic data of the population under study

	Group I	Group II	P- value
Age (years)	46.7 ± 4.8	46.3 ± 3.9	> 0.05
Body mass index (kg/m²)	31.4 ± 3.6	32.1 ± 3.1	> 0.05
Previous gravidity	4 ± 1.2	3.8 ± 1.4	> 0.05
Previous parity	3.2 ± 0.3	3 ± 0.1	> 0.05
Duration of marriage	23.8 ± 2.8	24.3 ± 2.5	> 0.05
Frequency of coitus per week	3.1 ± 0.4	2.9 ± 0.2	> 0.05
Mode of delivery **Vaginal** **Cesarean**	 42 23	 49 26	> 0.05
Education **≤High school** **>High school**	 27 48	 23 52	> 0.05
Occupation **House wife** **Employed/business Woman**	 61 14	 59 16	> 0.05
Previous use of IUCD	59	52	> 0.05
Previous use of hormonal contraception	62	65	> 0.05

In group I, 44 (58.7%) specimens were positive for Chlamydia and the other 31 (41.3%) specimens were negative for Chlamydia, while in group II only 17 (22.7%) specimens were PCR positive for chlamydia. There was a significant difference between the two groups about the prevalence of *C. trachomatis* among symptomatic peri-menopausal women complaining of abnormal uterine bleeding (**Table 2**).

Table 2. Number and percent of positive cases for *C. trachomatis* by PCR in the 2 groups of the study

	Group I	Group II	P value
Positive cases	44 (58.7 %)	17 (22.7 %)	< 0.05 (significant)
Negative cases	31 (41.3 %)	58 (77.3 %)	

There was no statistically significant correlation (P value > 0.05) between the presence of chlamydial endometritis in peri-menopausal women and the age of participants, parity, form of bleeding, history of abortion, type of infertility and duration of marriage (**Table 3-8**).

Table 3. Relation between the age and the presence of Chlamydial infection in perimenopausal women

Age (years)	PCR for Chlamydia					
	Negative		Positive		Total	
	No.	%	No.	%	No.	%
40 - 45 y	42	28	29	19.3	71	47.3
45 – 50 y	47	31.3	32	21.3	79	52.7
Total	89	59.3	61	40.7	150	100.0
P-value	> 0.05					

Table 4. Relation between parity and the presence of Chlamydial infection in perimenopausal women

Parity	PCR for Chlamydia					
	Negative		Positive		Total	
	No.	%	No.	%	No.	%
1- 3	45	30	30	20	75	50
4 or more	44	29.3	31	20.7	75	50
Total	89	59.3	61	40.7	150	100.0
P-value	> 0.05					

Table 5. Relation between the form of abnormal uterine bleeding (AUB) and the presence of Chlamydial infection in perimenopausal women

Uterine bleeding	PCR for Chlamydia					
	Negative		Positive		Total	
	No.	%	No.	%	No.	%
Regular	39	26	27	18	66	44
Irregular	50	33.3	34	32.7	84	66
Total	89	59.3	61	40.7	150	100.0
P-value	> 0.05					

Table 6. Relation between history of abortion and the presence of Chlamydial infection in perimenopausal women

History of abortion	PCR for Chlamydia					
	Negative		Positive		Total	
	No.	%	No.	%	No.	%
Positive	40	26.7	32	21.3	72	48
Negative	49	32.7	29	19.3	78	52
Total	89	59.3	61	40.7	150	100.0
P-value	> 0.05					

Table 7. Relation between infertility and the presence of Chlamydial infection in perimenopausal women

Infertility	PCR for Chlamydia					
	Negative		Positive		Total	
	No.	%	No.	%	No.	%
Fertile	52	34.7	32	21.3	84	56
Primary	23	15.3	19	12.7	42	28
Secondary	14	9.3	10	6.7	24	16
Total	89	59.3	61	40.7	150	100.0
P-value	> 0.05					

Table 8. Relation between duration of marriage and the presence of Chlamydial infection in perimenopausal women

	PCR for Chlamydia		P-value
	Negative	Positive	
	Mean ± SD	Mean ± SD	
Duration of marriage	24.9 ± 8.43	25.80 ± 8.19	> 0.05

DISCUSSION

AUB and C. trachomatis infection of the endometrium have a strong link as shown by the results of the current study. When plasma cells were absent, AUB alone lost its diagnostic value, while plasma cells alone without peri-menopausal bleeding remained predictive. This is possibly due to many factors linked to the diagnosis of bleeding. Histochemical staining with Syndecan-1 increases the plasma cells thus assisting in the diagnosis of endometritis. Syndecan-1 staining increases the identification rate of plasma cells between 10-50%, and is very useful to the less experienced pathologist allowing for detection of many plasma cells that are lacking the characters (clock-face chromatin, eccentrically placed nucleus and peri-nuclear halo) [12-14].

In the current study we found that Pipelle endometrial biopsies were collected and sent for detection of C. trachomatis by real time PCR. Both groups were comparable in terms of age, body mass index, gravidity, parity, duration of marriage, frequency of coitus per week, mode of delivery (vaginal or cesarean), level of education, occupation (house wife or employed/business woman) and previous use of IUCD or hormonal methods.

In group I, 44 (58.7%) specimens were positive for Chlamydia and the other 31 (41.3%) specimens were negative for Chlamydia, while in group II only 17 (22.7%) specimens were PCR positive for chlamydia. There was a significant difference between the two groups about the prevalence of C. trachomatis among symptomatic peri-menopausal women complaining of abnormal uterine bleeding.

There was no statistically significant correlation (P value > 0.05) between the presence of chlamydial endometritis in peri-menopausal women and the age of participants, parity, form of bleeding, history of abortion, type of infertility and duration of marriage.

In this group of patients, the presence of plasma cells correlated well with a positive C. trachomatis result but only at a higher number (>5 PCE) than expected. The presence of 'any number of plasma cells' was a much weaker predictor.

The number of C. trachomatis positive cases in this group of patients is quite high (48%). Selection bias may play a role. However, similarly high rates (38%) had been supported by Kiviat et al. in a group of women with suspected subclinical PID. Using species-specific monoclonal antibodies, these investigators also found C. trachomatis in 18% of culture negative specimens [15]. As it was stated before, close to one-third of our patients had at least previous evidence of a previous infection. Immunocytochemistry methods employing monoclonal antibodies on tissue samples appear to be superior to culture and/or to amplification assays. Antigen detection by monoclonal antibody in women who are culture or PCR negative likely represents a subclinical persistent infection that may have been undetected or inadequately treated [16,17]. Recent data confirmed that presumed recurrent infections by the same serovar of Chlamydia are indeed due to persistence as evidenced by outer membrane protein (Omp1) genotyping [18].

We believe that this study has identified a group of women with persistent C. trachomatis infection. These are relatively older women dealing with the late consequences of an earlier, missed or incompletely treated chlamydial infection.

The strong correlation between endometrial macrophage count and C. trachomatis infection is a novel finding. Macrophages have been described as the missing link in the pathogenesis of C. trachomatis induced (previously 'reactive') arthritis and are considered the vehicle by which C. trachomatis arrives to the synovial tissue from its point of

entry [19,20]. Chlamydia trachomatis infected macrophages can significantly induce the apoptosis of autologous T-cell lymphocytes, the most important defense line in C. trachomatis infection, allowing the bacterium to subvert the immune system and develop the persistent state [20].

The major limitations of this retrospective study are the lack of cervical/vaginal microbiology specimens and serum samples to confirm chlamydial infection. It is feasible that many of our study patients had had a negative lower genital tract C. trachomatis test.

Based on the data we suggest that the effect of C. trachomatis on women of reproductive age is overwhelmingly underestimated. An asymptomatic, preconceptional, persistent infection may lead to pregnancy complications in reproductive aged women. AUB in women of reproductive age should raise the suspicion of a persistent C. trachomatis infection. Endometrial specimens from these women should be examined for the presence of PCEs. Our data showed that Syndecan-1 could enhance PCE diagnosis in a clinical setting and that an elevated macrophage count might indicate the presence of C. trachomatis in the endometrium in a research setting. Large, prospective studies are needed to confirm a cause-effect relationship between C. trachomatis and AUB.

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