Diagnostic Procedures for Detection of Chlamydia Trachomatis Cervical Infections

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272 women complaining of vaginal discharge were examined in the outpatient department of LWH and Jinnah hospital Lahore, these included 122 pregnant and 150 non-pregnant. The cervical samples were taken for detection of Chlamydia trachomatis by various methods. Gram staining was used as a screening method and to rule out other bacterial and fungal causes. Positivity with this method for chlamydia was 78.68% in pregnant and 85.34% in non-pregnant women. Pap stained cervical smears revealed 18.85% positivity and 21.33% in the non-pregnant group. ELISA was used as the most specific diagnostic method for direct detection of chlamydial antigen from cervical secretions. Reactivity in the pregnant group was 8.19% and in the non-pregnant 12.66%. Results of the three compared and their correlation was found to be significant.

Key words: Chlamydia trachomatis cervical infection, diagnostic procedure

Chlamydia trachomatis is one of the most common sexually transmitted pathogen[1]. It is a major cause of cervicitis, urethritis, endometritis, and pelvic inflammatory disease (PID) in women[2]. Serious complications can result in salpingitis', infertility and ectopic pregnancy[3]. It is transmitted to infants during birth, chlamydia can cause conjunctivitis and pneumonia. 50-70 percent of infected women are asymptomatic, which makes diagnosis extremely important[4].

Chlamydia are related to gram negative bacteria. They are intracellular in nature and are unable to synthesize adenosine triphosphate (ATP). The extracellular elementary body form is infectious, while the intracellular reticulate form is metabolically active[5].

Epidemiological patterns indicate infections of Chlamydia trachomatis parallel or exceed those of Neisseria gonorrhoea and the two often occur together [6]. The disease cuts across the socioeconomic spectrum.

The primary methods for detection of chlamydia include staining, Grams and Papanicolaou stains and growth of the organism in cell culture[7]. Other methods include Direct Fluorescence Assays (DFA), Enzyme Linked ImmunoSorbent Assay (ELISA) and Polymerase Chain Reaction(PCR)[8 9].Cultures are not widely available as they are costly demand strict transport requirement and 5 days delay before the results are available. IF requires expensive reagents and immunofluorescent microscope. PCR also requires specialized skill and equipment[10]. Thus in our study endocervical swabs were examined for chlamydia by Grams, Pap staining and ELISA.

Material and Methods

The study was conducted on women attending the Gynaenocology and Obstetric outpatients departments of Lady Willingdon (LWH) and Jinnah Hospital Lahore. These included a total of 272 women out of which 150 were nonpregnant and 122 pregnant. A detailed general history and specific history was taken regarding cervicitis, cervical erosions, PID, ectopic pregnancy, habitual abortions, still births, PROM, and infertility. Relevant gynecological examination was carried out and smears taken from ectocervix by rotating cotton tipped applicator outside external os for Grams and Pap stain and STD EZE swabs for detection by ELISA using Chlamydiazyme kit by Abbott.

Grams staining was used as a criteria for selection of patients by counting the number of polymorphonuclear leucocytes (PMNL) per high power field (HPF). Our aim was to see whether cervical leucocytosis on Gram smear was a useful screening test before confirmation with Pap stain and ELISA, and to rule out bacterial causes of cervical infections. Smears with more than 10 WBC/H1PF on Gram stain were analyzed by Pap stain and ELISA.

Purpose of Pap stain of cervical smears was to see the various types of inflammatory cells, cellular inclusions and other cytological changes which support the diagnosis of Chlamydia trachomatis cervical infections. The ELISA uses an enzyme system to show the specific combination of an antigen with its antibody. Direct antigen, detection technique was used.

Results

A total of 272 women with vaginal discharge were assayed for the presence of chlamydial antigen in cervical smears by Grams, Pap staining and ELISA from obstetric and Gynaecological OPD of LWH and Jinnah hospital .The smears were tested in two batches 150 non pregnant and 122 pregnant. According to Gram's staining 96 (78.68%) women were positive for chlamydia whereas 23 (18.85 %were reactive on Pap staining: In the non pregnant 128 (85.34 %) were positive on Gram and 32 (21.24 %) on Pap staining. The correlation of Gram and pap staining between pregnant and non pregnant was highly significant ($P<0.05$) Table 1. Out of 122 pregnant women 10 (8.19 %) were reactive whereas in the 150 non-pregnant group 19 (12.66 %) were reactive for chlamydia by ELISA. The

correlation of Grams staining with ELISA in the groups studied was highly significant (P< 0.05) Table 2. 23 (18.85 %) of 122 pregnant women were positive on Pap staining and 10 (8.19 %) were reactive on ELISA.

In the 150 non-pregnant 32 (21.34 %) were positive on Pap's staining and 18 (12.66 %) were reactive on ELISA. The correlation between Pap staining and ELISA was highly significant (P<0.05) Table 3.

Table 1. Correlation of Gram and Papanicolaou stain

	Gram staining		Pap staining	
	Positive	Negative	Positive	Negative
Pregnant N=122%	96 (78.68)	26 (21.32)	23 (18.85)	99 (81.15)
Non pregnant N=150%	128 (85.34)	22 (14.66)	32 (21.33)	118 (78.66)

Table.2. Correlation of Grams stain with ELISA

	Gram staining		ELISA	
	Positive	Negative	Positive	Negative
Pregnant N=122%	96 (78.68)	26 (21.32)	10 (8.19)	112 (91.81)
Non pregnant N=150%	128 (85.34)	22 (14.66)	19 (12.66)	131 (87.34)

Table. 3. Correlation of Pap stain with ELISA

	Gram staining		ELISA	
	Positive	Negative	Positive	Negative
Pregnant N=122%	23 (18.85)	99 (81.15)	10 (8.19)	12 (91.81)
Non pregnant N=150%	32 (21.34)	118 (78.66)	19 (12.66)	131 (87.34)

Discussion

Chlamydia trachomatis infections are common in sexually active adolescence and young adults- more than 4million-chlamydial infection occur annually in U.S.A[1]. Infection by this organism is insidious and symptoms are minor among most infected men and women[2,3]. The large group of asymptomatic and infections persons sustain transmission within a community "The substantial long term morbidity from chlamydia in women, high prevalence of asymptomatic" infection and the availability of reliable screening tests and effective treatment suggest screening for chlamydial infection a useful strategy [11].

In the present study, Gram's staining was performed on all the 272 cervical smears. The most important aspect was looking for bacteria specifically Gram negative diplococci (Neisseria gonorrhoea) a pathogen likely to be found in the cervix[12] Gram's staining was also used as a criterion for detection of chlamydial infections by counting the number of PMNL/HPF. Gram staining of the cervical smears of our present study revealed 78.68% positivity in the pregnant group and 85.34 % in the non-pregnant group . This helps in the detection of such infections as mentioned by Binns et al 1988[13]. Our aim was to see whether cervical leucocytosis on Gram smear was a useful screening test before confirmation with Pap and ELISA and to rule out other bacterial causes of cervical infections .Comparing ELISA there is a large number of false positivity making it a non-specific diagnostic test for chlamydia .

In the present study cervical smears were also stained by Pap stain chlamydial infection was suspected if there was an increase in number of inflammatory cells. Slides with increased histiocytes lymphocytes were interpreted as positive for chlamydia .Using the above criteria our present study included 18.85 % in pregnant and 21.33 % in non-pregnant women which is similar to 15.5 % by Shafer et al 1985[15] and 33% by Quinn et al 1987[14]. Of the 122 pregnant women 23(18.85 %) were positive for chlamydia on Pap staining and 10(8.19 %). were interpreted as reactive by ELISA. The positivity in 150 non-pregnant was 10% higher (21.34%) on Pap staining as compared to ELISA (12.66%).

To find out reason for false positivity of 10 % on Pap staining cultures were performed on cervical swabs of women of same status. Growth of normal commensal 27 % and staphlococcus aureus in the rest of 73 %: Trichomnas vaginalis was also isolated. This gave an indirect explanation and a reason for 10 % more positivity on Pap. There were 2 false negative cases in the non-pregnant group by Pap smear, which may be either due to non-representative smears or because of some sub clinical infection, which failed to show changes on cervical smears. Pap smear is thus suggestive of Chlamydia trachomatis infection but is not a specific marker for final identification[16]. Simple methods like cervical cytology (Gram Pap) should by performed on all women with infections of the lower genital tract and where possible positive cases should be subjected to more accurate and specific methods like ELISA[17,18,19].

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