Efficacy of Cytology for the Diagnosis of *Chlamydia trachomatis* in Pregnant Women

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This study evaluated the effectiveness of Papanicolaou staining for the initial diagnosis of Chlamydial infection in pregnant women. A hundred thirteen patients were examined with a Papanicolaou test, independent of gestational age, parity or maternal age. Three endocervical samples were collected; the first two were collected with a brush (Cytobrush plus, Mediscand, Sweden) and the third with Ayre's spatula. The first specimen was used for McCoy cell culture and the other two were examined cytologically. Chlamydial infection was detected in 9 (7.9%) patients. Only one (0.8%) was diagnosed by cytological exam. The sensitivity and specificity of the cytological examination were 10 and 98%, respectively. The estimated positive predictive value was 33.3% and the negative predictive value was 92.7%. When Papanicolaou stain diagnosis suggests Chlamydia, a more specific complementary exam should be added to confirm infection; subsequently adequate treatment can be implemented, thereby preventing the frequent complications of untreated subclinical infections.

Key Words: *Chlamydia trachomatis,* pregnancy, infection, and cervical cytology.

Chlamydia trachomatis, an obligate intracellular pathogen, has been considered a bacterium since 1964. There are three biotypes: a rat pneumonia agent, a venereal disease agent and an eye-genital disease agent [1]. This species obtains its energy from host cells, and it preferentially infects the genital epithelia and the eyes [2]. This agent is responsible for more than three million cases of infection per year in the United States [3]. Chlamydial infection can be asymptomatic in women, as well as in men and newborns. In women, recurrent discharges, urethritis and cervicitis, pelvic inflammatory illness, ectopic pregnancy, infertility and chronic pelvic pain, suggest *C. trachomatis* infection [4,5]. An association between genital infection with Chlamydia and cervical intraepithelial neoplasia and cervix carcinoma has been reported [6]. This agent also increases the risk of infection by the human immunodeficiency virus [7]. In men, it can cause non-gonococcic urethritis, acute epididymitis, infertility, chronic prostatitis and other complications [8]. In pregnant women, *C. trachomatis* infection can affect pregnancy outcome, causing preterm labor, puerperal endometritis, low-weight in the newborn, as well as conjunctivitis and neonatal pneumonia [9-11]. New diagnostic methods have appeared in recent years; however, these are still inaccessible for most of the population. The available laboratorial tests for the detection of Chlamydia include McCoy cell culture, direct immunofluorescence, enzymatic immunoassay, hybrid capture, and polymerase chain reaction (PCR); the latter gives the greatest sensitivity. Indirect diagnosis tests, such as a search for specific

antibodies and Papanicolaou staining, though they have low sensitivity, are often the only available examination for many women [12,13]. The culture technique, which is a diagnostic method with high sensitivity and specificity (80% and 100%, respectively), is costly, time consuming, and requires considerable expertise, which limits its use in large healthcare centers [12]. The Papanicolaou stain, well established for the detection of pre-cancer cervix lesions, has been used as alternative method for the diagnosis of the *C. trachomatis* [14]. Considering that the Papanicolaou stain is routinely used in all the medical assistance services that attend woman, we evaluated its effectiveness for the initial diagnosis of Chlamydia infection in pregnant women.

Ethical aspects

The voluntary agreement of the patients was assured, as they signed an informed consent term. This research was approved by the Ethics Committee for research of the Federal University of São Paulo - UNIFESP. The authors affirm the inexistence of a conflict of interest (professional, financial and indirect or direct benefits) that could influence the results of the research.

Material and Methods

We studied 113 pregnant women in prenatal care at UNIFESP from October 1992 to September 1994. The patients were examined with a Papanicolaou test, whenever they had not been tested previously, independent of gestational age, parity and maternal age. Exclusion criteria were: obstetrics complications that could be aggravated by cervical manipulation or antibiotic use during the four preceding weeks. Three endocervical samples were collected; the first two with a brush (Cytobrush plus, Mediscand, Sweden) and the third with Ayre's spatula. The first specimen was used for culture

and the other two were examined cytologically. The specimens for McCoy cell culture were placed in test tubes containing glass pearls and were kept under refrigeration, while they were transported to the Adolph Lutz Institute Chlamydia and Rickettsiae Laboratory. They were prepared in petri dishes containing 2SP1 solution. The samples were then inoculated, in duplicate, into cell culture bottles containing prepared McCoy cells.

The culture bottles were centrifuged at 2500 x g at 30°C for one hour. After centrifugation, the inoculates were removed and 1ml of half minimum modified Eagle medium supplemented with cycloheximide (2μg/mL) was added. The identification of Chlamydia was made using an indirect immunofluorescence reaction (IFR). The antibody was human serum for LV (Lymphogranuloma venereum); human serum anti-IgG conjugated with fluorescein was used to reveal the reaction. The reaction was made in only one of the culture bottles; the other was used to process successive passage into new cell culture bottles. The cultures that were considered positive presented one or more typical inclusions, when the fluorescence intensity in the plates was two plusses (+ +) or more, on a scale of 0 to 4 plusses. The cultures were considered negative if no inclusion was found after the second passage. The Papanicolaou test was made on two previously identified samples; one had the endocervical specimens collected with the cytobrush and second was the specimen obtained with Ayre's spatula from the vagina and ectocervix, including the transformation zone. After fixing in 95% ethanol, the samples were stained with the Papanicolaou technique. The diagnosis of *C. trachomatis* infection was based on Gupta's alterations [15], which include the finding

of coccoid inclusion bodies in the vacuoles, in the perinuclear region or in the cytoplasm in a dense acidophilus aggregate, as well as irregular edge inclusions, encircled by a clear zone, presenting central condensation. The effectiveness of the cytological examination for *C. trachomatis* diagnosis was determined through the calculation of sensitivity, specificity, negative predictive values and false-negative probabilities.

Results

We detected endocervical and/or metaplasic cells in 31 samples (27.4%) collected by Ayre's spatula, while all the specimens obtained with the cytobrush showed these cellular types (Table 1). Cell culture was considered the gold standard diagnosis. Chlamydial infection was detected in 9 (7.9%) patients. Only one (0.8%) was diagnosed by cytological exam. The remaining 104 (92.1%) women did not have a positive culture for Chlamydia. The cytology presented similar negative values for 102 patients (90.3%) (Table 2). The sensitivity and specificity of the cytological examination were 10% and 98%, respectively. The estimated positive predictive value was 33.3% and the negative predictive value was 92.7%.

Discussion

Diagnosis of *C. trachomatis* infection is still critical, due to the high frequency of nonsymptomatic infections and the necessity of complementary exams. Screening tests for Chlamydia infection have reduced pelvic inflammatory disease incidence by 56% [16], confirming their importance in reducing morbidity due to Chlamydia infection in the genital tract. We

Table 1. Endocervical and/or metaplasical cell frequency in samples taken with Ayre's spatula and a cytobrush

Endocervical and/or metaplasical cells	Spatula		Spatula + cytobrush	
	N	**(%)**	**N**	**(%)**
Positive	31	(27.4)	113	(100)
Negative	82	(72.6)	0	(0)
Total	113	(100)	113	(100)

Table 2. Cytology and culture results for *Chlamydia trachomatis* in pregnant women

Cytology	Culture					
	Positive		**Negative**		**Total**	
	N	**(%)**	**N**	**(%)**	**N**	**(%)**
Positive	1	(0.8)	2	(1.8)	3	(2.6)
Negative	8	(7.1)	102	(90.3)	110	(97.4)
Total	9	(7.9)	104	(92.1)	113	(100)

used cell culture as a gold standard for Chlamydia infection. Advantages of cell culture include low probability of contamination and preservation of the microorganisms for future study [12]. The main disadvantage is the necessity of an expensive laboratorial infrastructure; it is also laborious and demands careful handling to maintain the microorganisms viable. However, this is compensated by 100% specificity and 80% sensitivity [12]. In our study the prevalence of Chlamydia infection among the pregnant women, determined by cell culture, was 8.0%, similar to literature values [7,17,18]. Some authors [18,19] have suggested that the cytological method can be used for screening of this infection, with the intention to use more specific tests later. The Papanicolaou stain is widely used in all medical assistance units that attend woman; that is why we evaluated the effectiveness of this cytological test for screening Chlamydial infection during pregnancy. To ensure that metaplasic and/or endocervical cells were available for cytological evaluation, the material was collected with a soft-bristle brush as well as with the traditional Ayre's spatula; this was done to avoid endocervical traumas. Due to the use of these brushes, endocervical and/or metaplasic cells were found in all samples, demonstrating the importance of using adequate tools for specimen collection, especially when looking for Chlamydia infection, which is preferentially found in endocervical cells (Table 1). This collection technique improved the vaginal samples, although it did not improve the sensitivity of the Papanicolaou test for Chlamydia infection diagnosis, as the prevalence indirectly diagnosed by Gupta's criteria was only 2.6% [15]. We found endocervical cells in 100% of the samples, while other studies [19,20] reported a lower percentage (64%) of satisfactory samples (with endocervical cells). The scarcity of endocervical cells in pregnant women samples could be explained by the extreme care of clinicians when manipulating the endocervical canal during collection. However, in ours and in other studies [21], "cytobrush" use did not induce any complications during gestation.

Using only Gupta's criteria [15] for the presumptive diagnosis of Chlamydia infection excludes the "search signals", which consist of eosinophilia, amphophilia, cytoplasm vacuolization and perinuclear halos [20]. These are also inherent to Chlamydia infection, which could have been present in some infected patients who were not identified. Other factors could also justify the low sensitivity of cytology that we observed in our study (10%), compared to other studies (62%) [13]; these include few infected cells in the samples and rupture of the inclusions at the time the samples were prepared [21, 22].

Conclusions

Considering that cytological exams are routinely made of women in all medical assistance services for, it would be useful to include a collection procedure directed towards the identification of Chlamydia infection, based on the high cytology specificity observed in our study (98%). When Papanicolaou stain diagnosis suggests Chlamydia, a more specific complementary exam should be added to confirm infection, so that adequate treatment can be implemented, avoiding the complications of untreated subclinical infections.

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