

PREVALENCE OF INFECTION WITH *NEISSERIA GONORRHOEAE* OR *CHLAMYDIA TRACHOMATIS* IN ACUTE MUCOPURULENT CERVICITIS

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The aim of this study was to determine the incidence of *N. gonorrhoeae* (NG) and/or *C. trachomatis* (CT) in acute mucopurulent cervicitis (MPC). The study included 617 non-pregnant women with MPC, who had not been receiving any antimicrobial treatment. The average age of patients was 22.2 years. There were no statistically significant differences according to place of residence, education, and marital status.

Samples for laboratory analysis were collected using a routine procedure; NG was identified using the cytochrome oxidase test and Gram staining. CT was isolated on McCoy cell culture and stained with Lugol solution.

NG was isolated in three women (0.8 %) and CT in 58 women (9.4 %). Fifty-six of the CT-positive patients were nullipara and only two were unipara. All NG-positive patients were also nullipara. The mean number of sexual partners was 2.2 in all study subjects, 2.4 in CT-positive subjects, and 2.9 in NG-positive subjects. Vaginal discharge purity according to Schröder was significantly deteriorated in CT-positive patients ($p=0.011$). When asked about the use of contraceptives, as many as 32.7 % patients answered that they did not use any protection, 39 % women used the rhythm method and *coitus interruptus*, 20 % were taking oral contraceptives, 6.1 % used mechanical devices, and 1.9 % used chemical protection. Previous acute and chronic pelvic inflammatory diseases correlated with MPC ($p<0.01$).

Our statistical analysis suggests that chlamydial infection significantly reduces the purity of vaginal discharge, which is more pronounced in nulliparae. Pap smear was not specific enough to demonstrate chlamydial infection. In view of the MPC findings, the prevalence of CT and NG infection is low.

KEY WORDS: *reproductive health, sexually transmitted diseases*

Mucopurulent cervicitis (MPC) is the most common inflammatory disease of the female genital system (1, 2). Although MPC is not reported in epidemiological surveys, it is perhaps the most widely spread infection of the female genital system. Cervicitis is quite difficult to diagnose because it is often asymptomatic. As aerobic and anaerobic bacteria are frequent in the cervical canal of women without symptoms and with normal cytological findings, it is often dubious whether a particular bacterium is the cause of the inflammation. Chronic cervicitis is histopathologically confirmed on almost every

cervical biopsy, and has no pathological significance. Simple criteria for the clinical diagnosis of MPC have only recently been developed. The inflammation is characterised by the presence of polymorphonuclear leukocytes (PMNL) in the cervical mucus (mucopus), which is of a characteristic yellowish colour. The cut-off point for the diagnosis of MPC is 30 PMNL per high-power field (HPF) (3). Cervicitis implies no special risk *per se*, unless caused by *Chlamydia trachomatis* (CT) or *Neisseria gonorrhoeae* (NG). These bacteria may cause endometritis, salpingitis, or perihepatitis by ascending migration and lead

to cervical intraepithelial neoplasia when infection becomes chronic (4-6). As many as 10 % of women with untreated cervicitis caused by CT or NG will develop pelvic inflammatory disease (PID) (7). In at least 15 % of all infertile women in the USA, their infertility is due to tubal lesion caused by PID resulting from an untreated sexually transmitted disease (STD). In pregnancy, chorioamnionitis, premature rupture of fetal membranes, and infection of the neonate may occur. MPC is most frequently associated with CT, *Gonococcus*, *Ureaplasma*, *Gardnerella*, and *Trichomonas* infection, bacterial vaginosis, and the use of oral contraceptives (1, 2). The real incidence of MPC is unknown; however, many studies show it to be quite common among young, sexually active women.

The aim of this prospective study was to determine the incidence of gonococcal and chlamydial infection in symptomatic cases of acute MPC diagnosed in a

female population of the Bjelovar area, Croatia, and to assess the presence and percentage of gonococcal and chlamydial coinfection.

MATERIALS AND METHODS

Subjects

This prospective clinical study included 617 non-pregnant women diagnosed with acute MPC in outpatient clinic. Their mean age was (22.2±4.2) years (range 16 to 40 years; Table 1). They had not been taking any systemic or local antibiotic therapy for six weeks before Pap smears.

Sample collection

Routine Pap smears were collected with patient's informed consent at the Department of Gynaecology

Table 1 Sociomedical variables of women with mucopurulent cervicitis

Variable	n	CT positive	NG positive	p value
Patients	617	58 (9.4 %)	3 (0.48 %)	
Age / Years	22.2 ± 4.2	20.3 ± 7.1	21.2 ± 2.1	> 0.5
Parity				
0	369 (59.8 %)	56		
1	160 (25.9 %)			
2	88 (14.2 %)	2	3	<0.01
Marital status				
Married	291 (47.1 %)	13	2	
Partner < 6 months	180 (29.1 %)	16		
Partner > 6 months	39 (6.3 %)	4		
Without actual partner	107 (17.3 %)	25	1	
Place of residence				
City	443 (71.7 %)	49	3	
Village	174 (28.2 %)	9		< 0.01
Education				
Primary school	99 (16 %)	7		
Secondary school	306 (49.5 %)	22		
University student	122 (19.7 %)	11		
University degree	90 (14.5 %)	18	3	
Number of sexual partners	2.2 ± 0.3	2.4 ± 1.0	2.9 ± 1.4	
History of diseases				
Acute PID	151 (24.4 %)	24	1	
Achronic PID	202 (32.7 %)	3		
Ectopic pregnancy	18 (2.9 %)	5		
Primary sterility	40 (6.4 %)	5		
Secondary sterility	59 (9.5 %)	1		
Without complications	147 (23.8 %)	20	2	

and Obstetrics, Bjelovar Community Health Center, Croatia from September 1998 to September 2001.

On endocervical culture collection, care was taken that the vaginal mirrors be free from any traces of disinfectant. Mucous discharge was removed from the exocervix using a sterile cotton wad to avoid specimen contamination with vaginal flora. The cervical canal then was wiped with a sterile cotton swab previously soaked in 0.9 % sodium chloride solution for about 15 seconds, which is long enough for the gonococci to absorb onto the cotton. The material from the swab was immediately applied evenly over a nutritive medium selective for NG (Gonoline, Biomerieux SA, France). Another sterile swab was then taken to absorb columnar cells from the endocervical canal. The swab was carefully taken out to avoid contact with the vaginal wall. Both specimens were referred to the Clinical Institute of Microbiology, Zagreb University Clinical Centre.

Methods

CT was cultured on McCoy cell medium (Thermo Fisher Scientific, Inc.), and the diagnostic slide was then stained with Lugol's solution. The transport medium used for gonococci was a modified Thayer-Martin medium (modified Mueller-Hinton agar with 5 % chocolate sheep blood and antibiotics, Sigma-Aldrich, Germany). It is used for culturing and primarily isolating *Neisseria* bacteria, including NG and *N. meningitidis*. After 48 hours of incubation, the gonococci were isolated by cytochrome oxidase reaction and stained according to Gram (8, 9). The Pap smear (vaginal, cervical and endocervical) was evaluated in the cytological laboratory of the General Hospital Bjelovar according to the Bethesda classification system (10).

Statistical analysis

We used Statistica® software for statistical processing, and chi-square test for data analysis.

RESULTS

Sociomedical variables of the women with acute MPC are presented in Table 1. NG was isolated in 3 (0.48 %) patients, whose mean age was 21.2 years. CT was isolated in 58 (9.4 %) patients, with mean age 20.3 years. The two groups did not significantly differ in age, place of residence, education, and marital

status. The mean number of sexual partners for the whole population studied was 2.2 (range 1 to 7). NG-positive patients had an average of 2.9 partners, while CT-positive subjects had 2.4 patients (Table 1). Vaginal discharge purity according to Schröder (6) was significantly deteriorated in CT-positive patients ($p=0.014$). Most study subjects (59.8 %) were nulliparae. Of the CT-positive patients 56 were nulliparae and two uniparae. All NG-positive patients were nulliparae. When asked about the use of contraceptives, 32.7 % of all subjects reported that they did not use any protection, while a similar proportion of patients used the rhythm (Ogino-Knaus) method and *coitus interruptus* (39 %). Others were using oral contraceptives (20 %), mechanical devices (6.1 %), or chemical protection (1.9 %). History of acute and chronic pelvic inflammatory diseases (PID) correlated with MPC ($p<0.01$). However, 23.8 % women with CT and two with NG reported no genital symptoms.

Cytological and microbiological findings of the vaginal-cervical-endocervical (VCE) smear are shown in Table 2.

DISCUSSION

According to the World Health Organization (WHO) data, some 250,000,000 new cases of STDs are recorded worldwide every year, with trichomonas infection accounting for almost 50 %, CT infections for 20 %, herpes simplex infection for 8 %, and HIV infection for 3 % of all cases (4). A 1995 pan-European study showed that the prevalence of chlamydial genital infection was 3.9 % (1.0 % to 7.4 %) (2, 11).

As our study included a selected population of women with a clear clinical picture of MPC, we expected a higher percentage of CT-positive findings than the 9.4 %. This low percentage is perhaps related to the use of the current Croatian "gold standard" - McCoy cell culture for CT isolation - with a sensitivity ranging between 50 % and 90 %, which is considerably lower compared to the methods of nucleic acid amplification with a sensitivity exceeding 95 %. Furthermore, CT was demonstrated upon staining with Lugol's solution (5, 9), which additionally reduces the method's sensitivity by about 10 % as compared with CT demonstration by a more expensive immunofluorescence method. The other reason for the unexpectedly low percentage of positive findings may be the fact that the study was conducted in Bjelovar, a

Table 2 Results of cytological screening in women with mucopurulent cervicitis.

Cytological finding	Microorganism								Total
	BV	MF	GV	HPV	HSV	Fungi	TV	CT	
Normal	12	-	-	-	-	-	-	-	12
Inflammation	106	289	28	13	2	30	46	16	530
ASCUS	1	4	1	9	1	1	12	1	30
CIN I	-	4	-	11	1	-	2	1	19
CIN II	-	4	-	2	-	-	-	1	7
CIN III	-	1	-	15	-	-	-	-	16
CIS	-	-	-	3	-	-	-	-	3
Total	119	302	29	53	4	31	60	19	617

Abbreviations: CT - chlamydia trachomatis; BV - Bacillus vaginalis flora; TV - Trichomonas vaginalis; MF - mixed vaginal flora; HSV - Herpes simplex virus; HPV - Human papilloma virus; GV - Gardnerella vaginalis; ASCUS - atypical squamous cells of undetermined significance; CIN - cervical intraepithelial neoplasia; CIS - carcinoma in situ cervicis uteri

town with a population of 40,000, who greatly differ in lifestyle from large cities of the world, with much higher prevalence of STDs.

In Croatia, the prevalence of CT infection was monitored in 1987 (18 %), 1988 (16.9 %), and 1992 (12.1 %) (12). Skerlev et al. (13) demonstrated CT infection in 240 men and 784 women in 1991, and in 272 men and 481 women in 1995. The female preponderance was attributed to better infection diagnosis on gynaecological examination rather than to the real sex distribution of genital chlamydia. The real incidence of CT in the population must be higher, but chlamydial infection is not always reported in practice.

In Austria, the prevalence of the disease is as much as 25 % in special population groups (14). In Sweden CT incidence declined from 107.2 per 1,000 in 1985 to 32.3 in 1993 (11). The prevalence of chlamydial infection was also investigated in Israel, but the female subjects examined were free from mucopurulent discharge. Chlamydial infection was demonstrated in 2.5 % of sexually active women, which was a significant decrease compared to the preceding two decades (15). A study of the presence of NG and CT infection in the lower genital system in women with salpingitis performed in Iceland included 225 women with laparoscopically or laparotomically verified salpingitis. NG was isolated in 18.9 % and CT in 38.5 % of the women (16).

Gonorrhoea incidence was monitored in nine western European countries between 1991 and 1996 (17). It dropped in respect to the preceding period, and the greatest drop was recorded in the Scandinavian countries. The study also found gonococcal infection to

be on the rise in England and Wales in age groups older than 25 years. In another study (18), the incidence of gonorrhoea showed a rising tendency all over England in 1998-1999 by 30 % among women and heterosexual men and by 10 % among homosexual men. The reason for this increase remains unknown. Poor sexual health, practicing unsafe sex, and increased antimicrobial resistance may have contributed to the phenomenon.

According to some other studies, the age group between 15 and 24 years is the major risk factor for chlamydial infection, irrespective of the presence of discomforts, previous infection, use of mechanical contraceptive devices, or number of sexual partners (11-14, 19-24). Our study showed that mucopurulent discharge was a discomfort generally associated with younger women (mean age 22.2 years), while the mean age of CT-positive patients was 20.1 years.

Another major risk factor for STD is the number of sexual partners (22, 23, 25). In our study, the mean number of sexual partners was 2.2 per subject. We believe that the real number is higher, as in our socio-cultural milieu women tend to under-report the number of their sexual partners. The mean number of sexual partners in CT-positive women was 2.4, and did not significantly differ from the whole. Louv et al. (23) compared the prevalence of chlamydial and gonococcal infection between women taking oral contraceptives and those who did not use them. The two groups were matched according to demographic characteristics and habits. The prevalence of infection was significantly higher in the group taking oral contraceptives, i.e. 70 % for either pathogen. The prevalence of gonococcal infection was considerably higher if the contraceptives contained more androgen

progestin. In contrast, in 1990 Paavonen et al. (26) reported that oral contraception reduced the risk of symptomatic PID, modifying it into milder clinical forms. A study comparing STDs and the use of intrauterine contraceptive device (IUD) included two groups of women without any major differences in age, education, number of partners, and frequency of sexual intercourse. The signs and symptoms of STD did not differ significantly between the two groups; only vaginal discharge was more common in the women using the IUD (27).

Vaginal discharge purity according to Schröder (6) is connected with sexually transmitted infections. Wathne et al. (27) studied 101 fertile women (15 to 50 years) with vaginal discharge. *Chlamydia trachomatis*, *Trichomonas vaginalis*, genital herpes virus, and *Neisseria gonorrhoeae* were established in 15 %, 9 %, 7 %, and 1 % of the women, respectively. Vaginal discharge purity was significantly deteriorated in CT-positive patients.

Some microorganisms can also be identified in VCE smears stained according to Papanicolaou, which is routinely obtained at periodical checkups for intraepithelial and initial invasive cervical lesions. Among nonviral pathogens, CT, *Gardnerella vaginalis*, *Trichomonas vaginalis*, and fungi (*Candida albicans*, *Candida glabrata*, and *Candida crusei*) have been described. However, a cytodiagnosis of CT based on the finding of intracytoplasmic inclusions accompanied by inflammatory exudate consisting of granulocytes, histiocytes, and transformed lymphocytes is not specific enough to allow for differentiation from other infections (sensitivity 0 % to 54 % and specificity 5 % to 95 %). Therefore, the Pap smear cannot be used as evidence of chlamydial infection, but can only serve as an indication that needs confirmation (27, 28). Similar was observed in our study where cytological findings indicated CT-infection in 19 patients only, while microbiological findings were CT-positive in 58. Therefore, VCE smear stained according to Papanicolaou is only one of the adjunct methods for assessing patient's condition, and can not be the sole basis for a diagnosis. Instead, the diagnosis should include the clinical picture expected for the type of infection and microbiological verification where appropriate.

CONCLUSION

The promising decline in STDs caused by CT and NG reported all over Europe and North America in the

1990s has been clouded by increasing microbiological findings of these agents in the same geographic areas only five to ten years later. The results obtained in our study showed low incidence of gonorrhoea and chlamydiosis in a female population of the Bjelovar area, Croatia. Considering that both diseases were predominantly diagnosed in younger women who had not given birth yet, our results point to the need of better education and implementation of adequate preventive measures by healthcare services.

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Sažetak

PREVALENCIJA INFEKCIJE *NEISSERIOM GONORRHOEAE* ILI *CHLAMYDIOM TRACHOMATIS*
KOD AKUTNOGA MUKOPURULENTNOG CERVICITISA

Cilj istraživanja jest utvrditi koincidenciju *N. gonorrhoeae* (NG) i/ili *C. trachomatis* (CT) u akutnim mukopurulentnim cervicitisima (MPC). Istraživanje je provedeno na populaciji 617 žena izvan trudnoće koje prethodno nisu uzimale antimikrobnu terapiju. NG i CT su izolirane iz obrisaka vrata maternice primjenom standardnih metoda. NG je transportirana na selektivnoj hranjivoj podlozi i identificirana citokrom-oksidadnim testom bojenjem po Gramu. CT je izolirana McCoyevom staničnom kulturom nakon bojenja Lugolovom otopinom.

NG je izolirana u tri ispitanice (0,8 %), a CT u 58 ispitanica (9,4 %). Srednja dob bolesnica iznosila je 22,2 godine. Školovanje i bračno stanje nisu statistički značajno utjecali na rezultate istraživanja. Među bolesnicama s izoliranom CT prevladavaju nulipare, a u slučaju NG sve su pozitivne bolesnice također bile nulipare. Srednja vrijednost broja partnera za čitavu populaciju iznosi 2,2. Bolesnice s potvrđenom CT imale su prosječno 2,4, a one s potvrđenom NG 2,9 partnera. Vrijednost određivanja stupnja čistoće rodničkog iscjetka po Schröderu značajno je povišena u CT-pozitivnih bolesnica ($p=0.011$). Utvrđeno je da 32,7 % ispitanica uopće nije koristilo kontracepciju, dok je većina (39 %) rabila ritmičke metode i prekinuti snošaj. Preostale ispitanice uzimale su oralne kontraceptive (20 %), mehaničku zaštitu (6,1 %) ili lokalne kemijske kontraceptive (1,9 %). Također je dokazano da prethodna akutna i kronična zdjelična upalna bolest korelira s MPC-om ($p<0.01$).

Zaključeno je da klamidijska infekcija statistički značajno korelira sa stupnjem čistoće rodničkog iscjetka, posebice u nulipara. Papanicolaouov razmaz nije specifičan u određivanju klamidijske infekcije. Rezultati istraživanja pokazuju da je učestalost klamidijske i gonokokne infekcije ipak relativno niska u odnosu na nalaz MPC-a.

KLJUČNE RIJEČI: *C. trachomatis*, mukopurulentni cervicitis, *N. gonorrhoeae*, prevalencija, reproduktivno zdravlje, spolno prenosive bolesti

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