Chlamydia trachomatis infection in women with CIN and invasive uterine cervix cancer. Significance of hormonal status

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Summary

In women with CIN at fertile age and those over 50 years of age, EGFR expression is lower in the presence of Chlamydia trachomatis (Cht) infection.

In all Cht infected women over 50 years of age expression of Ki 67 is higher; the increase is significant among women with invasive carcinoma. In these groups of women with CIN and invasive carcinoma TGF-α expression is insignificantly augmented.

Chronic Cht infection is associated with cervical hypertrophy.

Key words: Cht infection; CIN; Cervical cancer; Hormonal status.

Introduction

Some inflammatory conditions of the uterine cervix, induced by sexually transmitted pathogens, are suspected of CIN and invasive cancer promotion. The role of human papilloma viruses (HPV) seems to be well documented [10, 12]. In increasing numbers of studies, *Chlamydia trachomatis* (Cht) and serotypes G, I, and D of the bacteria in particular are also suggested to be associated with augmented risk of developing cancer of the uterine cervix [1, 6]. Infection with *Chlamydia trachomatis* affects young individuals (20-25 years of age) with significant sexual activity. However, similarly to anti-*Chlamydia* antibodies, the infection may persist for a long period of time [2, 8, 11].

No unequivocal data are available on the effects of female hormonal status on the incidence of Cht infection or on its course even if experimental data demonstrated that the presence of estrogens is required if the infection is going to take place [3, 4, 9]. The present study aimed at analyzing Cht infection in a group of women with CIN and invasive cancer of the uterine cervix in order to define the effects of the infection on growth and proliferation factors acting on uterine cervix cells, interaction of the infection with expression of human papilloma viruses and to define the effect of hormonal status on the studied parameters. Moreover, in a broader study we have planned to corroborate earlier data related to the effects of chronic infections with Cht on uterine cervix hypertrophy.

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Material and Methods

Uterine cervix samples were taken from 176 patients subjected to gynaecological surgery. In the samples, the presence of Cht was detected using in situ polymerase chain reaction (IS-PCR). Employing immunohistochemical techniques, expression of epidermal growth factor receptor (EGFR), transforming growth factor-α (TGF-α), Ki67 and the presence of human papilloma viruses, HPV16 and HPV18, were examined. In serum, IgG class anti-Cht antibodies were quantitated. The patients were included into the following groups:

– Patients operated on due to CIN or invasive cancer of the uterine cervix (11 patients with CIN I, 14 patients with CIN II, 41 patients with CIN III, 33 patients with cancer);

– Patients operated on due to uterine myomas or due to genital prolapse (66 patients, control group).

The studied groups were analysed for their hormonal function. In this way two additional groups were formed: one including 32 women at fertile age (< 40 years of age) and the other, which included 66 women over 50 years of age, in whom no hormonal substitution was applied. The analysis did include 67 patients between 40 and 50 years of age. Moreover, incidence of hypertrophic vaginal portion of the uterine cervix was examined in a proportion of the examined women, including 62 women in the control group, ten patients with CIN I, 12 patients with CIN II, 44 patients with CIN III, and 28 patients with invasive carcinoma.

Women in whom Cht was disclosed by the IS-PCR technique and in whom appropriate titres of specific antibodies were present were regarded as suffering from chronic Cht infection.

IS-PCR (in situ polymerase chain reaction)

For amplification of the Cht sequence the following PCR primers were employed: 5'GGA CAA ATC GTA TCT CGG-3' (T1, sense primer) and 5'GAA ACC AAC TCT ACG CGT-3' (T2, antisense primer). The primers were designed so as to amplify 517 bp sequence, typical of Cht.

Immunohistochemical technique

In paraffin sections HPV16, HPV18, Ki67 antigen, receptor for epidermal growth factor and transforming growth factor-α were investigated. Scores of the reactions reflected reaction intensity (0-3) and proportion of positive cells.

The fixed tissues were embedded in paraffin. In the sections, the markers were detected employing the three-stage ABC technique. The sections were incubated with specific monoclonal antibodies to HPV18, Ki67, EGFR (Novocastra), to HPV16 (Chemicon), TGF-α (Serotec). The Novostain Super ABC Kit (Novocastra) was applied in combination with antibodies to Ki67, HPV18, EGFR and TGF-α while the LSAB Kit (Dako) was used in combination with antibodies to HPV16.

Quantitation of serum IgG class antibodies to Cht using the immunoenzymatic technique

Serum anti-Cht antobodies bridged the solid phase antigen and anti-H-IgG, a chromogen yielded coloured complexes and colour intensity was established by spectrophotometry.

Statistical evaluation

The Whitney-Mann non-parametric test was used.

Results

Analysis of the data obtained for all the women, regardless of their hormonal status, demonstrated no significant differences between control patients, women with CIN or invasive cancer with or without Cht infection. A tendency existed for lowered expression of EGFR in women with invasive cancer accompanied by Cht infection (Table 1).

In women below 40 years of age expression of EGFR was significantly lower in Cht-infected patients with the diagnosis of CIN. Expression of HPV16 in the group of women was insignificantly lowered (Table 2).

In women above 50 years of age with CIN and invasive cancer, EGFR expression was lower in the presence of Cht infection while TGF-α expression was insignificantly augmented. Expression of Ki67 was higher in all Cht-infected women above 50 years of age and the increase was significant among women with invasive cancer (Table 3).

As proven by the data in Table 4, chronic infection with Cht was accompanied by hypertrophy of the uterine cervix.

Table 1. — *Values of EGFR, TGF-α, Ki67, HPV16, and HPV18 in women with or without infection with* Chlamydia trachomatis.

	Cht	No.	EGFR AVG (SD)	TGF-□ AVG (SD)	Ki67 AVG (SD)	HPV16 AVG (SD)	HPV18 AVG (SD)
CONTROL	(-)	32	11 (11)	0	34 (14)	18 (25)	11 (18)
	(+)	34	16 (16)	0.3 (2)	39 (22)	22.4 (26)	20 (25)
CIN I-III	(-)	26	68.8 (66)	22.6 (33)	109 (92)	35 (56)	12 (20)
	(+)	49	60 (52)	29 (40)	136 (108)	32 (43)	8 (19)
INVASIVE CARCINOMA	(-)	27	154 (77)	15 (28)	146 (88)	47 (48)	13 (23)
	(+)	7	121 (59)	24 (41)	157 (91)	31.6 (53)	16.4 (24)

AVG = average.

Table 2. — *Values of EGFR, TGF-α, Ki67, HPV16, and HPV18 in women below 40 years of age, with or without* Chlamydia trachomatis *infection.* ** p = 0.02*

< 40 yrs.	Cht	No.	EGFR AVG (SD)	TGF-□ AVG (SD)	Ki67 AVG (SD)	HPV16 AVG (SD)	HPV18 AVG (SD)
CONTROL	(-)	3	1.6 (3)	0	15 (7)	10 (10)	6.6 (11)
	(+)	3	16.6 (20)	0	26 (19)	16.6 (28)	6.6 (5)
CIN I-III	(-)	8	109 (88)	25 (42)	104 (87)	64 (86)	18 (24)
	(+)	12	37 (47)*	10 (13)	120 (93)	20 (35)	9.8 (19)
INVASIVE CARCINOMA	(-)	1	190 (62)	0	253 (69)	60 (19)	0
	(+)	5	107 (55)	2 (4)	170 (88)	98 (94)	21 (17)

Table 3. — *Values of EGFR, TGF-α, Ki67, HPV16, and HPV18 in women over 50 years of age, with or without infection with* Chlamydia trachomatis. ** p = 0.05* *** p = 0.03*

> 50 yrs.	Cht	No.	EGFR AVG (SD)	TGF-□ AVG (SD)	Ki67 AVG (SD)	HPV16 AVG (SD)	HPV18 AVG (SD)
CONTROL	(-)	11	1 1(10)	0	36 (17)	9.5 (15)	9 (15)
	(+)	11	11 (12)	0.8 (2.8)	40 (13)	17.5 (25)	16 (23)
CIN I-III	(-)	11	77.8 (57)	30 (33)	113 (104)	24 (24)	7 (14)
	(+)	17	40 (45) p=0.05*	53 (52)	158 (95)	43 (46)	3 (9)
INVASIVE	(-)	4	127.5 (69)	12.5 (25)	35 (70)	22.5 (38)	0
	(+)	12	107 (64)	40 (53)	160 (100) p=0.03**	12 (19.5)	15 (15)

AVG = average

Table 4. — *Uterine cervix (normal or hypertrophic) in women with or without* Chlamydia trachomatis *infection in the entire study.*

	ChT	Normal	Hypertrophy
CONTROL	(-)	22 (48.9%)	5 (29.4%)
	(+)	23 (51.1%)	12 (70.6%)
CIN I	(-)	4 (66.7%)	1 (25%)
	(+)	2 (33.3%)	3 (75%)
CIN II	(-)	4 (66.7%)	1 (16.7%)
	(+)	2 (33.3%)	5 (83.3%)
CIN III	(-)	8 (44.4%)	8 (30,7%)
	(+)	10 (55.6%)	18 (69.3%)
INVASIVE	(-)	4 (26.7%)	2 (15.4%)
CARCINOMA	(+)	11 (73.3%)	11 (84.6%)

Discussion

Chronic infection with Cht decreases EGFR expression in women with CIN and invasive cancer of the uterine cervix regardless of the hormonal status of the patients. No literature data are available which would relate the heretofore studied parameters to endocrine variables. Supplementation of medium with estrogens in cell cultures promoted infection with Cht by 50-60% [4].

In women over 50 years of age with CIN and invasive cancer, in whom Cht infection was disclosed, an insignificant increase in TGF-α and Ki67 has been noted (the increase was significant only in women with cancer), in line with our earlier studies [7]. This seems to contradict the data from a Finnish population of older women among whom levels of Cht infection remained without significance [5].

Cht infection remains related to uterine cervix hypertrophy in all groups of women: in the control population, among patients with CIN, and those with invasive cancer. This is consistent with our earlier observations [7]. Infection with *Chlamydia trachomatis* may augment expression of HPV16 in women over 50 years of age with CIN, however the hypotheses should be corroborated by studies on larger groups of patients.

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