Chlamydia trachomatis infection in cervical intraepithelial neoplasia and invasive carcinoma

N. Fischer, *M.D. Ph. D.*

Centre of Mother and Child Health Care, The Clinic of Holy Family, Poznań (Poland)

Summary

We analyzed 149 women (81 with cervical intraepithelial neoplasia and with invasive carcinoma of the cervix and 68 - as a control group). The influence of *Chlamydia trachomatis* (Cht) infection into expression of EGFR, TGF-α, Ki 67, HPV 16 and 18 was examined. IS-PCR was used to measure the level of antibodies in the serum. We detected that chlamydial infection may cause cervical hypertrophy in women with and without cervical intraepithelial neoplasia and invasive carcinoma. Infections of both Cht and HPV correlate with high expession of Ki 67 in epithelium. Cht infection also increased the expression of HPV16 in CIN I. These results suggest that Cht infection modifies the activity of viruses. In our research we have confimed that Cht infection increases the expression of EGFR and TGF-α. These facts may explain variants other than the HPV-mechanism of cervical carcinogenesis.

Key words: Chlamydia trachomatis; Cervical cancer; CIN.

Introduction

Cervical cancer supposedly has a relationship with sexually transmitted diseases. The role of herpes simplex type 2 (HPV2), Epstein-Barr virus (EBV), human papilloma virus (HPV) and cytomegalovirus (CMV) was investigated. The role of human papilloma virus seems to be well documented, [1-4] however the influence of Cht infection in cancer development is still being investigated. [5-11] Research conducted on a group of 530,000 women in Finland, Norway and Sweden shows that infection of serotype G of Cht is connected with cervical cancer development. Other serotypes (I and D) may also be involved in carcinogenesis [12].

This mechanism of proliferation is different than the HPV mechanism. Cht-antigen is recognized by macrophages which present as lymphocyte T. Macrophages release cytokines - tumor necrosis factor-a (TNF-a) and interleukin-1a (IL-1a). Cytokines induct synthesis of IL-6 in fibroblasts. IL-6 and its specific receptors on keratocytes activate the synthesis of growth factors (transforming growth factor-α,-TGF-α, amfiregulin). These factors start the autocrine stimulation of keratocytes. This mechanism has no place in the HPV inducting process of immortalisation [6, 13, 14]. Hyperexpression of TGF-α determines the critical moment of tumor generation [14-17].

Epithelial growth factor (EGF) may also take part in the proliferation process by growth stimulation of both normal and cancer cells. A specific surface receptor is necessary for EGF activities (EGFR) [18-22].

Normal cervical epithelium presents expression of EGFR mostly in basal and parabasal cells. Tissues with an average and considerable degree of dysplasia present continuous expression of EGF receptor [23], and expression in healthy tissue is considerably lower than in cervical dysplasia and cancer [24].

Bauknecht *et al's.* research [25] confirmed the presence of EGFR in 83% cases of cervical cancer. Dysplasia cells had increased the content of receptors in all cases of cervical intraepithelial neoplasia (CIN) with or without the presence of HPV.

In 66% of patients with cervical and endometrial carcinoma, excessive expression of TGF-α was found. These changes were often seen in HPV inducted pathological cervical epithelium. This fact indicates that TGF-α could be a determining factor which favors progression in these tissues [16]. Dellas *et al.* [14] in their investigations of CIN and cervical carcinoma ascertained the highest TGF-α expression in mild dysplasia. They compared HPV negative and HPV positive patients with pathological changes of the cervix. The HPV negative group presented a three times higher TGF-α expression than the HPV positive group. One question arises: if TGF-α is connected to these pathological changes of the cervix which Cht infection was it found in.

The relationship between Ki 67 and cervical cancer also appears to be very interesting. Expression of Ki 67 in CIN and invasive carcinoma is significantly higher in advanced dysplasia and invasive carcinoma than in lower degrees of CIN. Higher Ki 67 expression was found in positive HPV than in HPV negative carcinoma cases [14, 26].

HPV infections are not detected in all CIN and invasive carcinoma cases [7, 27, 28]. Thus HPV infection does not always determine the development of cervical pathology.

The basis of the argument is that Cht may also play a part in cervical carcinogenesis where clinical observations of women with cervical hypertropy and simultaneous Cht infection have been reported. The following questions arise:

Revised manuscript accepted for publication November 28, 2001

- Is there any relationship between Cht infection and cervical hyperthophy?
- Can we explain the mechanism of autocrine growth stimulation inducted by Cht infection (different than HPV); Or can we suggest the modifying influence on the mechanism of HPV?

Materials and Methods

Tissue were recovered from paraffin blocks. Material was received from 81 patients: nine patients with CIN I, 14 with CIN II, 36 with CIN III and 22 patients with invasive carcinoma. Investigated women were between 20-58 years old (average - 47.5) in the CIN I group, 21-58 (average - 40.5) in the CIN II group, 25-77 (average - 46.6) in the CIN III group, 30-81 (average - 50.3) in the invasive carcinoma group.

The control group included women who underwent surgery (52 patients with leiomyomatosis, 16 with descensus of genital organs) between the ages of 33-71 (average - 48.4). Six women from the control group did not have the cervix measured because of past surgical procedures on the cervix; 3 ml of blood was taken from each patient, using enzyme immuno assay (EIA) and antibodies against Cht were detected in the serum. A level of antibody IgG against Cht above 9 AIU/m (Arbitrary International Unit) was acknowledged as a positive result. Measurement of the cervix was administered with an instrument manufactured by a medical equipment company. Hypertrophy was accepted as above 2-2.5 cm in nulliparous and 3-3.5 cm in multiparous females.

Chlamydia trachomatis was detected in paraffin blocks using in situ PCR (IS-PCR). HPV 16, HPV 18, antifen Ki 67, EGFR and TGF-α were detected using immunohistochemical methods. The Mann-Whitney, χ^2 and Fisher tests were used to estimate significance with $p \leq 0.05$ being significant.

Results

Cht in IS-PCR was detected inside and outside cells - both in the epithelium and in its borders. Detection of antibodies in serum did not result higher than 20 AIU/ ml in the investigated group. Detection of a positive result in IS-PCR together with the presence of antibodies in the serum was accepted as persistent Cht infection.

We tried to confirm the influence of Cht infection on the pathology of the cervix based on the hypothesis that Cht infection is found more often in women with cervical hypertrophy.

Table 1. We reviewed women with Cht infection in the control, CIN and invasive carcinoma groups. We proved that cervical hypertrophy was statistically present ($p = 0.05$) in the group with Cht infection. Among 62 women with Cht infection, 19.3% of the cases presented hypertrophy, while only 8% of the women with hypertrophy did not show Cht infection. In the CIN group we detected 35% of hypertrophy cases in women with Cht infection and only 9% of hypertrophy cases without this infection. The invasive carcinoma group illustrates the greatest difference in percentage of cervical hypertrophy (45% with infection and 10% without Cht infection). We found no differences in percentage between the control and the CIN group in women with a normal cervix.

The expression of EGFR and TGF-α was detected in cytoplasm; HPV 18 and Ki 67 in the nucleus and HPV 16 both in the nucleus and cytoplasm.

Table 2. We found differences in expression of EGFR, TGF-α, Ki 67, HPV 16 and 18 with and without Cht infection in all investigated groups. With Cht infection expression of HPV 16 in the CIN I group significantly increased and expression of HPV 18 in the CIN III group significantly decreased. We also observed a tendency for decreased expression of EGFR in CIN I and CIN II;

Table 1. — *Cht Infection in women with normal and hypertrophic cervixes.*

	Cht	Normal	Hypertrophy
Control (62)	(+)	23 (37.1%*)	12 (19.3%*)
	(-)	22 (35.5%)	5 (8%)
CIN (56)	(+)	16 (28.6%*)	20 (35.6%*)
	(-)	15 (26.8%)	5 (9%)
CA (20)	(+)	8 (40%)	9 (45%)
	(-)	1 (5%)	2 (10%)

Table 2. — *Espression of TGF-alfa, Ki 67, HPV 16 and 18 in the control group and CIN I, CIN II, CIN III and carcinoma groups with and without Cht infection (* p = 0.05) (scorequotient of reaction intensity and percent of cells).*

	Cht	N	Control group	N	CIN I	N	CIN II	N	CIN III	N	Carcinoma
EGFR AVG±SD	(-)	32	14±21 (10)	5	30±28 (20)	6	39±29 (27)	11	84±74 (50)	4	156±100 (155)
	(+)	36	16±16 (10)	4	70±74 (55)	8	63±62 (40)	25	76±66 (60)	18	130±56 (150)
TGF-alfa AVG±SD	(-)	32	0,7±2 (0)	5	17±25 (5)	6	18±18 (15)	11	42±43 (40)	4	18±35 (0)
	(+)	36	0±0 (0)	4	18±24 (10)	8	23±35 (5)	25	36±44 (20)	18	34±47 (15)
Ki 67 AVG±SD	(-)	32	38±30 (36)	5	86±67 (70)	6	116±111 (72)	11	173±75 (190)	4	201±62 (212)
	(+)	36	41±22 (38)	4	140±63 (150)	8	110±71 (98)	25	213±81 (196)	18	202±56 (190)
HPV16 AVG±SD	(-)	32	18±18 (13)	5	*8±11 (0)*	6	41±42 (30)	11	71±73 (30)	4	48±62 (30)
	(+)	36	24±30 (10)	4	*39±25 (38)**	8	11±19 (0)	25	41±50 (20)	18	40±62 (15)
HPV18 AVG±SD	(-)	32	13±20 (0)	5	54±59 (45)	6	22±23 (18)	11	*27±35 (0)*	4	8±17 (0)
	(+)	36	18±25 (5)	4	11±16 (5)	8	16±33 (3)	25	*5±12 (0)**	18	24±27 (20)

Table 3. — *Expression of EGFR, TGF-alfa, Ki 67 in cases with and without Cht infection in women without viral infection (scores).*

	Cht	N	AVG ± SD	Median
EGFR	(-)	13	25 ± 32	10
	(+)	21	47 ± 60	20
TGF-alfa	(-)	13	3 ± 11	0
	(+)	21	17 ± 39	0
Ki 67	(-)	13	60 ± 52	8
	(+)	21	112 ± 91	72

Table 4. — *Expression of EGFR, TGF-alfa, Ki 67 in cases with and without Cht infection and HPV 16 and HPV 18 infection (scores).*

	EGFR AVG ± SD (MEDIANA)	TGF-alfa	Ki 67 AVG ± SD (MEDIAN)
Cht(-), HPV16 (-), HPV18 (-)	14 ± 32	3 ± 11	60 ± 52
(13)	(10)	(0)	(44)
Cht(+), HPV16 (+), HPV18 (-)	72 ± 65	25 ± 41	151 ± 86
(28)	(50)*	(5)**	(176)****
Cht(+), HPV16 (-), HPV18 (+)	61 ± 75	16 ± 38	138 ± 103
(14)	(15)	(0)	(140)***
Cht(+), HPV16 (+), HPV18 (+)	63 ± 63	17 ± 30	121 ± 106
(28)	(40)	(0)	(90)***

**** p = 0.002 *** p = 0.05
** p = 0.02 * p = 0.01

TGF-α in CIN II; Ki 67 in CIN I, CIN III, HPV 16 in the control group and HPV 18 in the group of women with invasive carcinoma.

Table 3. We examined the influence of Cht infection on EGFR, TGF-α and Ki 67 in the group of 34 women without HPV infection. We proved that all investigated factors had a tendency for growth in the group of Cht infected women.

Table 4. We qualified values of EGFR, TGF-α and Ki 67 in the entire investigated group of women. The influence of Cht infection and infection of one or both types of HPV has been demonstrated. The co-existence of Cht and HPV 16 influences the values of EGFRn TGF-α and Ki 67 expression. The presence of Cht infection co-existing with only one type of HPV significantly amplified the expression of Ki 67, EGFR and TGF-α; Ki 67 expression is higher with the presence of Cht than without it.

Discussion and conclusions

HPV infection has been detected in 61,1-90% of cases of CIN and invasive carcinoma [7, 9, 28, 29, 30]. Investigations concentrated on other sexually transmitted diseases. Chlamydial infection and its influence on the development of carcinoma was also investigated [6, 7, 8, 9, 11, 31].

The influence of chlamydia on cervical carcinogenesis is controversial. Schmauz *et al.* [20] examined women in Uganda. In 60% of the carcinoma cases a significant level of antibodies was found, while in a control group only 28.7% of the women presented such level. Antibodies against Cht were not detected in 59,4% of the carcinoma group and 77,8% of the control group. A passive haemolysis test used in this research did not show a difference between previous and active infection. Koutsky *et al.* [9] based on their investigations of 241 women believe that the relationship between Cht infection and cervical carcinogenesis is not clear. According to the calculations of De Sanjose *et al.* [7] the presence of Cht infection increases the risk of CIN and carcinoma development. Ferrara *et al.* [31] believe that the risk with Cht infection is twice as high. According to Hakama *et al.* [32] even fime times higher. A controlled clinical investigation of 530,000 Scandinavian women shows a correlation between Cht-serotype G infection, (but also I and D-Cht) and development of cervical cancer [12]. Other authors [33, 34] do not confirm such a relationship. Takać and Gorisek examined the presence of Cht infection in 423 patients with histologically confirmed CIN. They used the method of immunofluorescence in cytological swabs and did not find any relationship between Cht presence or even CIN promotion [34].

In response to this research we conducted our own investigation.

Detection of a positive result in IS-PCR (as information about existing Cht infection) together with the presence of antibodies in the serum (as information about Cht infection in the past) was accepted as persistent Cht infection. We promote such discrimination with the argument that a past or persistent inflammable process is necessary for the long-lasting process of carcinogenesis. Clinical observations show that Cht infection was found in a considerable percentage of women with hypertrophy of the cervix. Therefore we suspect that infection causes changes in the fibroblasts of the cervix via IL-1α activity and TNF-α. These factors are released from macrophages after fagocytosis of chlamydia. Woodworth *et al.* [35] have detected that both these cytokines significantly stimulate proliferation of HPV 16 and 18 immortalized cervical cell lines. Another well known fact is that both IL 1-α and TNF-α are often expressed in infected epithelium cells. It seems obvious that IL1-α and TNF-α is inducted in the border cells of cervix IL-6 synthesis. Then IL-6, joining the paraepidermal cells, induces synthesis specific factors such as TGF-α. This triggers the autocrine mechanism of epithelial cell stimulation. Enlarged synthesis of TGF-α in CIN cases wher HPV genomes are not found can prove this mechanism [36].

This hypothesis may be proved by comparing values of EGFR, TGF-α and Ki 67 and their distribution in cervical epithelium among a group of women with CIN, invasive carcinoma and a control group, with or without HPV infection. Higher expression of these factors in women with Cht infection may testify to an autocrine way of stimulation in pathological cells of the cervix.

Table 1 shows that Cht infection more often accompanies hyperthophy (19 cases, 3% with and 8% without Cht infection). Additionally, in CIN I, CIN II and invasive carcinoma cervical hypertrophy occurs more often when Cht infection exists (Table 3). With Cht infection HPV 16 expression is statistically higher in CIN I and expression of HPV 18 is unexpectedly lower in CIN III (Table 2). The fact that HPV 18 is not as oncogenous as HPV 16 can explain these results. Cht infection may also amplify suppressor proteins in viral infections. This observation demands further investigation. Our initial report suggested that the presence of Cht enlarges the expression of HPV in CIN and invasive carcinoma groups. This was connected with a methodological error – we did not use the IS-PCR method at that time [36].

We have observed that a Cht positive and HPV negative group of 34 women showed enlarged (however not statisti-

cally) expression of EGFR, TGF-α and Ki 67 (Table 3). This statistically insignificant result may be caused by high median but average (AVG) values of EGFR, TGF-α and Ki 67 which were 1.5-5 times higher in the presence of Cht.

We have detected a statistically important increase of EGFR, TGF-α an Ki 67 in all women with HPV 16 infection in comparison with Cht negative and HPV negative groups (Table 4) which proves the influence of Cht infection. A statistically important increase of Ki 67 expression was also detected in Cht positive and HPV 18 positive groups, and HPV 16 positive and HPV 18 positive groups. We concluded that Cht positive women are often HPV-infected. Thirteen women were not Cht and HPV infected, while 70 women showed Cht infection and one or both types of HPV infection. Also Lehmann *et al.* [37] detected a more frequent existence of Cht infection in a group with HPV infection.

The literature does not show an analysis of the parameters presented above which limits more extensive debate.

References

[1] Zur Hausen H.: Cervical carcinoma and human papillomavirus: on the road to preventing a major human cancer". *J. Natl. Cancer Inst.*, 2001, *93 (4)*, 252.

[2] Aquilar-Lemarroy A., Kirchoff S., Whitaker N. *et al.*: "Differential sensitivity of human papillomavirus type 16(+) and type 18(+) cervical carcinoma cell to CD-95-mediated apoptosis". *Int. J. Cancer*, 2001, *93 (6)*, 823.

[3] Zur Hausen H.: "Papillomaviruses causing cancer: evasion from host-cell control in early events in carcinogenesis". *J. Natl. Cancer Inst.*, 2000, *92 (9)*, 690.

[4] Howley P. M., Scheffner M., Munger K.: "Oncoproteins encoded by the cancer-associated human papillomavirus target the products of the retinoblastoma and p53 tumor suppressor genes". Cold Spring Symp. *Quant. Biol.*, 1991, *56*, 149.

[5] Zur Hausen H.: "Papillomaviruses in human cancer". *Cancer*, 1987, *59, 10*, 1692.

[6] Schmauz Z. R., Okong P., DeVilliers E. M. *et al.*: "Multiple infections in cases of cervical cancer from a high incidence in tropical Africa". *Int. J. Cancer*, 1989, *43*, 805.

[7] De Sanjose S., Munoz N., Bosch F. X. *et al.*: "Sexually transmitted agents and cervical neoplasia in Colombia and Spain". *Int. J. Cancer*, 1994, *56*, 358.

[8] Zhang Z. F., Graham S., Yu S. Z. *et al.*: "Trichomonas vaginalis and cervical cancer. A prospective study in China". *Ann. Epidemiol.*, 1995, *5*, 325.

[9] Koutsky L. A. *et al.*: "A cohort study of the risk of cervical intraepithelial neoplasia grade 2 or 3 in relation to papillomavirus infection". *N. Engl. J. Med.*, 1992, *327*, 1272.

[10] Stone K. M., Zaidi A., Rosero-Bixby L. *et al.*: "Sexual behavior, sexually transmitted diseases and risk of cervical cancer". *Epidemiology*, 1995, *6*, 409.

[11] Schlott T., Ruda G., Hoppert M. *et al.*: "The in situ polymerase chain reaction for detection of Chlamydia trachomatis". *J. Histochem Cytochem*, 1998, *46*, 1017.

[12] Antilla T., Saiku P., Koskela P. *et al.*: "Seotypes of Chlamydia trachomatis and risk for development of cervical squamous cell carcinoma". *JAMA*, 2001, *285 (1)*, 47.

[13] Pfeiffer D., Stellwag B., Pfeiffer A. *et al.*: "Clinical implications of epidermal growth factor in the squamous cell carcinoma of the uterine of cervix". *Gynecol. Oncol.*, 1989, *33*, 146.

[14] Dellas A., Schultheiss E., Almendral A. C. *et al.*: "Assessment of EGFR and TGF-alpha expression in relationship to HPV status and Ki 67 distribution in cervical intraepithelial neoplasms". *Int. J. Cancer*, 1996, *69*, 165.

[15] Todaro G. J., Fryling C., De Larco J. E.: "Transforming growth factors produced by certain human tumor cells: polipeptydes that interact with epidermal growth factor receptors". *PNAS 77*, 1980, 5258.

[16] Ynos F. P., Klos D. J., Hamilton P. D. *et al.*: "Expression of transforming growth factor alpha mRNA in benign and malignant tissues derived from gynecologic patients with various proliferative conditions". *Anticancer Res.*, 1992, *12*, 1151.

[17] Konishi I., Ishikava Y., Wang S. Y. *et al.*: "Expression of transforming growth factor α in the normal cervix and in benign and malignant lesions of uterine cervix". *Br. J. Obstet. Gynecol.*, 1994, *101*, 325.

[18] Stoscheck C. M., King L. E.: "Role of epidermal growth factor in carcenogenesis". *Cancer Res.*, 1986, *46*, 1030.

[19] Carpenter G., Cohen S.: "Epidermal growth factor". *Ann. Rev. Biochem.*, 1979, *48*, 193.

[20] Goustin A. S., Leof E. B., Shipley G. D., Moses H. L.: "Growth factors and cancer". *Cancer Res.*, 1986, *46*, 1015.

[21] Heldin C. H., Westermark B.: "Growth factors: mechanism of action and relation to oncogenes". *Cell.*, 1984, *37*, 9.

[22] O'Keefe E., Hollenberg M. D., Cuatrcasas P.: "Epidermal growth factor characteristics of specific binding membranes from liver, placenta and other target tissue". *Arch. Bioch. Biophys*, 1974, *164*, 518.

[23] Berchuck A., Rodriguez G., Kamel *et al.*: "Expression of epidermal growth factor receptor and HER-2/Neu in normal and neoplastic cervix, vulva and vagina". *Obstet. Gynec.*, 1990, *76*, 381.

[24] Kim J. W., Kim Y. T., Kim D. Y. *et al.*: "Expression of epidermal growth factor receptor in carcinoma of the cervix". *Gynecol. Oncol.*, 1966, *60*, 283.

[25] Bauknecht T., Kohler M., Janz I., Pfeiderer A.: "The occurence of epidermal growth factor receptors and the characterisation of EGF-like factors in human ovarian, endometrial, cervical and breast cancer". *J. Cancer Res. Clin. Oncol.*, 1989, *115*, 193.

[26] Al-Saleh W., Delvenne P., Greimers R. *et al.*: "Assessment of Ki 67 antigen imunostaining in squamous intraepithelial lesions of the uterine cervix". *Anta. Pathology*. 1995, *104*, 154.

[27] Zur Hausen H.: "Papillomaviruses in human cancers". *Proc. Assoc. Am. Phys.*, 1999, *111 (6)*, 581.

[28] Durst M., Gissmann L., Ilenberg H., Zur Hausen H.: "A papillomavirus DNA from cervical carcinomas and its prevalence in cancer biopsy samples from different geografic regions". *Proc. Nat. Acad. SU*, 1983, *80*, 3812.

[29] Bosch F. X., Munoz N., Shah K. V., Mehens A.: "Second international workshop on the epidemiology of cervical cancer and human papillomavirus". *Int J. Cancer*, 1992, *52*, 171.

[30] Tabrizi S. N., Fairley C. K., Chen S. *et al.*: "Epidemiological characteristics of women with high grade CIN who do and do not have human papillomavirus". 1999, *101*, 252.

[31] Ferrara A., Baay M. F. D., Herbrink P. *et al.*: "A sero-epidemiological study of the relationship between sexually transmitted agents and cervical cancer in Honduras". *Int. J. Cancer*, 1997, *73*, 781.

[32] Hakama M., Lehtinen M., Knekt P. *et al.*: "Serum antibodies and subsequent cervical neoplasm: a prospective study with 12 years of follow-up". *Am. J. Epidemiol.*, 1993, *137*, 166.

[33] Takać J., Gorišek B.: "The frequency of Chlamydia trachomatis infection in women with intraepithelial neoplasia of the uterine cervix". *Eur. J. Gynaecol. Oncol.*, 1998, *19, (5)*, 492.

[34] Takać J., Gorišek B.: "Chlamydia trachomatis in women with and without cervical intraepithelial neoplasia". *Int. J. STD. AIDS*, 1999, *10 (5)*, 331.

[35] Woodworth C. D., Mcmullin E., Iglesias M., Plowman G. D.: "Interleukin 1α and tumor necrosis factor α stimulate autocrine amphiregulin expression and proliferation of human papillomavirus-immortalized and carcinoma-derived cervical epithelial cells". *Proc. Nat. Acad. Sci.*, 1995, *92*, 2840.

[36] Markowska J., Fischer N., Filas V. *et al.*: "The role of Chlamydia trachomatis infection in cervical cancer development". *Eur. J. Oncol.*, 1998, *20, (2)*, 144.

[37] Lehmann M., Groh A., Rodel J. *et al.*: "Detection of Chlamydia trachomatis DNA in cervical samples with regard to infection by human papillomavirus". *J. Infect.*, 1999, *38 (1)*, 12.

Address reprint requests to:
N. FISCHER, M.D.
Centre of Mother and Child Health Care
The Clinic of Holy Family
Ul Jarochowskiego 18
60-235 Poznań (Poland)