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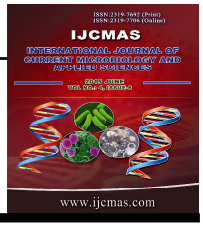

Case Study

Chlamydia trachomatis and *Human Papilloma Virus* (HPV) infection in Egyptian Patients with Invasive Cancer Cervix - A Case Control Study

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Keywords

Chlamydia trachomatis, HPV, Genotypes, Cervical cancer

A B S T R A C T

Human Papilloma Virus (HPV) is crucial to the pathogenesis of cervical cancer. *Chlamydia trachomatis* (CT) is considered a potential cofactor for development of cervical intraepithelial neoplasia. The objective of this case-control study was to detect the association between CT infection and invasive squamous cell carcinoma of the cervix taking into account the central role of HPV infection. Cervical biopsies from both groups were tested for HPV and *Chlamydia trachomatis* by PCR followed by genotyping by restriction fragment length polymorphism (RFLP). HPV and CT are significantly positive in 49(98%) and 20(40%) of the 50 cancer patients and in 9(30%) and 4(13%) of the 30 control cases respectively. Co-infection of HPV and CT is positive in 19/49(38.8%) HPV-infected cancer patients and in 2/9(2.2%) of HPV-infected control cases. The overall prevalence of HPV in CT positive samples is 19/20(95%); 17/19(89.5%) with single carcinogenic genotype (HPV 16) and 2/19 (10.5%) multiple carcinogenic genotypes HPV 16 and 18. In conclusion, a high prevalence of high risk HPV was associated with C. trachomatis infection in cervical cancer which acts as a cofactor. For prevention of cancer cervix, screening and early treatment of CT and HPV infection is recommended.

Introduction

Human Papilloma Virus (HPV) infection by the high-risk types is crucial to the pathogenesis of invasive cervical cancer (ICC), but other co-variants/cofactors as chronic infection with Herpes simplex virus, Chlamydia trachomatis or other cofactors must be present for the development of malignancy (Bellaminutti et al., 2014; Seraceni et al., 2014).

Chlamydia trachomatis (CT) genital infections have been identified as a major health concern. It is the most common bacterial cause of sexually transmitted diseases which is associated with adverse effects on women reproductive system. It is also associated with cervical hypertrophy and induction of squamous metaplasia with subsequent cervical intraepithelial neoplasia

grade 3 or worse (CIN3+) which provides a possible environment for induction of HPV infection (Jensen et al., 2014).

A significant association between CT and HPV positive has been reported (Tavares et al. 2014). CT oncogenic effect may damage the genital mucosal barrier enhancing HPV infection, or may interfere with the immune response and viral clearance supporting the persistence of HPV infection. Moreover, CT-related chronic cervical inflammation, decrease of the lower genital tract antigen-presenting cells, inhibition of cell-mediated immunity, and anti-apoptotic capacity may influence the natural history of HPV infection namely persistence, progression or resolution. Although several epidemiological studies have stated a positive association involving CT and HPV-related cervical neoplastic lesions and/or cervical cancer (CC), the specific role of this bacterium in the pathogenesis of cervical neoplasia has not been completely clarified specially in developing countries (Silva et al., 2014).

The aim of this study was to investigate the association between *Chlamydia trachomatis* infection and invasive squamous cell carcinoma of the cervix taking into account the central role of HPV infection.

Subjects and Methods

Subjects

This case-control study included 80 females attending the Obstetrics and Gynecology Department of Ain Shams University Hospitals, which covered the catchment area of greater Cairo and nearby provenances that represent a population from different social strata. The study was conducted in the Central Microbiology Lab., Ain Shams University Hospitals over a period of 2 years. The study included 2 groups of women:

1- The patient group:
Included 50 patients who had invasive cervical cancer selected from the Gynecology Oncology Unit, Department of Obstetrics and Gynecology. They were diagnosed by histopathology as having squamous cell carcinoma (adenocarcinoma was excluded).

Exclusion criteria: They had no previous treatment of cervical neoplasia.

2- The control group:

Included 30 control patients who underwent any gynecologic surgery for benign condition (fibroid, genital prolapse, etc.). They had normal looking cervix and normal cervical cytology.

Exclusion criteria:

Women were not eligible to participate as control patients if they had received any previous cervical cancer treatment (as treatment of previous squamous intraepithelial lesion) or they had chronic cervicitis or they had disease possibly sharing risk factors with cervical cancer e.g. cardiovascular or cerebrovascular disease, chronic bronchitis or organ neoplasia **(Smith et al., 2002)**.

After taking an informed consent from every patient, all participants (of both groups) were subjected to the following:

I. Full detailed history (Medical - Surgical - Sexual and Reproductive) with special reference to the risk factors of cervical cancer.
II. Full general, abdominal, vaginal and Cusco speculum examination.

III. Cytological examination (PAP smear) to exclude the presence of abnormal cells in the control group.

Cervical biopsies: were taken by one of the following methods:

1. Colposcopy directed punch biopsy.
2. During examination under general anesthesia for staging of cervical cancer.
3. From the surgical specimen if the patient underwent surgery (radical hysterectomy).
4. Intraoperative cervical biopsy in the control group.

All biopsies were immediately stored at -70°C without additives until processing to detect DNA of HPV and Chlamydia trachomatis by PCR. Positive samples were subjected to restriction fragment length polymorphism analysis (RFLP) for genotyping.

Materials and Methods

3- **HPV DNA detection by PCR**: was carried out according to Zhang et al.(1995):

- Extraction of DNA from cervical biopsies and the positive control was done using ZR viral DNA kit (Zymo Research Corp, USA).
- The extracted DNA was ultimately suspended in distillated water at a concentration of 50 ng/µl and stored at -20°C to be used as template DNA.
- Two consensus primers were used for detection of high risk HPV types:

- Sense: PU-IM: '5-TGTCAAAAACCGTTGTGTGTGCC-3'.
- Antisence: PU-2R:′5-TGTCAAAAACCGTTGTGTCC-3'.

- The positive control was Hela cells for each type of HPV supplied by cancer institute. To determine the quality for PCR amplification an internal control of 268bp human β globin gene was used in separate reaction to control the integrity of chromosomal DNA and detect the presence of PCR inhibitors, the primers used were:

- Sense: '5-GAAGAGCCAAGGAC AGGTAC-3'.
- Antisence: '5-CAACTTCATCCACG TTCACC -3'.

- Sterile nuclease free water was used as negative control.
- The amplification was performed by including the reaction mix for 40 cycles in a thermocycler (Uno II, Biometra, Germany). Each cycle consisted of denaturation at 94°C for 30 sec followed by annealing at 51°C for 1 min and extension at 72°C for 1 min, the amplified product(s) were detected by gel electrophoresis according to the type of HPV (figure.1).
- HPV genotyping was carried out using restriction fragment length polymorphism (RFLP) using four restriction enzymes (Ava II, Rsa I, BgL II and ACC I) (figure.2).

4- **Chlamydia trachomatis DNA detection**: was done according to Singh et al., (2003):

- Positive samples in the β globin PCR were used for *C. Trachomatis* detection using a plasmid PCR as "screening PCR". The plasmid primers P3 (sense, 5'GAA CAA ATC GTA TCT CGG) and P4 (antisense, 5'GAA ACC ACC TCT ACG TCG) generated a fragment of 517 bp in the *C. trachomatis*-positive samples.
- Positive cases by plasmid PCR were further examined by Major Outer Membrane Protein (MOMP) PCR for confirmation and genotyping by RFLP using AluI to differentiate serovars of *C. trachomatis* **(Yang et al., 1993)**.

The MOMP primers used were 2 pairs (nested PCR):

- P1 sense: GCC GCT TTG AGT TCT GCT TCC TC
- P2 antisense: ATT TAC GTG AGC AGC TCT CTC AT
- P3 sense: T GAC TTT GTT TTC GAC CGT GTT TT
- P4 antisense: TTT TCT AGA TTT CAT CTT GTT CAA T/CTG.

- The amplification was performed by including the reaction mix for 40 cycles; denaturation at 94°C for 1.5 min followed by annealing at 60°C for 2.5 min and extension at 72°C for 3 min and final extension for 10 min.The amplified product(s) were detected by gel electrophoresis according to the type of HPV.
- Detection of the amplification products were made by electrophoresis onto 4% agarose gel electrophoresis then stained with ethidium bromide and exposed by UV 320 nm. The amplification product generated a fragment of 879 bp (figure.3, 4, 5).

Results and Discussion

Risk factors for cancer cervix:

There are no significant differences between patient and control groups as regards risk factors of cervical cancer as age, educational level, parity, sexarche, total life time sex partner, smoking and contraceptive history (Table 1).

The HPV DNA is significantly positive in 49/50 (98%) of cancer patients and in 9/30 (30%) of control cases. The odds ratio (OR) of cancer cervix for HPV infection is 114 (95% CI=13.6-960.5, P<0.001). Also, the CT DNA is significantly positive in 20/50 (40%) of cancer patients and in 4/30 (13%) of control cases. The odds ratio (OR) of cancer cervix for HPV infection is 4.33 (95% CI=1.31-14.32, P<0.05) (Table 2).

No significant association is found between C. trachomatis DNA positivity and the stage of cervical cancer (P >0.05) (Table.3).

Genotypes of HPV and CT:

The genotype of HPV 18 and 52 are significantly higher in patient group (OR 10.19, 95% CI 1.28-2.14, P<0.05 and OR 7.25, 95% CI 0.88-59.83 respectively). Although HPV 16 is the most commonly detected type (17/50, 34.6%) and a positive trend is found with cancer cervix group, it is not found significant (Table 4).

The most common CT genotypes is D in patient and control groups (40% and 75%), but there is no significant difference in CT genotype distribution between the patient and control groups (table 5).

Co-infection of HPV and CT:

The co-infection of HPV and CT is positive in 19/49 (38.8%) of HPV-infected cancer patients and in 2/9 (2.2%) of HPV-infected control cases. The odds ratio (OR) of cancer cervix for HPV and CT infection is 2.2 (95% CI=0.42-11.81, P>0.05) (Table 6).

Among the patient group, the overall prevalence of HPV DNA in *C trachomatis* positive samples is 19/20 (95%) in which 17/19 (89.5%) of samples HPV is present as a single high risk genotype (HPV16) while 2/19 (10.5%) shows detection of multiple high risk genotypes HPV 16 and 18 (table 7).

The risk factors of cervical cancer in the current study, showed no statistically

significant difference between patient and control groups as regards the age distribution, educational level, sexarche, life time sexual partner, smoking and parity (P<0.05). Similarly, Giuliano et al. (2004) found that there was no association between smoking, pills or parity with cancer cervix. However, these results were in disagreement with the studies conducted by Smith et al. (2002) and Smith et al. (2004) who reported that exogenous hormones, multiparity, smoking, sexarche at early age were cofactors acting in conjunction with HPV, but large population size is required to sort out these factors..

In the present study, HPV DNA showed high significant association with cervical cancer in Egyptian patients. HPV DNA was positive in 49/50 (98%) of cancer patients and 9/30 (30%) of control group with significantly higher risk in patient group (OR 114, 95% CI=13.6-960.5, P<0.001). This is in agreement with Bhatla et al. (2013) who stated that HPV DNA has been detected in 99.7% in all Geographic areas. Golijow et al. (2005) and Geraets et al. (2014) reported that HPV DNA prevalence increased with increasing severity of the cervical lesion, ranging from 30% among women with normal cytology to 99-100% among women with squamous cervical cancer. Giuliano and his coworkers (2004) and Bellaminutti et al. (2014) reported that the exposure to high risk HPV genotypes was not enough for cervical carcinogenesis that need several risk factors mainly high viral load, exposure to non-European variants of HPV and concurrent cervical inflammation by *C. trachomatis* infection in cancer high risk areas. All these factors have demonstrated significantly the elevated risk of neoplasia.

Also in the present study C. *trachomatis* detected by PCR showed a statistically significant association and higher risk of cervical cancer in patients than controls (40% and 13.3% respectively) (OR 4.33, 95% CI=1.31-14.32, P<0.05). These results are in agreement with previous studies as Markowska et al. (2001) who found that *C. trachomatis* DNA was significantly higher in cancer patients (85% and 52%) than control group (55% and 30%) respectively using insitu PCR technique (IS-PCR). Moreover, Geraets et al. (2014) reported that *C. trachomatis* was one of the determinants independently associated with carcinogenesis, (OR 1.89; 95% CI 1.32 to 2.70). *C. trachomatis* as a co-factor for cervical neoplasia is attributed to epithelial damage by allowing easier HPV entry, inflammation that releases high levels of reactive oxygen species, inhibition of cell division and metaplasia and reduction of host cell mediated immunity (Bhatla et al., 2013).

In the present study, the prevalence of *C.* trachomatis DNA did not differ significantly by the clinical staging of cervical cancer: stage I (40%), stage II (20%), stage III (40%), this result was in agreement with Smith et al. (2002). On the other hand these results were in disagreement with Belland et al. (2001) and Golijow et al. (2005) who reported that the prevalence of *C. trachomatis* DNA in invasive cervical cancer (ICC) has been generally low (3-22%) compared to controls (11%) suggesting that Chlamydial DNA does not persist in ICC tissue. But the prevalence was higher in high-grade squamous intraepithelial lesions (HSIL) (47%) which represent the precancerous lesion of the cervix. However, Singh et al. (2003) did not find chlamydial infection associated with an increased risk of progression of inflammatory smear to squamous intraepithelial lesions (SIL).

Table.1 Clinical history as regards risk factors of cervical cancer among patient and controls

Risk Factor	Patients No. = 50	Controls No. = 30	OR	RR	95% CI	Statistical test value	P. value	S
1) Age (mean)	50.8±11.8	50.5±11.2	NA	NA	NA	0.141*	>0.05	NS
2) Educational level								
low	29 (58%)	17 (56.7%)	1.06	1.02	0.72-1.44			
intermediate	15 (30%)	10 (33.3%)	0.67	0.87	0.26-1.74	0.139**	>0.05	NS
high	6 (12%)	3 (10%)	1.23	1.08	0.28-1.77			
3) Parity (mean)	3.6±1.9	3.4±1.0	NA	NA	NA	0.529*	>0.05	NS
4) Sexarche								
<17 year	23 (46%)	11 (36.7%)	1.47	1.15	0.58-3.72			
17-21 year	18 (36%)	9 (30%)	1.31	1.1	0.5-3.47	2.44**	>0.05	NS
>21	9 (18%)	10 (33.3%)	0.44	0.7	0.15-1.25			
5) Total life time sex partner								
1 partner	43 (86%)	27 (90%)	NA	NA	NA	0.274**	>0.05	NS
≥2 partner	7 (14%)	3 (10%)						
6) Smoking								
smoker	2 (4%)	-	0.68	0.88	0.16-2.87	1.231**	>0.05	NS
non smoker	48 (96%)	30 (100%)						
7) Contraceptive history								
non user	28 (56%)	17 (56.7%)	0.97	0.99	0.39-2.42			
OCP	7 (14%)	3 (10%)	1.47	1.14	0.35-6.16	0.373**	>0.05	NS
IUD	13 (26%)	9 (30%)	0.82	0.93	0.3-2.24			
Injectable	2 (4%)	1 (3.3%)	1.21	1.07	0.1-13.92			

*: t-test; **: Chi-square test (X^2); N: number; OCP: oral contraception; IUD: intrauterine device; OR: Odds Ratio; RR: Relative risk; CI: confidence interval; P: probability; HS: Highly significant; S: Significant; NS: non-significant.

Table.2 The association between HPV andC. trachomatis with cervical canceramong study and control groups

Risk	Patient group (N=50)	Control group (N=30)	X^2	OR	RR	95% CI	P
HPV DNA positive	49 (98%)	9 (30%)	43.49	114.3	18.6	13.6-960.5	<0.001 (HS)
CT DNA positive	20 (40%)	4 (13%)	6.35	4.33	1.56	1.31-14.32	<0.05 (S)

OR: Odds Ratio; RR: Relative risk; CI: confidence interval; HS: Highly significant; S: Significant

Table.3 The association between C. trachomatis DNA positivity and the stage of cervical cancer

Cancer Cervix Stage	C. Trachomatis PCR		X^2	OR	RR	95% CI	P
	Patient (N=50)	Control (N=30)					
I	8	1	3.013	5.52	1.50	0.66-46.58	>0.05 (NS)
II	4	0	2.635	NA	NA	NA	
III	8	3	0.569	1.71	1.19	0.42-7.04	

OR: Odds Ratio; RR: Relative risk; CI: confidence interval; NS: Non significant

Table.4 Genotypes of HPV among positive cases in study and control groups

Genotype	Patient (N = 50)	Control (N = 30)	X^2	OR	RR	95% CI	P
HPV 16	17 (34.6%)	5 (55.5%)	2.8255	2.58	1.36	0.99-1.87	>0.05 (NS)
HPV 18	13 (26.5%)	1 (11.1%)	6.672	10.19	1.66	1.28-2.14	<0.05 (S)
HPV 52	10 (20.4%)	1 (11.1%)	4.392	7.25	1.57	0.88-59.83	<0.05 (S)
HPV 31	9 (18.3%)	2 (22.2%)	2.03	3.07	1.38	0.62-15.31	>0.05 (NS)

OR: Odds Ratio; RR: Relative risk; CI: confidence interval; S: Significant; NS: Non significant

Table.5 Genotypes of C. trachomatis among positive cases in the study and control groups

CT Genotype	Patient (N=50)	Control (N=30)	X^2	OR	RR	95% CI	P
D	8 (16%)	3 (10%)	0.5692	1.71	1.19	0.42-7.04	>0.05 (NS)
G	5 (10%)	-	3.2	NA	NA	NA	>0.05 (NS)
E	4 (8%)	-	2.5263	NA	NA	NA	>0.05 (NS)
I	2 (4%)	1 (3%)	0.0231	1.21	1.07	0.10-13.9	>0.05 (NS)
K	1 (2%)	-	0.6076	NA	NA	NA	>0.05 (NS)

OR: Odds Ratio; RR: Relative risk; CI: confidence interval; NS: Non significant

Table.6 The association between HPV and C. trachomatis with cervical cancer among study and control groups

Risk	Patient group (N=49)	Control group (N=9)	X^2	OR	RR	95% CI	P
Co-infected HPV and CT	19 (38.8%)	2 (22%)	0.902	2.22	1.12	0.42-11.81	>0.05 (NS)

OR: Odds Ratio; RR: Relative risk; CI: confidence interval; NS: Non significant

Table.7 HPV co- infection distribution in the patient group withpositive CT DNA

CT DNA positive	HPV co- positivity	Single genotype	Multiple genotype
20/50 (40%)	**19/20 (95%)**	**17/20 (85%)**	**2/20 (10%)**

Figure.1 Agarose gel electrophoresis of the amplified products of HPV; Lane 1: Molecular weight marker (50 to 2642bp); Lane 2: Positive control (238 pb); Lane 3: Negative control; Lane 4, 7: Positive cases; Lane 5, 6, 8: Negative cases

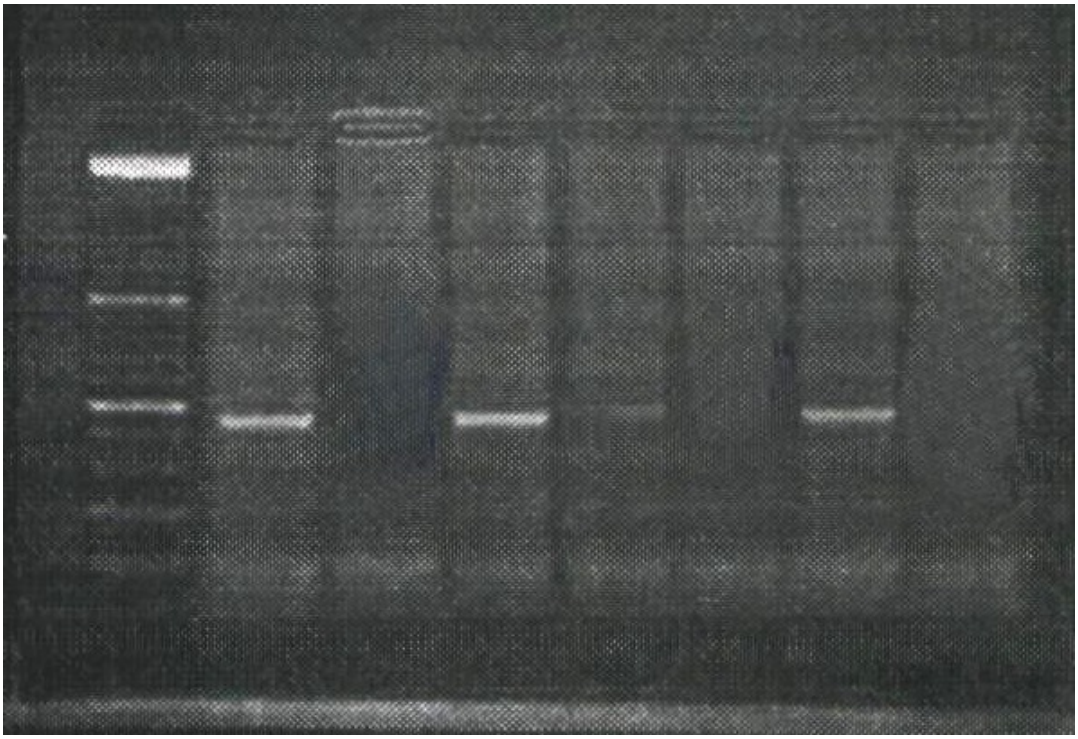


Figure.2 Gel electrophoresis showing different HPV genotypes by RFLP analysis; Lane 1:Molecular weight marker (50 to 2642bp); Lane 2, 5, 7: HPV 16 (157-81); Lane 3: HPV 31 (119-114); Lane 4: HPV 18 (172-96); Lane 6, 8: HPV 52 (176-55)

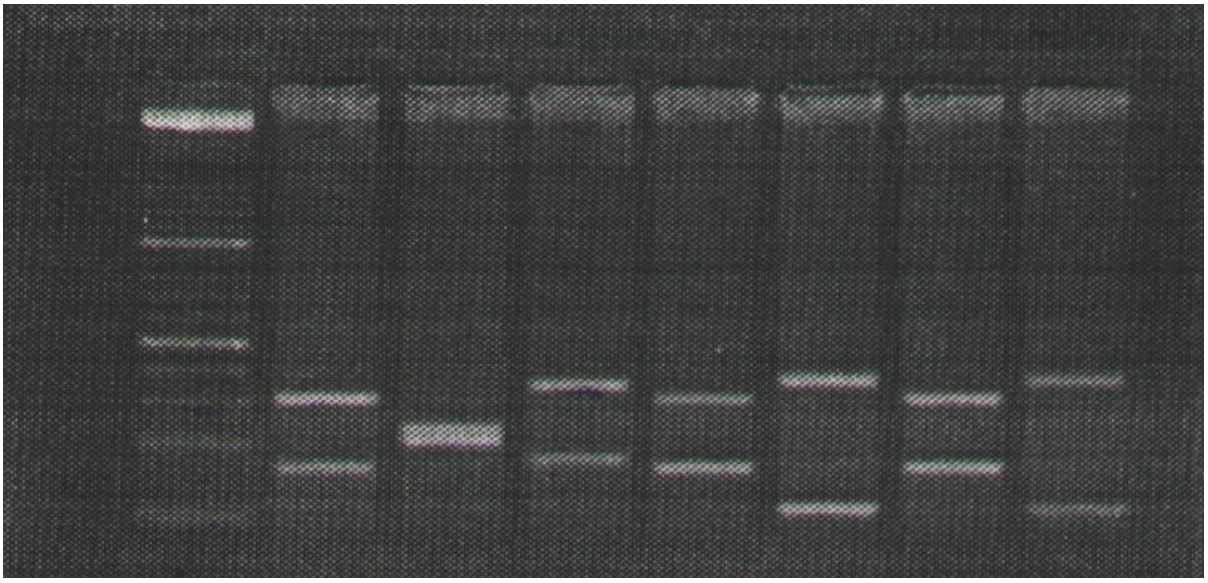


Figure.3 Electrophoresis separation of plasmid PCR amplified product of C. trachomatis
Lane 1: DNA molecular weight marker; Lane 2: Positive control; Lane 3: Negative control
Lane 4, 6, 8: Positive cases; Lane 5, 7: Negative cases

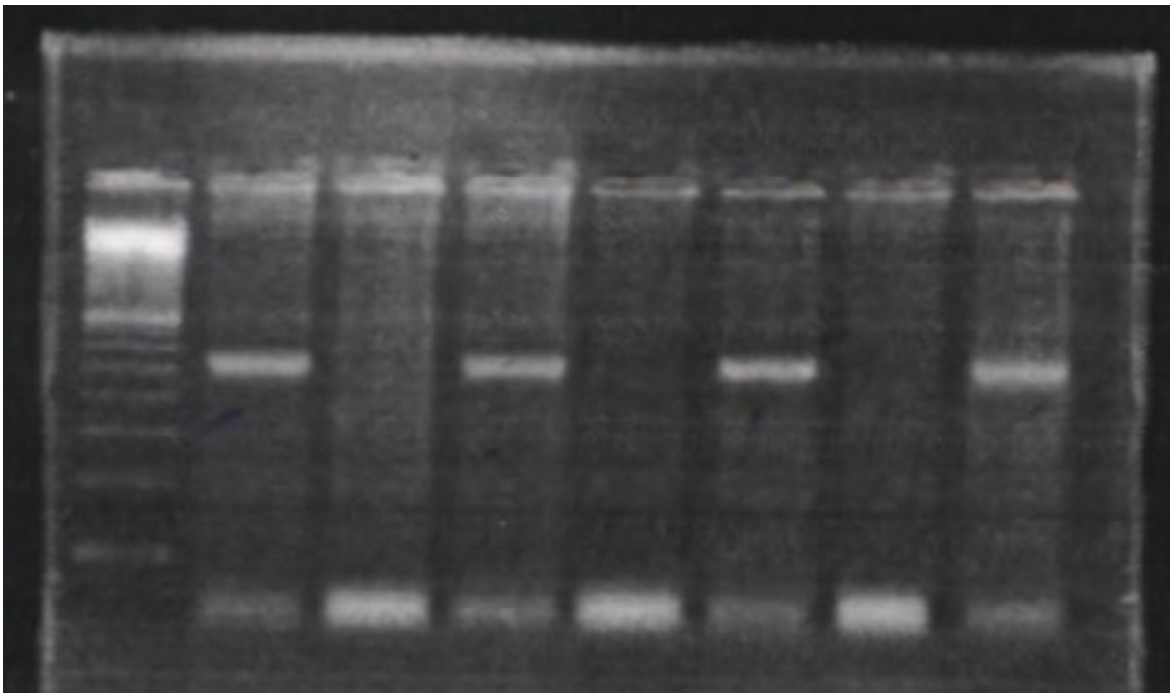


Figure.4 Gel electrophoresis separation of MOMP PCR amplified product of C. trachomatis
Lane 1: DNA molecular weight marker; Lane 2: Positive control; Lane 3: Negative control; Lane 4, 6, 8: Positive cases; Lane 5, 4: Negative cases

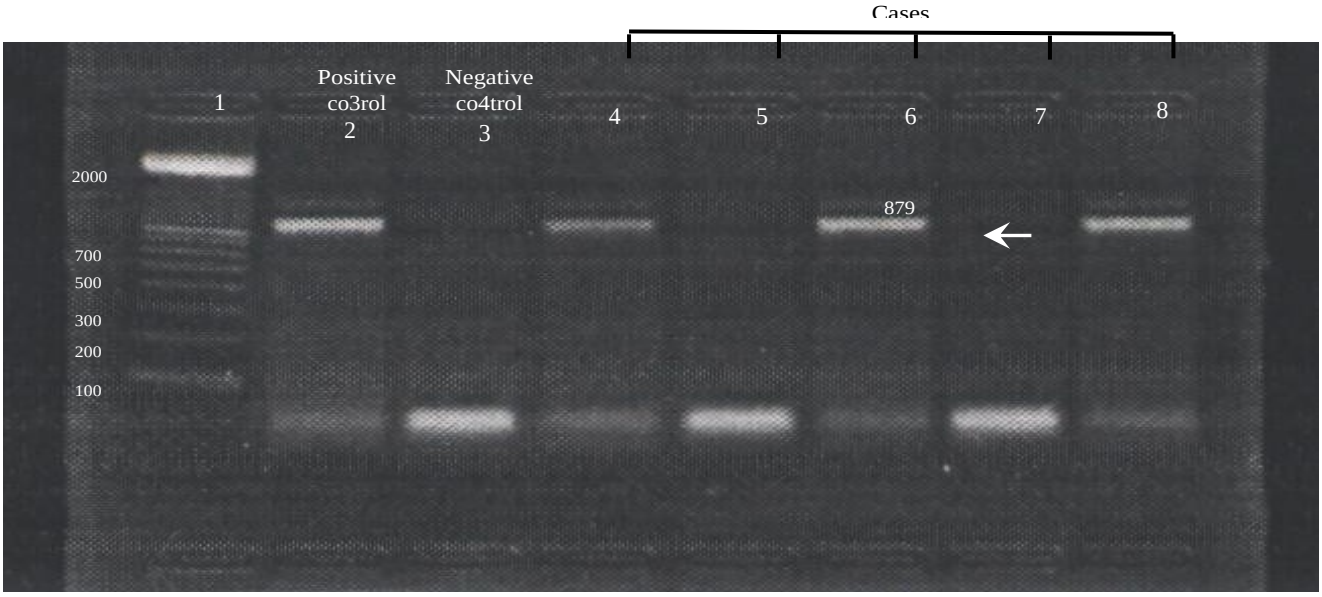
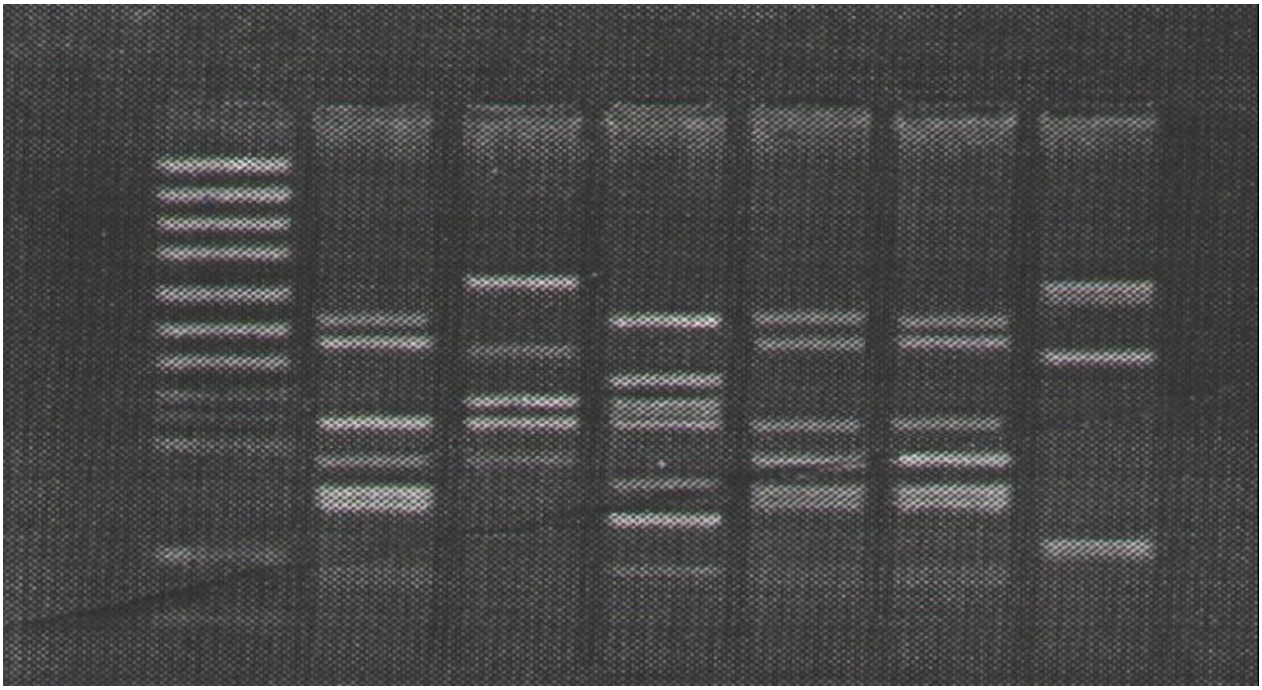


Figure.5 RFLP patterns of MOMP amplified product of C. trachomatis by gel electrophoresis Lane 1: Molecular weight marker; Lane 2, 5, 6: genotype (D); Lane 3: genotype (G); Lane 4: genotype (E); Lane 7: genotype (I)



The genotype of HPV 18 and 52 were significantly higher in the patient group than the control group (OR 10.19, 95% CI 1.28-2.14, P<0.05 and OR 7.25, 95% CI 0.88-59.83 respectively). The most commonly detected HPV genotypes were HPV 16 (34%) and HPV 18 (26%) in cancer patients but HPV 16 (55.5%) and HPV 31 (22.2%) in controls. This is in agreement with Golijow et al. (2005), Schimtt et al. (2013) and Geraets et al. (2014) who reported that HPV types 16 and 18 appeared to be the most common viral types detected in the population studied in Argentina. Tavares and coworkers (2014), reported that the most prevalent HPV types in cervical cancer were HPV 16 and 18 with significant association between CT positive and HPV 16 infection was found (p<0.0106; OR=5.31; 95% IC1.59-17.67).)

Chlamydia trachomatis Genotype D was the most common genotype observed in both cancer patients (16%) and control group (10%) followed by genotype G (10%) in cancer patients and genotype I (3.3%) in control group. However genotypes G, E and K were found only in cancer patients. Markowska et al. (2001) observed that genotypes (G, D and I) were associated with cervical cancer development. At the same time Singh et al. (2003) reported that serovars D and E were more frequently found in symptomatic patients in India and the developing countries. Anttila et al. (2007) reported that serovars G is the most strongly associated with the development of cervical squamous cell carcinoma.

This study did not prove the association between HPV DNA positivity and *C. trachomatis* DNA positivity. This was in agreement with Molano et al. (2003). However, a positive association was found by Giuliano et al. (2001), Tamim et al. (2002) and Deluca et al. (2006) who concluded that *C. trachomatis* acts as

essential cofactor of HPV for the development of cervical neoplasia.

As regards the co-infection of HPV and CT among the patient group, it was positive in 19/49 (38.8%) HPV-infected cancer patients and in 2/9 (2.2%) of HPV-infected control cases (OR 2.2, 95% CI=0.42-11.81, P>0.05). Several epidemiological studies have stated a positive association between *C trachomatis* and HPV–related cervical diseases. The co-presence of *C trachomatis* and HPV was reported in cervical precancerous and cancerous lesions. High levels of specific IgG antibodies or DNA of *C trachomatis* were recovered in HPV positive patients (Jensen et al., 2014; Seraceni et al., 2014). Also Paba and coworkers (2008) reported that CT infection leads to persistence of HPV infection.

The overall prevalence of HPV DNA in *C trachomatis* positive samples was 19/20 (95%) in which 17/19 (89.5%) of samples HPV was present as a single carcinogenic genotype (HPV 16) while 2/19 (10.5%) showed detection of multiple carcinogenic genotypes HPV 16 and 18. These results were in agreement with Seraceni and his co-workers (2014), who analyzed the distribution of HPV DNA among chronic chlamydia cervicitis with neoplastic lesions and reported that the co-presence of both was found in 60% of positive CT DNA. However, different genotypes were found as HPV 42 and 31 presented the most frequently detected genotypes. This variation may be explained by different sample sizes as they studied 1071 patients. Bellaminutti et al. (2014) reported that the co-infection of HPV and CT was found significantly associated to the presence of intraepithelial lesions in younger patients when compared to older females (20% vs. 1%; P□<□0.001). They recommended the use of a high sensitive molecular technique exhibiting higher analytical sensitivity as sequencing method for the diagnosis of *Chlamydia trachomatis* and HPV co-infection. This is important for active screening and timely treatment of *Chlamydia trachomatis* infection in young females to decrease the incidence of pre-cancer intraepithelial lesions. Moreover, Jensen et al. (2014) reported that some authors recently detected a high-risk for the development of cervical cancer in patients with HPV infection and history of *C. trachomatis*. They recommended the detection of *C. trachomatis* DNA by a sensitive PCR assay in cervical screening programs for measurement of potential risk factor for cervical neoplasia in younger age using liquid–based cytology (liquid PAP smear) as a golden specimen for preservation of the cells. They suggested that repeated CT infections increased the risk of cervical intra epithelial neoplasia 3+ among women with prevalent as well as persistent high-risk HPV infection.

In conclusion, a high prevalence of HPV infections has been associated with C. trachomatis infection in cervical cancer patients. In addition, specific HPV and CT genotypes seem to be more frequently associated with cervical cancer. For prevention of cancer cervix, further prospective epidemiologic studies on large scale are needed to clarify the occurrence of *C. trachomatis* in younger age group in conjunction with HPV infection in the screening and treatment of pre-cancerous cervical lesions.

References

Anttila, T., Saikku, P., Koskela, P., Bloigu, A., Dillner, J., Ikaheimo, I., Jellum, E., Lehtinen, M., Lenner, P., Hakulinen, T., Narvanen, A., Pukkala, E., Thoresen, S.,

Youngaman, L., Paavonen, J. (2007): Serotypes of Chlamydia trachomatis and risk for development of cervical squamous cell carcinoma. JAMA; 285:47-51.

Belland, R.J., Scidmore, M.A., Crane, D.D., Hogan, D.M., Whitmire, W., McClarty, G. and Caldwell, H.D. (2001): *C. trachomatis* cytotoxicity associated with complete and partial cytotoxin genes. Proc. Nat. Acad. Sci. USA; 98:13984-9.

Bellaminutti, S.1., Seraceni, S., De Seta, F., Gheit, T., Tommasino, M., Comar, M. (2014): HPV and *Chlamydia trachomatis* co-detection in young asymptomatic women from high incidence area for cervical cancer. J. Med. Virol.; 86 (11):1920-5

Bhatla, N., Puri, K., Joseph, E., Kriplani, A., Iyer, V.K. and Sreenivas, V (2013): Association of *Chlamydia trachomatis* infection with *Human Papillomavirus* (HPV) and cervical intraepithelial neoplasia - A pilot study. Indian J. Med. Res.; 137: 533-9.

Deluca, G.D., Marin, H.M., Schelover, E., Chamorro, E.M., Vicente, L., Albhom, M. and Alonso, J.M. (2006): *Chlamydia trachomatis* and *papilloma virus* infection in women with cytohistological abnormalities in uterine cervix. Medicina; 66(4):303-6.

Geraets, D.T., Grünberg, A.W., van der Helm, J.J., Schim van der Loeff, M.F., Quint, K.D., Sabajo, L.O., de Vries, H.J. (2014): Cross-sectional study of genital carcinogenic HPV infections in Paramaribo, Suriname: prevalence and determinants in an ethnically diverse population of women in a pre-vaccination era. Sex Transm. Infect. 90(8):627-33.

Giuliano, A.R., Papenfuss, M., Abrahamsen, M., Denman, C., de Zapien, J.G., Henze, J.L., Ortega, L., Brown de Galaz, E.M., Stephan, J., Feng, J., Baldwin, S., Garcia, F. and Hatch, K.. (2001): HPV infection at the United States-Mexico border: implications for cervical cancer prevention and control. Cancer Epidemiol. Biomarkers Prev.; 10:1129-36.

Giuliano, A., Papenfuss, M., Brown, E.M., Feng, J., Abrahamsen, M., Denman, C.A., de Zapien, J.G., Henze, J.L.N., Hatch, F.G.yK. (2004): Risk Factors or Squamous Intraepithelial Lesions (SIL) of the Cervix Among Women Residing at the US-Mexico Border. International Journal of Cancer 109 (1): 112-18.

Golijow, C.D., Abba, M.C., Mouron, S.A., Laguens, R.M., Dulout, F.N. and Smith, J.S. (2005): *Chlamydia trachomatis* and *Human Papilloma Virus* infection in cervical disease in Argentina women. Gyne. Onco; 96 (6) 181-6.

Jensen, K.E., Thomsen, L.T., Schmiedel, S., Frederiksen, K., Norrild, B., van den Brule, A., Iftner, T., Kjær, S.K. (2014): *Chlamydia trachomatis* and risk of cervical intraepithelial neoplasia grade 3 or worse in women with persistent Human Papillomavirus infection: a cohort study Sex Transm. Infect.; 90 (7):550-5.

Markowska, J., Fischer, N., Warchol, J.B., Fischer, Z. (2001): Detection of *Chlamydia trachomatis* infection in women with CIN and invasive carcinoma: Eur. J. Gynaecol-Oncol.; 22 (2):134-6.

Molano, M., Weiderpass, E., Posso, H., Morre, S.A., Ronderos, M., Franceschi, S., Arslan, A., Meijer,

C.J.L.M., Muñoz, N., van den Brule, A.J.C. and the HPV Study Group (2003): Prevalence and determinants of *Chlamydia trachomatis* infections in women from Bogota, Colombia. Sex Transm. Infect.; 79:474-8.

Paba, P., Bonifacio, D., Di Bonito, L., Ombres, D., Favalli, C., Syrjänen, K. and Ciotti, M. (2008): Co-expression of HSV2 and *Chlamydia trachomatis* in HPV-positive cervical cancer and cervical intraepithelial neoplasia lesions is associated with aberrations in key intracellular pathways. Intervirology; 51: 230-4.

Schimtt, M., Depuydt, C., Benoy, I., Bogers, J., Antoine, J., Arbyn, M., Pawlita, M., VALGENT Study Group (2013): Multiple human papillomavirus infections with high viral loads are associated with cervical lesions but do not differentiate grades of cervical abnormalities. J. Clin. Microbiol.; 51 (5):1458–64.

Silva, J., Cerqueira, F., Medeiros, R. (2014): Chlamydia trachomatis infection: implications for HPV status and cervical cancer. Arch. Gynecol. Obstet.; 289 (4):715-23.

Singh, V., Salhan, S., Das, B.C., Mittal, A. (2003): Predominance of Chlamydia trachomatis serovars associated with urogenital infections in females in New Delhi, India. J. Clinical. Microbiol.; 2700-2.

Seraceni, S., De Seta, F., Colli, C., Del Savio, R., Pesel, G., Zanin, V., D'Agaro, P., Contini, C., Comar, M. (2014): High prevalence of HPV multiple genotypes in women with persistent chlamydia trachomatis infection Infectious Agents and Cancer, 9:30.

Smith, J.S., Munzo, N., Herrero, R., Neto, J.E., Ngelangel, C., Franceschi, S., Bosch, F.X., Walboomers, J.M.M. and Peeling, R.W. (2002): Evidence for Chlamydia trachomatis as a human papilloma virus cofactor in the etiology of invasive cervical cancer in Brazil and Philippines. J. Infect. Dis.; 185:324-31.

Smith, J.S., Bosetti, C., Munoz, N., Herrero, R., Bosch, F.X., Eluf-Neto, J., Meijer, C.J., Van Den Brule, A.J., Franceschi, S., Peeling, R.W.; IARC multicentric case-control study (2004): Chlamydia trachomatis and invasive cervical cancer: A pooled analysis of the IARC multicentric case-control study. Int. J. Cancer; 111: 431-9.

Tamim, H., Finan, R.R., Sharida, H.E., Rashid, M. and Almawi WY. (2002): Cervicovaginal coinfections with HPV and C. trachomatis. Diagn. Microbiol. Infect. Dis.; 43:277-81.

Tavares, M.C., de Macêdo, J.L., de Lima Júnior, S.F., de Andrade Heráclio, S., Amorim, M.M., de Mascena Diniz Maia, M., de Souza, P.R..(2014): *Chlamydia trachomatis* infection and Human Papillomavirus in women with cervical neoplasia in Pernambuco-Brazil. Mol. Biol. Rep.; 41(2):865-74.

Yang, C.L., Maclean, I. and Bronham, R.C. (1993): DNA sequence polymorphism of *Chlamydia trachomatis* omp1 gene. J. Infect. Dis.; 6 (168): 1225-30.

Zhang, J., Rose, B.R., Faiml, S., Thompson, C.H., Jarrett, C., Russell, P., Houghton, R.S. and Cossart, Y.E. (1995): Associations between oncogenic human papilloma viruses and local invasive patterns in cervical cancer. J. Gyn. Oncology; 57: 170-7.