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ORIGINAL ARTICLE

CORRELATION OF CERVICAL DYSPLASIA WITH SEROLOGICAL AND MICROBIOLOGICAL TESTS FOR GENITAL INFECTIONS

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ABSTRACT: AIMS: Prevalence of Sexually transmitted infections (STIs) tends to be high which constitutes an important public health problem at present worldwide and in India, being associated with Human Immunodeficiency virus and predisposing to cervical dysplasia and cancer. There is a need to overcome the STI infections throughout the nation by cytological and microbiological screening. **SETTING AND DESIGN:** The present study was undertaken to investigate the association of Chlamydia trachomatis (CT), Herpes simplex virus 2 (HSV 2), Cytomegalovirus (CMV), Hepatitis B (HBV), Hepatitis C virus (HCV), *Trichomonas vaginalis* (TV) and *Candidiasis* with cervical cytological changes in symptomatic female in West Bengal. **METHODS:** Sera and vaginal samples from 431 syndromic female patients with cervico-vaginal discharge were tested for detection of STIs. The study period extended over two years. Concomitantly, study of cervical cytology on Papanicolaou stained smears was performed in all cases. ELISA was performed for detection of CT IgM, HSV2 IgM, CMV IgM, HBsAg and HCV IgM in sera. Candida spp. and TV were identified by culture of vaginal samples. Cytological findings were interpreted according to Bethesda 2001 classification. Statistical analysis was performed using MedCalc for Windows, version 13.3.1.0 (MedCalc Software, Ostend, Belgium). **RESULTS:** Out of 431 samples, majority of the patients belonged to the age group of 29 to 42 years, however the mean age of patients presenting with HSIL on PAP was 45 ± 7.93 years. CT, HSV2, TV and Candida spp. were associated with cervical cytological changes of ASCUS or worse. Age greater than 40 years and infection with CT emerged independent risk factors associated with cervical dysplasia on univariate and multivariate analysis with $p < 0.05$ in both. **CONCLUSION:** C. trachomatis is a risk factor for cervical dysplasia. Early detection and treatment will prevent progression of STIs and development of cervical dysplasia and neoplasia.

KEYWORDS: Cervical cytology, dysplasia, sexually transmitted infections.

INTRODUCTION: Sexually transmitted infections (STIs) are a diverse group of infections which are caused by different micro-organisms such as bacteria, viruses, protozoa, yeasts, ectoparasites and even nematodes, whose common characteristic is that they are transmitted from person to person by sexual contact. Studies in literature indicate that prevalent sexually transmitted infections (STIs) for genital area are C. trachomatis, Herpes simplex virus type 2 (HSV 2) and CMV in women.(1) Some STIs such as Herpes simplex virus type 2, Chlamydia trachomatis, *T. vaginalis*, Candida spp. in women are associated with cervical dysplasia. These organisms have been found commonly in women attending STI clinics.(2) Studies show that HSV 2 and C. trachomatis infected person demonstrate cervical cytological abnormalities. In recent years, CDC estimates that 19 million new infections occur each year, almost half of them among young people aged 15 to 24 years. In United States, infection by Chlamydia remains the most common disease. It is reported that every year approximately 2.8 million new cases of Chlamydia infection occur in the United States.(3) Women, especially of young

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age, are hit hardest by Chlamydia.[4] Gerbase et al., 1998 reported that 70-75% of women infected with C. trachomatis are symptom free.[5]

Sexually transmitted infection have been considered as possible factors in the pathogenesis of cervical epithelial lesions such as ASCUS, Low grade squamous intraepithelial lesion (LSIL) and High grade squamous intraepithelial lesion (HSIL).[6] Cervical cytological abnormalities are associated with C. trachomatis, HSV 2 infections, *Tricomoniasis* and *Candidiasis*.[7]

Chlamydia is a Gram negative intracellular, obligate bacteria and Chlamydia trachomatis is one of the major causative agent of STI in female. It is reported that infection by C. trachomatis is frequently associated with cervical dysplasia[8] with increased risk in unhealthy cervix.[9] Singh et al previously reported that chlamydia infection was not associated with squamous intraepithelial lesions (SIL).[10] Herpes Simplex Virus Type 2 is a DNA virus which is a common genital infection. HSV-2 infects oral and genital sites with increased prevalence in the genital region. This infection is most common in those people who are HIV positive.[11] HSV 2 may have a relationship with cervical abnormalities.[12] Cytomegalovirus is a member of the family of herpesvirus. This virus frequently infects cervix of adult population as well as the new born infants during delivery in infected women.[13]

The present study was undertaken to investigate the association of Chlamydia trachomatis, HSV 2 and cytomegalovirus (CMV), Hepatitis B and Hepatitis C, *T. vaginalis* and *Candidiasis* with cervical dysplasia in symptomatic Indian women.

MATERIALS AND METHODS: To determine the prevalence STIs in hospitals, serum and genital discharge samples were collected from outpatient clinics of Department of Gynecology and Obstetrics of R. G. Kar Medical College & Hospital and Kolkata Medical College & Hospital and from STI patients from different regions of West Bengal and all the tests were conducted in the Regional STI Reference Research Teaching and Training Centre at Institute of Serology, Kolkata from April 2012 to June 2014 to facilitate control of STIs. Patients complained of vaginal discharge during some months, bleeding, itching.

A total of 431 female patients with history of genital discharge were enrolled for this study. Sterile swab sticks and cytobrush were used for vaginal and cervical specimen collection to detect the cervical abnormalities. Serum samples were collected for serology to determine the STIs following Standard Laboratory Techniques.[14]

Discharges from vagina and cervix were collected for Pap smear, Gram stain and culture. Pap smear was reported following Bethesda Classification, 2001.[15] For determination of *T. vaginalis*, vaginal swab was used to make a thin smear in one drop of normal saline over a clean glass slide and observed microscopically under 40X within 5-8 minutes of collection. Another swab was inoculated in Kupferberg medium, incubated at 37^0C and observed for rippling motility up to 7 days for identification of *T. vaginalis*. To detect *Candidiasis*, specimen from vaginal swab was inoculated on Sabouraud Dextrose Agar (SDA) plate. The SDA plate was incubated at 25^0C for 24-48 hours for appearance of typical cream coloured large colonies followed by Gram staining on smear from colonies for morphological identification of Candida spp. Such colonies were inoculated on to HiCrome medium (Himedia), incubated at 25^0C for appearance of colonies of different colours for species identification.[16]

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Serum samples from all clients were collected to determine IgM antibody to C. trachomatis, Herpes simplex virus type 2 (HSV 2), Cytomegalovirus (CMV) and Hepatitis C by Enzyme-linked Immunosorbent assay (ELISA) techniques. ELISA for Hepatitis B surface antigen was done in all cases.

ELISA TESTS: Antibody against C. trachomatis, HSV 2 and CMV were detected using the commercially available IgM kits DSI S.r.l. which were mamufactured by Saronno (VA), Via A. Volonterio, 36a, 21047, Italy and HBs Ag and HCV kits were from Qualpro Diagnostics, Verna Industrial Estate Goa, 403722, India. Microtiter wells were coated with antigen which was used for antibody determination. Diluted patient's serum was added and specific antibodies present to the samples bound to these coated antigen and the non-specific antibodies were removed by washing. Conjugated horseradish peroxidase (HRP) was added and incubated. Now HRP-conjugate was attached to the pre-bound antigen-antibody complex and unbound conjugate was removed by washing. The bound enzyme was detected by adding the substrate and the total reaction was stopped by adding the stopping reagent. Results were recorded by microplate reader at wavelength of 450nm, with reference filter at 620-680 nm. Detection of HBsAg antigen was done by antigen coated 96 wells plated microplates.

STATISTICAL ANALYSIS: Statistical analysis was performed using MedCalc for Windows, version 13.3.1.0 (Med Cale Software, Osland, Belgium).

ETHICS: All the mentioned experiments were carried out in Institute of Serology, Kolkata, India. The study was approved by Institutional Ethical Committees of Medical College & Hospital and R. G. Kar Medical College & Hospital, Kolkata, prior to initiation of the study. After proper counseling and extensive clinical observation speculum examination was done for 431 symptomatic female patients with complaints of STDs.

RESULTS: Four hundred thirty one syndromic female patients were enrolled in this study. All of these females had cervical and or vaginal discharge syndrome. Among 428 clients, median age for population without STI was 32 (range 15-70) that is 41.82% people were treated as normal patient; whereas 58.17% cases showed cervical cytological abnormalities and their median age was 36 (Range 15-70).

Among the 431 women, 179 samples showed no cytological abnormalities on PAP smear. Among the abnormal PAP smear, 90 cases were ASCUS, 158 cases were LSIL and only 4 cases were HSIL. Table 1 represents the demographic characteristic, the mean age of normal population was 34.6, for ASCUS mean age was 42 and for HSIL mean age was 45, whereas for LSIL mean age were 29. Out of 431 patients, 95 were post-menopausal. In this population, 69.37% were Hindu whereas 30.62% was Muslim. Prevalence of C. trachomatis, HSV type 2, *T. vaginalis Candida spp*, HBsAg, HCV and CMV were higher in cases with ASCUS and above on PAP smear than normal population. Based on ELISA, C. trachomatis was detected in 104/431 (24.12%) patients (Table 2). Most of the patients with Chlamydia had LSIL (7.8%) on PAP smear. Significant differences existed (P= 0.001) between prevalence of Chlamydia in cases with cytological abnormalities and normal patients. The other STIs such as HSV 2, *trichomoniasis* and candidiasis were higher in case of ASCUS and above than normal population; but the difference was not significant (Table 2). HCV were detected 11.9% and CMV

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15.87% in case of ASCUS and above. But HBsAg did not show any significant prevalence in case with ASCUS and above. Results of univariate and multivariate analysis are described in Table 3.

Multiple infections with C. trachomatis, HSV 2, *T. vaginalis* and candidia spp. were noted (Fig. 1) in case of women with cervical cytological abnormalitis. C. trachomatis co-infection rate in *T. vaginalis* infected subjects was 32.91%, 11.11% in *Candidiasis* infected cases and 20.58% in HSV2 positive patients. There was no association between HBsAg, HCV and CMV and C. trachomatis. Most of the patients with LSIL were infected more than one organism (Fig. 1).

DISCUSSIONS: C. trachomatis, HSV type 2, *Trichomoniasis* and *Candidiasis* are the most frequent STIs worldwide.[17,18] Genital infection caused by C. trachomatis is chronic and asymptomatic.[19] In this study among patients with STIs, prevalence of C. trachomatis infection is 24.12% which is higher than the previous study in Mumbai where the prevalence of 8.8% was reported.[20] Smith et al 2004 reported that women below 35 age groups are affected with STIs mainly C. trachomatis and the infection is associated with cervical cancer.[21] Based on cervical PAP smear finding, all STI infections in the present study coexisted with cervical dysplasia. In this, mean age of the STI infected cases is 35.There are association of C. trachomatis with cytopathology of ASCUS and above. Chlamydial infection is associated with cervical abnormalities and co-infection was noted with trachomatis, candidiasis, bacterial vaginosis, HSV type 2 infections.[22] Based on study by Malhotra et al., 2011; chlamydia coexisted with STIs such as *Trichomoniasis*, *Candidiasis*, gonorrhea, HIV. Chlamydial infection occurs 48.1% in cases of ASCUS and above, while in normal women 13.96% are infected with multiple organisms. Previous reports indicate that infection by *T. vaginalis* increased the risk of cervical dysplasia and infertality.[23] In the present study age above 40 years and Chlamydia trachomatis infection were found to be independently associated with cervical dysplasia on both univariant and multivariant logistic regression analysis.

In addition to chlamydial infection cases with cervical cytological abnormalities were co-infected with HSV 2, HBsAg, HCV, *T. vaginalis* and *Candidiasis* according to the observation of this study. Duru et al, 2014 reported that antibody against chlamydial and HSV infections were seen concomitantly in same patients.[24] It is noted that, in cases with normal PAP smear, HSV 2 infection rate 23 out of 179 cases (12.84%) whereas multi-infections are observed 13 out of 34 cases (38.13%). This difference is not statistically significant. An in vivo experiment in mouse model showed that dysplasia in cervix persisted after repeated cervical infection by cytomegalovirus together with HSV-1 and HSV-2.[2] Morse et al, 1974 found concomitant co-infections of the cervix uteri by HSV and CMV.[25] Our study showed that 34 patients were infected by HSV 2 and 4 were infected by CMV in case of ASCUS and above out of 252 women.

CONCLUSIONS: In conclusion, observations of this study showed that co-infection between C. trachomatis, HSV 2, CMV, *T. vaginalis* and *Candidiasis* infections occurred in hospital-based female patients with cervical dysplasia, Chlamydia trachomatis was found to be an independent risk factor of cervical dysplasia.

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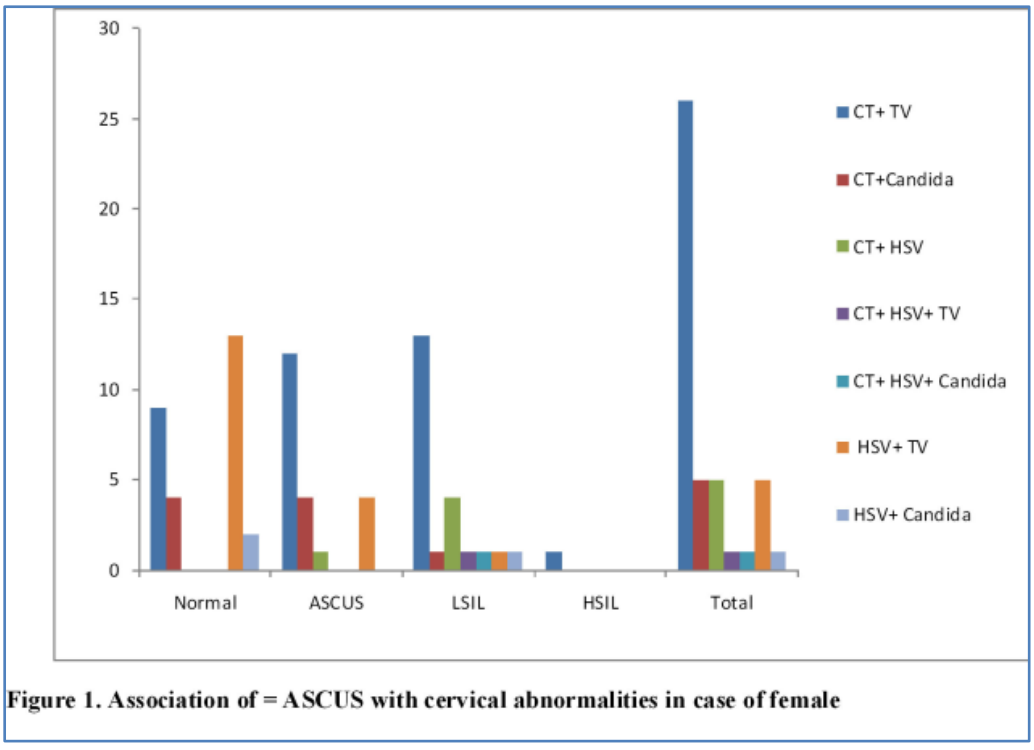

Figure 1. Association of = ASCUS with cervical abnormalities in case of female

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Table 1. Demographic characteristics of the study population

DEMOGRAPHIC PARAMETERS	CYTOLOGICAL DIAGNOSIS			
	NILM	ASCUS	LSIL	HSIL
Mean age	34.6 ±11.96	42±11.38	29±11.87	45±7.93
Hindu	113	71	112	3
Muslim	66	19	46	1
Menopause attained	20	22	51	2

Table 2. Association of different STIs with cytological diagnosis

STIs	NILM	ASCUS	LSIL	HSIL	Total positive	=ASCUS
C. trachomatis	25	20	34	25	104	79
HSV 2	23	9	25	0	57	34
T. vaginalis	76	35	53	3	167	91
Candida spp.	32	18	26	1	76	45
HBsAg	1	0	0	0	1	0
HCV	1	0	3	0	3	3
CMV	2	0	4	0	4	4

Table 3. Univariate analysis in cases with = ASCUS and comparison with P value of multivariate on Pap smear

PARAMETERS	OR	95% C I	STD error	P values	
				Univariate	Multivariate
AGE >= 40 YEARS	1.85	1.15 to 2.99	0.24	0.01	0.001
RELIGION	1.25	0.78 to 2.01	0.24	0.35	0.76
MENOPAUSE	1.56	0.91 to 2.65	0.27	0.10	0.87
TRICHOMONAS	0.68	0.43 to 1.08	0.23	0.10	0.11
CANDIDA	0.93	0.53 to 1.63	0.28	0.81	0.49
CHLAMYDIA	2.66	1.42 to 4.98	0.32	0.001	0.002
CMV	0.63	0.09 to 4.52	1.006	0.64	0.54
HBsAg	1.27	0.11 to14.12	1.23	0.84	0.99
HCV	0.63	0.09 to 4.52	1.006	0.64	0.48
HSV2	0.85	0.47 to 1.54	0.30	0.61	0.60

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