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Prevalence of High Risk Human Papillomavirus (type-16 and 18) in High-Grade Cervical Intraepithelial Neoplasia (CIN) and Cervical Cancer in a Tertiary Hospital of Bangladesh

SM SHAHIDA[a], NP ANSARI[b], A BEGUM[c], MA ISLAM[d], ZA RIFAT[e]

Summery:

Background: Persistent infection with high-risk Human Papilloma Virus (HPV) causes development of cervical cancer. Among the high risk group, HPV-16 accounts for 50% & HPV-18 accounts for 12% of cervical cancer.

Objective: The current sudy aimed to evaluate the prevalence of HPV genotype 16 and 18 in high grade cervical intraepithelial neoplasia (CINII&III) and cervical cancer.

Methods: This descriptive type of cross-sectional observational study was carried out in colposcopy clinic of Mymensingh Medical College Hospital (MMCH), Bangladesh, from July 2012 to June 2013. Women with colposcopically diagnosed high-grade CIN and clinically diagnosed cervical carcinoma were enrolled. Pregnant & menstruating women were excluded from this study. Colposcopy guided cervical biopsy were taken from high grade CIN with loop electrosugical excision procedure (LEEP) and wedge biopsy were taken from cervical cancer. All the specimen were reviewed by histopathologist to confirm the diagnosis. Extra sample of specimen were sent to the Department of Microbiology and Hygiene of Bangladesh Agriculture University, Mymensingh for the detection of HPV-16 & 18 DNA by Polymerase Chain Reaction (PCR).

Result: Out of 71 samples, histopathologically 4.2% was diagnosed as chronic cervicitis, 8.5% CIN I, 26.8% CIN II, 16.9% CIN III, 40.8% squamous cell carcinoma and 2.8% adenocarcinoma of cervix. The results revealed that 30 cases were positive for HPV-16, 06 cases for HPV-18 and 04 cases for both. In this study, the prevalence of HPV-16 & 18 infection was 56.3%. Infection found to be higher in women aged between 45 and 54 (P<0.01). Most of them were from low socioeconomic status (P<0.01) and married at an early age ranging from 11 to 15 (P<0.01). It was found that multiparity correlated higher rate of HPV positivity than only one or two pregnancy, but statistically it was not significant (P>0.05).

Conclusion: The high prevalence of HPV-16 and 18 in high grade CIN and cervical cancer samples suggests that vaccination against HPV-16 and 18 may be effective in bringing down the cervical cancer incidence in Bangladesh.

Key Words: Prevalence, HPV-16 & 18, High-Grade Cervical Intraepithelial Neoplasia, Cervical Cancer.

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Introduction:

Persistent HPV infection is the essential cause of cervical cancer and its precursor cervical intraepithelial neoplasia[1]. The presence of HPV has been implicated in >99% of cervical cancer cases worldwide.

HPV is a small, nonenveloped, double-stranded DNA virus, with a genome of approximately 8,000 nucleotides. Over 100 HPV genotypes have been isolated to date. Among these more than 40 have been shown to infect the genital tract, and 12 of them (genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 and 59) have been found to be associated with cervical cancer or high-grade cervical intraepithelial neoplasia and therefore classified as high-risk types[2].

Furthermore, HPV types 16 and 18 have been regarded as the genotypes most closely associated with progression to cervical cancer. HPV-16 is the most carcinogenic and is associated with approximately 60% of all cervical cancers, while HPV-18 accounts for approximately 10% to 15% of cervical cancers[1].

a. Dr. S. M. Shahida, Associate Professor, Department of Obstetrics & Gynaecology, Dhaka Medical College, Dhaka, Bangladesh.

b. Dr. Nazma Parvin Ansary, Asst. Professor, Dept. of Pathology, Community Based Medical College, Mymensing, Bangladesh.

c. Dr. Afrina Begum, Associate Professor, Department of Obstetrics & Gynaecology, Dhaka Medical College, Dhaka, Bangladesh.

d. Dr. Lt. Col. Md. Anisul Islam, Anaesthesiologist, Combined Millitary Hospital, Sayedpur Cantt. Nilphamary, Bangladesh.

e. Dr. Zannat Ara Rifat, Honorary Medical Officer, Dhaka Medical College Hospital.

Address of Correspondence: Dr. S. M. Shahida, Associate Professor, Department of Obstetrics & Gynaecology, Dhaka Medical College, Dhaka, Bangladesh; e-mail: smshahida827@gmail.com. Phone: +8801714085105.

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However, cervical cancer remains a leading cause of cancer related death for women in the developing countries. In Asia, cervical cancer is more widespread, where it records around half of the world's new cases of cervical cancer each year.

HPV infection is the most frequent sexually transmitted disease worldwide, and up to 60% of sexually active women will have HPV infection in the genital tract[3]. The prevalence of HPV infection has shown to vary by region, country and within a country by population sub-groups[4]. While the estimated global HPV prevalence is reported to be 11.7%, country specific prevalence ranges between 1.6% and 41.9%[3]. HPV prevalence is higher (14.3%) in developing regions than developed regions (10.3%). Type-specific distribution of HPV infection also varies by geographic region[5]. Some HPV types are more prevalent in the Asia Pacific region than other regions[6].

While some epidemiological research on HPV has been conducted in the South Asian region[7], only a few studies have reported HPV prevalence among selected populations in Bangladesh such as female sex workers[8] and cancer patients in tertiary level hospitals[9,10]. Moreover, there have been no systematic population-based study to estimate HPV prevalence or risk factors for HPV infection in Bangladesh[11].

With regard to these facts, the purpose of this study was to detect prevalence of high risk HPV subtypes 16 and 18 among the patients of cervical cancer and colposcopic diagnosed CIN II & CIN III in MMCH, Bangladesh.

Methods:

This descriptive type of cross-sectional observational study was planned and conducted at Obstetrics & Gynaecology Department of Mymensingh Medical College Hospital, Mymensingh, Bangladesh. The study was done after getting ethical clearance from Ethical Committee of Mymensingh Medical College Hospital during the period of July 2012 to June 2013.

Women with colposcopically diagnosed high grade CIN and clinically diagnosed cervical carcinoma were included in this study. Pregnant & menstruating women were excluded from this study. Informed written consent was obtained from patients before taking biopsy. Colposcopy guided cervical biopsy were taken from high grade CIN with loop electrosugical excision procedure (LEEP) and wedge biopsy were taken from cervical cancer in general operation theature.

The Biopsy specimens were sent to histopathology department for confirmation of diagnosis. Specimen were fixed in 10% buffered formalin; routine paraffin sections were taken for processing and stained with hematoxylin and eosin. The samples were graded as Normal cervicitis, CIN I, II, III, squamous cell carcinoma and adenocarcinoma.

Extra sample of specimens were sent to the Department of Microbiology and Hygiene in Bangladesh Agriculture University, Mymensingh for the detection of HPV-16 & 18. The DNA was extracted and then was subjected to PCR for amplification of HPV DNA type 16 and 18 using type specific primer.

Statistical analysis were performed by using the SPSS version 18. Chi-squire test was done and $P<0.05$ was considered as statistically significant.

Results:

The study population consisted of 71 colposcopically diagnosed high grade CIN and cervical carcinoma. All of them were married, parous, non pregnant and in the age range of 25 to 60 years (34.26± 9.33years). Most (58.33%) of the patients of high grade CIN were between 25-34 years and most (62.85%) of the cervical carcinoma were between 45-54 years (Fig-1). It was found that most (52.11%) of the patients belonged to low socioeconomic classes (Fig-2). Majority (36.61%) of them were married at an age between 16-20 years (Fig-3) and mean age of marriages were (18.72±5.58). Fig-4 shows that 31(43.66%) are multiparous, having para 4 and above.

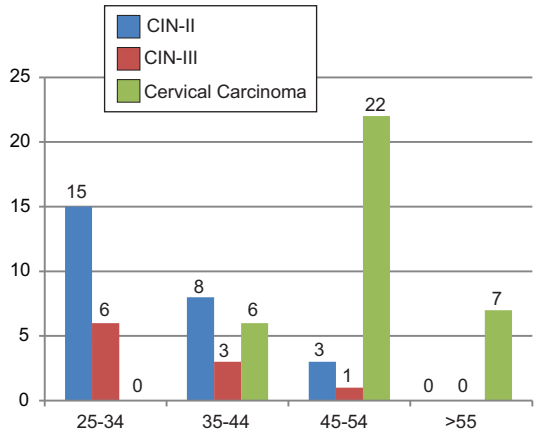

Fig-1: *Age Group Distribution of Studied Patient (n= 71)*

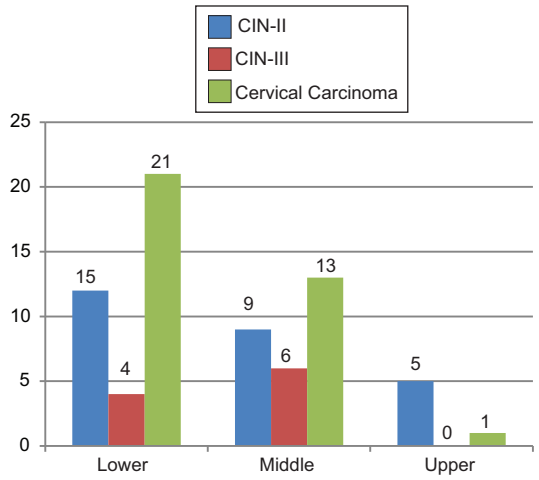

Fig-2: *Distribution of Patient According to Socio-Economic Condition (n=71)*

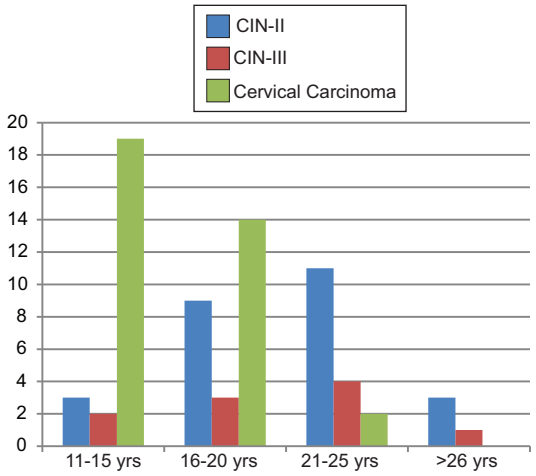

Fig-3: *Distribution of Patient According to Age at Marriage (n=71)*

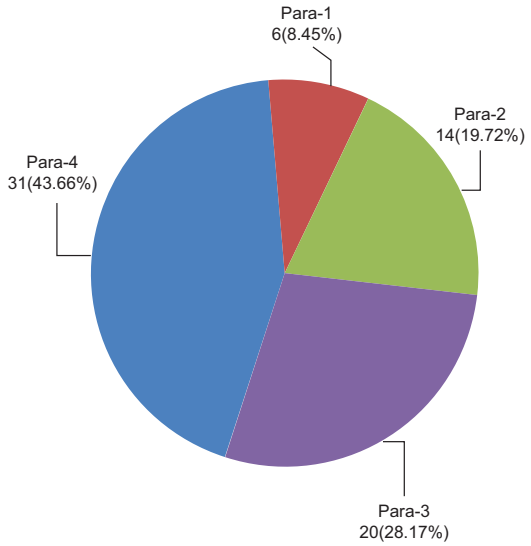

Fig-4: *Distribution of Patient According to Parity*

Table-I represents comparison of colposcopic, clinical and histopathological findings of cervical examination. According to biopsy reports, among 26 colposcopically diagnosed CIN II cases, 3 biopsy specimen were diagnosed as Chronic Cervicitis, 4 cases CIN I, 15 cases CIN II and 4 cases CIN III. Among 10 colposcopically diagnosed CIN III cases, 2 case were diagnosed as CIN I, 2 cases CIN II, 5 cases CIN III and 1case was diagnosed as squamous cell carcinoma. Among 35 cases of clinically diagnosed cervical carcinoma 2 biopsy samples were diagnosed as CIN II, 3 cases were CIN III, 28 cases were squamous cell carcinoma and 2 cases were diagnosed as adenocarcinoma.

Table-II reveals that out of 26 colposcopically diagnosed CIN-II cases, 7 cases were positive for HPV-

Table-I

Histopathological findings of studied samples (n=71)

Colposcopic & Clinical Diagnosis of Patient	Histopathological Findings					
	Chronic Cervicitis	CIN-I	CIN-II	CIN-III	Squamous Cell Carcinoma	Adeno Carcinoma
CIN-II(n=26)	3	4	15	4	0	0
CIN-III(n=10)	0	2	2	5	1	0
Cervical Carcinoma(n=35)	0	0	2	3	28	2
Total n (%)	3(4.2%)	6(8.5%)	19(26.7%)	12(16.9%)	29(40.8%)	2(2.8%)

Table-II

HPV 16 and/18 genotype distribution in studied samples (n=71)

Colposcopic & clinical Dx	HPV 16	HPV 18	Both 16&18	Positive	Negative	P value
CIN II (n=26)	7	1	-	8	18	0.001
CIN III(n=10)	3	1	-	4	6	0.739
Cervical Carcinoma(n=35)	20	4	4	26	7	0.001
Total: n(%)	30(42.2%)	06(8.45%)	04(5.63%)	40 (56.3%)	31(43.7%)	-

16 and 1 cases was positive for HPV-18. Among 10 colposcopically diagnosed CIN III cases, 3 cases were positive for HPV-16 and 1 cases was positive for HPV-18. Among 35 clinically diagnosed Cervical Cancer, 20 cases were positive for HPV-16, 4 cases were positive for HPV-18 and 4 cases were positive for both. From these findings, HPV-16 was found to be more prevalent than HPV-18 among CIN and Cervical cancer cases.

Association between HPV infection and age group of patients were shown in table-III. Among 40 positive cases, 20(50%) cases were found to positive between age group 45-54. Table-IV shows the relationship between HPV infection and some major characterstics. Of the 37 patients from low socioeconomic conditions 26(96.29%) were positive for high risk HPV. Among 24 patients having history of early age at marriage 15(62.5%) patients were found to have high risk HPV infection. Multiple pregnancies correlated higher rate of HPV positivity (56.25%) than only one or two pregnancy.

Table-III

HPV type 16 and/18 DNA status according to age group of patients (n=71)

Age in years	Positive	Negative	P value
25-34 (n=18)	2	16	0.001
35-44 (n=19)	13	6	0.034
45-54 (n=26)	20	6	0.006
≥55	5	3	0.480

Table-IV

Risk factors associated with HPV positive cases

Risk factors	HPV Positive	HPV Negative	P value
Low socioeco-nomic condition(n=37)	26	11	0.001
Early age at marriage (n=24)	15	09	0.001
High Parity(n=32)	18	14	0.102

Discussion:

The present study was carried out to detect prevalence of high risk HPV i.e. subtypes 16 and 18 among the patients with cervical cancer and colposcopically diagnosed high grade CIN in the department of Obstetrics and Gynaecology of Mymensingh Medical College Hospital, Mymensingh, Bangladesh. MMCH is one of the biggest tertiary hospital of Bangladesh. The patients attending the MMCH are from 13 subdistricts of Mymensingh division.

The peak incidence of Carcinoma in Situ (CIS) cervix is in women aged 25-35, while the incidence of cervical cancer rises after the age of 40[12]. Similar to that study, most of the patients with high grade CIN were between 25-34 years and cervical carcinoma were between 45-54 years in this study. In our series there are significant association of cervical precancers and cancer cases with poor socioeconomic conditions, early age at marriage and high parity. These sociodemographic profile is comparable with other studies[13,14]

During the last decade there has been an increasing interest in the use of colposcope[15]. Colposcopy guided biopsy is described as the reference investigation or "gold standard" for the diagnosis of cervical precancer[16]. In this study, we did LEEP biopsy because simultaneously we could treat the patient & confirm diagnosis by sending adequate specimen for histopathological examination.

In the present study colposcopic diagnosis and histopathological correlation was 26 out of 36 (72%) for the detection of cervical precancers which is not comparable to the study done in Korea, where the detection rate is 89.3% to 91.5%[16]. The result of this study stress the need for quality assurance in colposcopic diagnosis and also in histopathologic diagnosis.

In this study, the prevalence of HPV-16 and/ HPV-18 was found in 56.3% of cases. Our result does not correlate the previous studies performed by other countries like Norway (92%)[18], Australia (90%)[19] and China (75-83%)[20]. Their results have shown the higher HPV prevalence because their study included detection of all high risk HPV Genotypes.

HPV 16 was found to be universally common but HPV 18 was particularly common in South East Asian countries[2]. Current study shows that, HPV-16 were more common among high grade CIN and cervical cancer than HPV-18 (P<0.01). This is in consistent with the results of other authors who have mentioned HPV-16 as the main oncogenic type of HPV associate with cervical cancer[21,22].

We also found that both HPV-16 and HPV-18 infections were present in about 10% of our samples. An international survey of invasive cervical carcinomas by the International Biological Study on Cervical Cancer revealed that 4% of specimens harbored both HPV 16 and 18 type of high-risk HPV[23]. The proportion of multiple HPV genotypes in cervical carcinomas in several countries, including Morocco (9.0%)[24], Thailand (3.7%)[25], Brazil (5.1%)[26], varied among countries and in relation to the HPV detection method used.

Among 71 cases, we found higher prevalence (36.61%) of oncogenic HPV among women aged (45-54). Our findings are not consistent with those from a recent large population based multicenter survey of HPV prevalence in China that concluded the prevalence of oncogenic HPV DNA was high among women aged 20-25 and again in women aged 40-45[27].

A significant association of cervical carcinoma was observed with early age at marriage (<18 years), high parity, and low literacy level[28,29,30]. These observations are similar to the observation of our study. In this study, risk factors associated with HPV+ve cases were low socioeconomic condition (P<0.01), early age at marriage (P<0.01). It was found that multiple pregnancies correlated higher rate of HPV positivity than only one or two pregnancy, but statistically it was not significant (P>0.05).

Present study adds immense value to the existing knowledge of the burden of high risk HPV-16 and 18 infection among selected female population in a tertiary care hospital. Due to limited resource this study had some limitations: single center based study, small sample size and all high risk HPV genotyping was not done. It urges the need for detection of all high risk HPV genotyping to determine the type-specific HPV distribution among cervical cancer and precancers.

Conclusion:

Data is not yet available on the HPV burden in the general population of Bangladesh. Based on these results, it can be concluded that HPV-16 and HPV-18 are the most prevalent HPV genotypes among Cervical Intraepithelial Neoplasia and invasive squamous cell carcinoma. Current HPV vaccination by bivalent vaccine containing attenuated 16 & 18 type of HPV antigen is expected to trim down the burden of cervical cancer in our population.

Conflict of interest:

No conflict of interest is declared by the authors.

References:

1. Saslow D, Solomon D, Lawson HW, et al: American Cancer Society, American Society for Colposcopy and Cervical Pathology, and American Society for Clinical Pathology Screening Guidelines for the Prevention and Early Detection of Cervical Cancer. J Low Genit Tract Dis 2012;16(3):175-204.

2. Munoz N, Bosch FX, de Sanjose S, Herrero R, Castellsague X, Shah KV, Snijders P J, Meijer CJ. Epidemiologic classification of human papillomavirus types associated with cervical cancer. N Engl J Med 2003; 348:518-527.

3. Mayrand M, Coutlee F, Hankins C, Lapointe N, Forest P, Ladurantaye M, Roger M Detection of human papillomavirus type 16 DNA in consecutive genital samples does not always represent persistent infection as determined by molecular variant analisys. J Clin Microbiol.2000; 38: 3388-93.

4. Smith JS, Melendy A, Rana RK, Pimenta JM. Age-specific prevalence of infection with human papillomavirus in females: a global review. The Journal of adolescent health: official publication of the Society for Adolescent Medicine. 2003; 43: S5–25, S25 e21–41.

5. De Sanjose S, Diaz M, Castellsague X, Clifford G, Bruni L, et al. Worldwide prevalence and genotype distribution of cervical

human papillomavirus DNA in women with normal cytology: a meta-analysis. The Lancet infectious diseases. 2007; 7: 453–459.

6. Garland SM, Cuzick J, Domingo EJ, Goldie SJ, Kim YT, et al. Recommendations for cervical cancer prevention in Asia Pacific. Vaccine 26 Suppl. 2008;12: 89–98.

7. Bhatla N, Lal N, Bao YP, Ng T, Qiao YL (2008) A meta-analysis of human papillomavirus type-distribution in women from South Asia: implications for vaccination. 2008; 26: 2811–17.

8. Huq M, Nahar S, Alam A, Imam H, Rahman M. The prevalence of selected high-risk human papillomavirus infections among female sex workers in Dhaka, Bangladesh. International journal of STD & AIDS.2012; 23: 151–152.

9. Banik U, Ahamad MS, Bhattacharjee P, Adhikary AK, Rahman Z. High risk human papillomavirus type 16 and 18 infection in the cervical lesions of women with epithelial cell abnormality in Pap smear: A cytohistomorphologic association in Bangladeshi women. CytoJournal.2013; 10: 14.

10. Khatun S, Syed MAH, Hossain F, Choudhury A. Human Papillomavirus and other risk factors in carcinoma cervix. Bangladesh Med J. 2009; 38. 18–23.

11. Sankaranarayanan R, Bhatla N, Gravitt PE, Basu P, Esmy PO, et al. Human papillomavirus infection and cervical cancer prevention in India, Bangladesh, Sri Lanka and Nepal. Vaccine 26 Suppl.2008;12: 43–52.

12. Christine H. Holschneider, MD., 2003. Premalignant & Malignant Disorders of the uterine cervix. In: Alan H. DeCherney, MD , Lauren Nathan, MD. Editors. Current Obstetric & Gynaecologic Diagnosis & Treatment. 9th edition. The McGraw-Hill Companies.2003; PP.894.

13. Indian journal of Public Health: A cytoepidemiological study on married women in reproductive age group (15-49) years regarding reproductive tract infection in a rural community of west Bengal.Tear 2012,vol 56 issue3 page 204-9.

14. Dutta P K, Upadhyay A, Dutta M, Urmil AC,Thergaonkar MP, Ganguly SS. A case control study of cancer cervix patients attending Command Hospital, Pune. Ind. J Cancer. 19990;27: 101-8.

15. T.J.M. Helmethorst, G.H.Dijkhuijen, J.J.Calme, H.J.Kiwikkel, J.G. Stolk: The accuracy of colposcopically directed biopsy in diagnosis of CIN: European Journal of Obstet & Gynecol: March 1987;24(3): 221-229.

16. Singer a, Monaghan J. Lower Genital Tract precancer colposcopy, Pathology and treatment. 2nd edition. Blackwell Science.2000; Oxford.

17. Kim SJ, Namkoong SE, Lee JM,Ahn WS, Park JS, Kim JW. Cervical cancer control in Korea.Colposcopy/Cervicography/ Cytology.1994;8(1-2):101-11.

18. Kraus I, Molden T, Holm R, Lie AK, Karlsen F, Kristensen GB, Skomedal H. Presence of E6 and E7 m RNA from Human Papillomavirus Types 16, 18, 31, 33 and 45 in the Majority of Cervical Carcinomas, J Clin Microbiol. 2006;44:1310-17.

19. Liu J, Rose B, Huang X, Liao G, Carter J, Wu X, Thompson C. Comparative analysis of characteristics of women with cervical cancer in high versus low-incidence regions Gynecol Oncol 2004;94:803-10.

20. Qiu AD, Wu EQ, Yu XH, Jiang CL, Jin YH, Wu YG Chen Y, Shan YM et al.HPV Prevalence, E6 sequence variation and physical state of HPV 16 isolates from patients with cervical cancer in Sichuan , China, Gynaecol Oncol. 2007;104:77-85.

21. Garland SM, Cuzick J, Domingo EJ, Goldie SJ, Kim YT, et . Recommendation for cervical cancer prevention in Asia Pacific. Vaccine 26 Supple.208;12: 89-98.

22. Ma CL, Li YJ, Zhang FC, Wang GQ, Zheng YJ, Kai LM, Re XD, Han Y, Patiguli. Detection of HPV 16 E6 gene in cervical tissues by quantitative polymerase chain reaction. Zhonghua Yi Xue Za Zhi 204;8:469-73.

23. Bosch FX, Manos MM, Munoz N, Sherman M, Jansen AM, Peto J, et al. Prevalence of human papillomavirus in cervical cancer: a worldwide perspective. J Natl Cancer Inst 1995;87: 796–802.

24. Chaouki N, Bosch FX, Munoz N, Meijer CJ, El Gueddari B, El Ghazi A, et al. The viral origin of cervical cancer in Rabat, Morocco. Int J Cancer 1998;75:546–54.

25. Chichareon S, Herrero R, Munoz N, Bosch FX, Jacobs MV, Deacon J, et al. Risk factors for cervical cancer in Thailand: a case-control study. J National Cancer Inst 1998;90:50–57.

26. Eluf-Neto J, Booth M, Munoz N, Bosch FX, Meijer CJ, Walboomers JM. Human papillomavirus and invasive cervical cancer in Brazil.Br J Cancer 1994;69:114–9.

27. Qiao YL, Franceschi S, Belinson JL, et al. HPV prevalence in Chinese women : A population-based multiple center survey in mainland Chaina. Oral presentation 1B-01, 24th international Papillomavirus Conference infection and Clinical Workshop; 2007 Nov 3-9; Beijing, China.

28. Indian journal of Public Health: A cytoepidemiological study on married women in reproductive age group (15-49) years regarding reproductive tract infection in a rural community of west Bengal.Tear 2012,vol 56 issue3 page 204-9.

29. Dutta P K, Upadhyay A, Dutta M, Urmil AC,Thergaonkar MP, Ganguly SS. A case control study of cancer cervix patients attending Command Hospital, Pune. Ind. J Cancer. 19990;27: 101-8.

30. Pravakar AK,Menon GR,Age at marriage and cervical cancer incidence.Indian J Cancer.1995;2:63-8.