Cervical cancer is a preventable disease unlike other cancers. HPV infections have a long incubation period to cause cervical cancer. Other risk factors like early age of conception, illiteracy, low socioeconomic status, use of OCPs for a long period of time, low folate level, high parity and smoking predisposes to cervical cancer. Materials and Method: Present study aimed to identify the risk factors responsible for HPV infections and its association with development of cervical carcinoma in women of age group 20 to 65 years with history of white discharge, blood-stained discharge; heavy menstrual flow, intermenstrual spotting, and postcoital bleeding attending Gynaecology OPD samples of cervical scraping, Pap smear, Visual Inspection with Acetic Acid (VIA) and Visual Inspection with Lugols Iodine (VILI) was taken for detection for high-risk groups for cervical cancer and its association with different risk factors. Results: HPV infection prevalence in our study was 20% among total of 100 patients. PCR positivity was highest among the age group 30-39 years. HPV infection was highest among the age group 19-20 years. Multiparous women having 3 or more children had shown high positivity for HPV- DNA. Illiterate women and women with only up to primary education had shown the highest HPV infection and it was 18 %. Nonvegetarian women had highest HPV infection. Major symptom of the PCR positive cases was white discharge. PCR for HPV-DNA was 100% positive in women who had cervical growth. Conclusion: Our study shows the importance of Pap smear and HPV-DNA testing of women in our country where prevalence and risk factors are extremely high. It can identify and help in preventing or treating the condition at exceedingly early stage. Keywords: Cervical Carcinoma Risk Factors, HPV, PAP Smear, VIA, VILI.
Cervical cancer is of two types – squamous cell carcinoma (85%), adenocarcinoma, less commonly mixed carcinoma (Adeno-squamous). Incidence of adenocarcinoma is increasing in young women because adenocarcinoma is often missed by cytology method [5]. Hence Pap smear combined with VIA/VILI; HPV-DNA detection can increase the chances of detecting preinvasive lesions. Control of cervical cancer is by primary and secondary prevention. Primary prevention involves healthy sexual practice and vaccination against HPV high risk genotypes. Secondary prevention involves screening procedures like Pap smear, VIA/VILI, and HPV-DNA detection for high-risk genotypes [6]. The aim of secondary prevention is screening for precancerous lesion and treating them.

MATERIALS AND METHODS

The purpose of this study was to identify the risk factors responsible for HPV infections and its association with development of cervical carcinoma. It was carried out in Department of Microbiology, Stanley Medical College, in association with department of Gynaecology, RSRM hospital, Stanley Medical College, Chennai between August 2012 to September 2013. During this study period women attending Gynaecology outpatient Department of age group 20 to 65 years with history of white discharge, blood-stained discharge; heavy menstrual flow, intermenstrual spotting, and postcoital bleeding were included in the study while pregnant women and with history of hysterectomy were excluded. Permission to conduct the study was obtained from the Institutional Ethical Committee of Stanley Medical College and respective hospital authorities. Informed consent was obtained from the patients before enrolling in the study.

To obtain cervical scrapping sample the cervical os and surrounding ectocervix area were cleared of excess mucus using a cotton swab and then the cervix was washed with normal saline. Using Ayers spatula cervical cells from ectocervix was obtained. The cells were immediately transferred to 2ml aliquot containing cold phosphate buffer saline (PBS) and then the aliquot was transferred to cold storage box containing ice packs. Strict cold temperature was maintained throughout the transportation of the sample and they were stored in deep freezer at -70°C until they were processed. For the selection of subjects, preferably a woman was tested only two weeks after the first day of her last menstrual period (LMP) and not when she was menstruating. Vaginal contraceptives or douches were not used during the 48 hours before examination. Woman who abstained from intercourse 24 hours prior to examination were selected.

For Pap smear sample, the exfoliated cervical cells were collected. In this procedure a small spatula was gently rotated at the cervical os. Then the tissue obtained was smeared and spread on a glass slide and fixed using a cytotoxicative and then sent to the pathology laboratory for analysis of the cells.

For the Visual Inspection with Acetic Acid (VIA) with Magnification, patient was kept in lithotomy position and cervix was visualised using Cusco’s speculum. After cleaning the os and ectocervix with normal saline, 3% acetic acid was applied to cervix and after one minute observed for acetowhite area under bright illumination using ring lens magnification. For Visual Inspection with Lugol’s Iodine (VILI) under Magnification, 5% Lugol’s Iodine was applied instead of 3% acetic acid. The normal epithelium was mahogany brown in colour and if precancerous cells were present it would appear as saffron yellow in colour.

Statistical Analysis was done with PASW (Predictive Analysis Software), Statistics-18 version by statistician. P value obtained by Chi Square test and p<0.05 was considered significant.

RESULTS

The prevalence of Human papillomavirus infections in our study was 20% from total of 100 patients. PCR positivity was highest among the age group 30-39 years. It was 9 cases (20 %). HPV infection was highest among the age group 19-20 years.

| Age in years | Number of women | Number of women for positive for HPV DNA |
|--------------|-----------------|------------------------------------------|
| No. %        | No %            |                                          |
| 17-18        | 10 % 1         | 1                                        |
| 19-20        | 55 % 11        | 11                                       |
| 21-23        | 27 % 8         | 8                                        |
| >24          | 8 % 0          | 0                                        |
| Total        | 100 % 20       | 20                                       |

Statistical analysis of above data showed that there is a significant difference in the HPV infection among different age groups (p<0.05). The highest HPV-DNA positivity was seen in multiparous women having 3 or more children had shown high positivity for HPV- DNA by PCR.
Table 2: Parity with PCR positivity

| Parity | Number of women | Number of women for positive for HPV DNA |
|--------|-----------------|------------------------------------------|
| No     | %               | No | %                          |
| 0      | 2               | 0  | 0                          |
| 1      | 15              | 0  | 0                          |
| 2      | 59              | 8  | 8                          |
| >3     | 24              | 12 | 12                         |
| Total  | 100             | 20 |                            |

Illiterate women and women with only up to primary education had shown the highest HPV infection and it was 18%.

Fig-1: Literacy Rate & Socio-Economic status of the patients with PCR positivity

Nonvegetarian women had highest HPV infection. Two women who were positive for HPV infection had Family history of cervical cancer and they were asymptomatic. Among the asymptomatic three were positive for HPV -DNA. Major symptom of the PCR positive cases was white discharge.

Fig-2: Symptoms of the patients with PCR positivity

PCR positivity was 11.9 % among women who had healthy cervix. PCR for HPV-DNA was 100% positive in women who had cervical growth. They harboured HPV -16 and 18.
PCR for HPV-DNA was positive (13%) among patient who had normal Pap smear. Among the women who had HSIL in Pap smear report had HPV-DNA positivity of 7%. Women who had squamous cell carcinoma were positive for HPV-DNA 16 & 18.

DISCUSSIONS
Among the symptomatic patients the commonest age group was between 30-39 years with mean age of 38.83 years. This is well correlated with study of Aggarwal et al 2006 where the mean age is 37.5 years [7].

Early age of marriage (19-20) years in this study shows highest HPV positivity (20 %). This correlates with the study done by Anna R Giuliano et al., [8] which shows 21.1 %. HPV infection in our study is highest in multiparous women parity >3 shows (50- %). This correlates with the study done by Aggarwal et al., 2006.

In this study Women had an illiteracy rate of 70% have shown positivity for HPV infections. This correlated with Franceschi et al., [9] and Aggarwal et al., 2006. The study shows HPV positivity is associated with 90% belonging to low socioeconomic class. This is in correlation with Burd et al., 2003 [10].

In this study association with family history of CA cervix with PCR positivity is 2%. But the study conducted by Shankar Narayana et al., [11] showed 9.8%. Use of OCP in this study did not show association with cervical patients. Use of OCP for 5 years longer is associated with 3-fold risk of cervical cancer. Nonvegetarian diet associated with 100% positivity for HPV infection. This is in correlation with study of Sedjo et al., 2002 [12] and Rao et al., 2010 [13].

In this study Women had an illiteracy rate of 70% have shown positivity for HPV infections. This correlated with Franceschi et al and Aggarwal et al 2006. PCR positivity was 11.9% among women who had healthy cervix for HPV-DNA was 85.7 % positive in women who had cervical growth. They harboured HPV-16 and 18.HR-HPV in CA cervix is 100% in correlation with Sahar Elderdiri et al., 2013 (99.7%) [14].

PCR for HPV-DNA was positive (13%) among patient who had normal Pap smear. Among the women who had HSIL in Pap smear report had HPV-DNA positivity of 7%. Women who had squamous cell carcinoma were positive for HPV-DNA 16 & 18had 100%. This correlates with the study done by Karolina et al., [15] where it was 98.7%.

CONCLUSION
In India, every seven minutes a woman dies due to cervical cancer. Pap smear and HPV-DNA testing increases the sensitivity of diagnosing precancerous lesions of cervix before they progress to frank cancer. Pap smear and HPV –DNA testing if done together it increases the screening interval to 5 years instead of 3 every year. This study shows the importance of Pap smear and HPV-DNA testing of women in our country where prevalence and risk factors are extremely high. It can identify and help preventing or treating the condition at exceedingly early stage.

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