CHAPTER 5: DISCUSSION

Human papillomavirus (HPV) plays a central role in the development of cervical cancer and is considered to be a important, although, not always a sufficient cause. According to the literature, persistent high risk HPV infection increases the risk of cervical precancer or invasive cervical cancers. The distribution of HPV varies geographically; however, HPV 16 is the commonest type, followed by HPV 18. There is plenty of data available on the prevalence of HPV in women with cervical cancer, but, there is a paucity of the data on HPV prevalence in women, with clinically normal cervix from India.

Post-treatment, HPV testing for confirmation of successful cervical treatment, may allow women to return to routine and extended cervical cancer screening intervals. However, it is important to distinguish between the newly detected HPV genotypes from the recurrent lesion associated HPV genotypes. There is paucity of the data available regarding the frequency of newly detected HPV genotypes, following treatment for cervical precancer and cancer. These estimates are important, for determining the optimal use and interpretation of post-treatment HPV screening results, and may also aid in determining, whether, vaccination may have any benefit in these women.

In developing countries, like India, cervical cancer is one of the most common malignancies among middle aged women, especially in rural areas. Therefore, the development and availability of HPV vaccine will definitely help in limiting the cervical cancer incidence, and may also bring down the cost burden for cervical cancer screening programmes in India. To maximize the cost effectiveness of the HPV vaccination programmes in India, it is important to understand the distribution of the major HPV types in various geographical regions.

5.1. Demographic distribution

As shown in Table 4.1, majority of the participants were Hindu 58.1% (90/155), followed by the Muslims 32.2% (50/155), and Christians 9.7% (15/155). None of the participants were from any other community except these three communities, which was similar to the study done by Duttagupta et al., (2004), from East India, in which they have reported that, 51.3%, 45.6%, 3.1% were, Hindu, Muslim and Christian participants, respectively.
Table 4.1 also shows that, 73.5% (114/155) of the study participants from the below poverty category (which was considered on the basis of having a ration card), yet most of them, i.e., 65.8% (102/155) were educated. However, in contrary to a study from Kashmir, which reported that, 51.9% of the study participants were from effluent backgrounds, yet 41% subjects had a school education up to 6th standard, and 37.6% subjects had education in between 6-12th standard. Abulizi et al., (2017), reported that, higher HPV infection was observed in participants with a higher family income. While, present study reports that, the maximum frequency of HPV infection, 80.8% (21/26), was observed in low income families.

After the assessment of the questionnaire, it was observed that, even though the subjects were classified based on literacy based on the classification given by Arora et al., (2017), none of the study participants, i.e., 100%, were aware of HPV infection or HPV mediated malignancies. However, according to Ganju et al., (2016), the awareness regarding cervical cancer from India, Nepal and Sri Lanka, was 66%, 58.8% and 57.7%, respectively.

The mean age of the participants was 43 ± 10.3 years, of which 40% (62/155) were in the age group of 40-50 years, followed by 30-40 years, 27.7% (43/155), which was in accordance with the previous studies done in Asia pacific region. However, studies done by Vince et al., (2001), and Asiaf et al., (2012), reported that, the major age groups for HPV isolation, was 18-57 years, which was not in accordance with the present study. Persistence of virus plays a key role in the course of HPV infection, which was observed in the present study, as the maximum number of HPV DNA positive samples, were obtained from the patients who were in the age group of 40-50 years, 34.6% (09/26).

According to literature, the peak prevalence rate of HPV, is in the range of 20 to 24 years of age, with a steady decline as age progress. However, as shown in Table 4.2 and Fig. 4.1, the incidence of HPV was low in the age group of 20-40 years, higher in the age group 40-60 years, and lesser in patients age ≥60 years of age. Similar results were reported by Abulizi et al., (2017), that the incidence of HPV infection in women >45 years of age was significantly higher. However, on the contrary, it was reported by the Asiaf et al., (2012), that, the prevalence of HPV was highest (33.3%) in women aged 18-27 years, which gradually decreased to 7.9% in women aged 38-47 years, and then again showed a rise to 13.5% in women aged 4-57 years.
5.2. Human papillomavirus type distribution among women with cervical abnormalities

Prevalence of Human papillomavirus, in women with normal cervical cytology was estimated between 1.4-25.6%.\textsuperscript{288} Epidemiological studies throughout the world, suggest that, the prevalence of high-risk HPV variants varies geographically.\textsuperscript{285} The International Agency for Research in Cancer, survey in Asia, reported a prevalence of 9.6%, of which 5.4% were high-risk types, among which HPV16 was twice as frequent as any other high-risk type, in all the regions.\textsuperscript{289,290}

Present study shows that, the occurrence of HPV 16, among invasive carcinomas and cervical intraepithelial neoplasias, was found to be 29.4% (10/34, n=79), as compared to women with chronic cervicitis and other non inflammatory symptoms, which was found to be 13.3% (06/45, n=79). Similarly, studies from Andhra Pradesh (2005), and Brazil (2009), reported that, the most prevalent HPV types found in the invasive cervical cancers, were HPV 16, followed by HPV 18.\textsuperscript{85,291}

As given in Table 4.5, among women with negative cytology results as for intraepithelial lesion/malignancy (NILM) and inflammatory smear, HPV occurrence was 11.43% (08/70, n=76), whereas, among women with cytology results as atypical squamous cells of undetermined significance (ASCUS), and inflammatory smear with reactive atypia, was 33.3% (02/06, n=76), which is in accordance with the study from Pakistan.\textsuperscript{211} However, the occurrence of HPV infection in the present study, was found to be low, i.e., 26/155 (16.8%), which was in accordance with a study by Chakravarty et al., (2016),\textsuperscript{292} from East India. In which, reported that the prevalence of HPV among HIV positive subjects was 26.9%, which was according to the author, was the high prevalence as compared to the general population. Similarly, Asirf et al., (2012),\textsuperscript{277} also reported that, the overall prevalence was 13.8%. Whereas, results from western countries, where HPV prevalence was found to be quite high, are contradictory to the present study.\textsuperscript{293,294}

Similar to the present study, a study done in Morocco, which studied, tissues biopsies and swab samples for HPV infection. With PCR, HPV was detected in 91/147 (62%) patients. The high-risk HPV types 16 and 18 were found in 45% of the cases (41/91), and HPV type 18 was found in 19% of the cases (17/91). Mix infection with HPV types 16 and 18 were found in 3 cases. Whereas, among 447 swabs tested, only 28 (6%) were positive for HPV infection.\textsuperscript{293} However,
results of the present study, among tissue biopsies, are not in accordance with this study, as only 20.1% (16/79) biopsies were positive for HPV DNA, whereas, among cytobrush samples, the results are in accordance, as only 13.2% (10/76) samples were positive for HPV DNA.

In the present study, 96.2% (25/155) HPV DNA positive samples were positive for only HPV type 16. Whereas, only one sample was co-infected with, both, HPV type 16 and 18. Similarly, in a study by Klaes et al., (1999), 200 high-risk HPV infections were detected in 242 samples (44.1%), of which 155 were positive for either HPV16 or 18. In another study from Korea by Cho et al., (2015), also reported that, although, the most prevalent genotype was HPV 53, HPV 16 was the most persistent HPV type, and together, HPV 16 and HPV 18 account for 35% of HPV positive samples.

5.3. Detection of E6/E7 oncogenes of HPV 16/18 from HPV DNA positive samples

In the present study, E6/E7 was detected in 38.46% (10/26) of HPV DNA positive cervical samples. In which, HPV 16 E6/E7 was detected in 90% of the samples (09/10), and HPV 18 E6/E7 in 10% of the samples (01/10). However, in a study by Fontecha et al., (2016), overall E6/E7 was detected in 68.3%, among which, HPV 16 E6/E7 was detected in 71.4% and HPV 18 E6/E7 was detected in 10.7% of the samples.

As shown in Table 4.6 and 4.7, among HPV DNA positive, tissue biopsies 16/26, and cytobrush samples 10/26, only six and four samples were positive for E6/E7 oncogenes respectively. Therefore, in the present study, only these 10, E6/E7 positive samples, were considered positive for HPV mediated malignancy, and the rest 16 samples, which were only HPV DNA positive and negative for oncogenes, were considered negative for HPV mediated malignancy. The reason being, during carcinogenesis process, over expression of oncoproteins, E6 and E7, is a necessary factor in the development of cervical neoplasia. Moreover, according to Holm et al., (2008), based on the individual results of type specific PCR and in situ hybridization (ISH), the presence of HPV DNA was not found to be a prognostic factor.

As shown in Table 4.6, among eight samples of cervical cancer, only four samples were positive for E6/E7 oncogenes, and the remaining four samples were negative for HPV mediated malignancy. However, presence of cervical malignancy suggests that there must be some other reason for the malignancy among these four patients. Similarly, among HPV16 DNA positive samples with, CIN 2 (2/16), cervical inflammation (5), and mild dysplasia (1), only one each of
CIN 2 and chronic cervicitis are positive for HPV 16 E6 oncogene. However, biopsies with, chronic non specific cervicitis and mild dysplasia, were just HPV infected. Therefore, these patients still have a chance of recovery from HPV infection, as a result of immune response of the body. \(^{295}\) Nevertheless, according to Discacciati et al., (2014),\(^{297}\) E6/E7 oncogene is not a long-term significant and static prognostic factor, since women who were positive for HPV DNA and negative for E6/E7 oncogene, may later become E6/ E7 oncogene positive, which would change the pathology results.

Similarly, among ten HPV DNA positive cytobrush samples, with the cytological reports as NILM (07), ASCUS (01) and inflammatory smears (01), only 03/07 of NILM and 01/01 of ASCUS were positive for HPV16 E6 oncogene. As discussed earlier, the reason could be the same in the prognosis of the disease.

### 5.4. **Comparison of cytology/ histopathology results with the PCR results**

Pap smear examination is a simple, cost effective and sensitive tool, for the early detection of premalignant and malignant changes in the cervical cells. The efficiency, sensitivity and specificity of the Pap smear depends on, frequency of cervical cancer screening programs, adequate sample collection, and the quality of laboratory analysis.\(^{298}\)

In a study on HPV infection among head and neck cancer from Rome, (2014), has reported that, comparison of the HPV status of cytology and histology of samples from patients with head and neck squamous cell carcinoma, shows that 90.4\% of the cases were concordant. \(^{299}\) As given in Table 4.8, the results of the present study, were contradictory, as among tissue biopsies, out of 43 histopathologically positive samples, only 11/43 (25.6\%) were PCR positive for HPV DNA, and remaining biopsies were negative. Whereas, 31 histopathologically negative biopsies 31/36 (86.1\%) were negative by PCR also. Similarly, among 06 cytologically positive cytobrush samples, only 02/06 (33.3\%) samples were positive by PCR, and 62 cytologically negative samples 62/70 (88.6\%), were negative by PCR also.

A study published from north India, has reported that, the Pap smear had sensitivity of 80\%, with a positive predictive value of 49\%, and a negative predictive value of 88.2\%.\(^{297}\) As it is well known that, the infection in cervix with HPV is reversible,\(^{295}\) this could be one of the reasons for the discordance between cytology and molecular detection, or the result might be influenced by the self-clearance of the virus, during the interval between Pap test and molecular assay.
5.5. DNA sequencing of HPV 16/18 and their E6/E7 oncoproteins

As already reported in other studies, despite its limitations, sequencing has been considered the gold standard for HPV genotyping, due to the possibility of identifying virtually all virus types without any false classifications due to cross-reactions among similar types, which can occur in tests, based on hybridization. Till date, Sanger sequencing is the gold standard, but it lacks the resolution, generated by next-generation sequencing (NGS) techniques.

By molecular detection (with one set of consensus general primers) of HPV DNA in clinical specimens, for high sensitivity and efficiency, the specificity is sacrificed, for the detection of all possible clinically relevant genotypes, including the less frequently encountered variants as well. The target DNA can be validated, by DNA sequencing of the inter-primer segment of the PCR amplicon. Therefore, in the present study, to validate the results of PCR, randomly, HPV DNA positive samples (08) were selected, and outsourced for sequencing. Comparison with the known alleles in the NCBI’s GeneBank, showed that in the present study no novel mutations were evident among all alleles, as all the nucleotide sequences showed 100% to 99% (Fig 4.4) similarity with the other sequences.

5.6. Predisposing risk factors for the acquisition of HPV infection

The frequently cited risk factors for HPV infection are: multiple sexual partners, use of oral contraceptives, early marriage and early sexual debut, number of parities, and smoking. Findings from the current study suggest that HPV infection was significantly associated with, early marriage/sexual debut \((p=0.01)\) and use of cotton cloth/pads \((p=0.03)\). According to the literature, high parity and early age at first pregnancy have been also described, as important risk factors for cervical cancer. With respect to prior immunization against HPV, a study from Germany, (2014), reported that, among 223 vaccinated women, prevalence of HPV 16/18 was significantly lower as compared to non-vaccinated women \((13.9\% \text{ vs. } 22.5\%, p = 0.007)\).

In the present study, 91\% \((141/155)\) participants were monogamous, among them only 16.3\% \((23/141)\), participants had HPV infection. Whereas, 9.03\% \((14/155)\) participants had \(\geq 2\) sexual partners, among them 21.4\% \((03/14)\) participants had HPV infection. However, according to the literature, having multiple sexual partners was the main risk factor, in the acquisition of HPV infection. Several studies have shown that, cervical infections occur shortly after sexual debut, emphasizing the importance of sexual activity in the transmission of HPV, which is in
accordance with the present study, as the age at marriage/sexual debut (considering the fact that according to the Indian culture making sexual contacts before marriage is prohibited) is significantly associated ($p=0.01$) with HPV infection. The reason for this association could be justified with the fact that, during puberty the cervix undergoes cellular changes at the transformation zone, known as ectopy. During ectopy, the cervical cells are at a higher risk of acquiring HPV infection, and to long lasting damage from an infection due to the viral persistance. Almonte et al., (2011), observed that, early sexual debut, and multiple partners, during a lifetime increases the risk of acquiring high-risk HPV in the near future. In a meta-analysis on sexual behavior from 29 countries, showed that women from Latin America and Caribbean (2016), tend to remain monogamous once married or cohabiting, while men do not. Therefore, the risk of contracting HPV infection among women also depends upon, to a great extent on the sexual behavior of her male partner(s).

In the present study, risk factors, such as, sexual partners, age at first delivery, number of parities, immune status or systemic disease, have no statistically significant association with HPV infection. Similar reports have been published earlier, with negative association between these risk factors and HPV infection. Few studies have addressed, the risk of HPV infection and smoking, and found a significant correlation, attributed to a local impairment of cell-mediated immunity, but the effect of this was small. On the other hand, in the present study, as shown in Table 4.10, risk factors like, use of oral contraceptives, social habits (smoking/alcohol/ chewing tobacco), personal hygiene, nutrition and prior HPV immunization, were excluded from the statistical analysis, as out of two options for the assessment of these risk factors, either the same option was selected by all the participants, or no HPV infection was detected. So, the $p$ value could not be calculated. However, according to literature, the use of oral contraceptives and smoking were the two principal factors found to accelerate maturation, and are known to be important risk factors for cervical neoplasia, which is not in accordance with the present study.

The present study also reveals, that the women using or used cotton cloth in the past during menstruation, were significantly associated ($p=0.03$) with HPV infection, than those who were using sanitary napkins. The reason for this association could be attributed to, use of poor quality of cloth, or by not maintaining proper hygiene while washing, and re-using the same cloth, during menstruation. However, results of the present study, contradict the results published by
Abulizi *et al.*, (2017), \(^{282}\) where they reported that, women using sanitary napkin or toilet paper during menstruation, had higher chance of HPV infection, than those using regular cloth.

5.7. **Knowledge and awareness regarding HPV infection and cervical cancer**

If detected early, cervical carcinoma is preventable as well as curable. The incidence rates are almost five-times greater in developing countries than, developed nations. \(^{316}\) A lack of effective screening programs, is an important reason, for the much higher cervical cancer incidence in developing countries. \(^{317}\) It has been estimated that, about 5% of women in developing countries are screened for cervical dysplasia in the past 5 years, compared to the 40-50% of women screened in developed countries. \(^{318}\)

Till date, Pap test is the standard method, which is widely used for the cervical cancer screening in India. However, there are very few organized screening programs available in India. Despite the availability of Pap testing, the incidence of invasive cervical cancer remains high, especially in rural India. \(^{85}\) Similar results were obtained in the present study (Table 4.11) that, even though there are screening facilities available, yet 70.3% (109/155) of the subjects had no idea regarding the cervical cancer screening, and therefore 94.8% (147/155) participants, never had undergone any screening for cervical cancer. On the other hand, none of the participants (155/155) ever heard of HPV infection, and therefore, never had any HPV test done before.

Although, vaccines are available for 2 major high risk oncogenic HPVs, i.e., type 16 and 18, the major problem in vaccinating women in developing countries is, the lack of knowledge, or awareness and the high cost of vaccination. \(^{313}\) However, a study from North India by Hussain *et al.*, (2014), \(^{320}\) on the willingness to take HPV vaccination (after educating, about the risks of HPV infection and consequences related to this virus), it was found that, the willingness was 70% and 64%, in rural and urban populations respectively.

As shown in Table 4.11, in the present study it was found that, none of the participants had any idea regarding HPV infection, and HPV mediated malignancy. However, 29.7% (46/155) participants have heard about cervical cancer, but had no idea about its causes, and there were common knowledge gaps concerning the risk factors of cervical cancer and HPV infection. However, in a qualitative study, by Modibbo *et al.*, (2015), \(^{321}\) on the barriers to cervical cancer screening, reported that, most of the women were aware of cervical cancer, but only one third of them had ever been screened, and only one participant had ever heard of the association between...
HPV and cervical cancer. They have also reported that, their findings were similar to findings from other low-resource settings, in which, they found that the knowledge of women about cervical cancer is elementary at best, and that there was a high prevalence of ignorance about causes, symptoms and treatment options, for cervical cancer.\textsuperscript{321}

From western countries, many studies are reported on knowledge of cervical cancer, and HPV and its health effects, but as such from India, there are very few studies available. One such community based study from southern India, on rural women was conducted in 2014,\textsuperscript{322} in which they have reported that, none of the participants have heard of HPV, and are unaware of its health effects. They concluded that, there is a total ignorance about HPV and its health effects, among rural women, which may be due to the absence of organized cervical screening programmes in India. As shown in Table 4.11, in the present study, similar results were obtained, regarding, both, knowledge and awareness of HPV infection and its health effects.

Unlike, studies from western countries, with the increase in education, the knowledge and awareness also increases, but in the present study, education does not correlate with the knowledge of cervical cancer, and HPV and its health effects. However, earlier studies have shown an important association, between health behavior and educational level.\textsuperscript{323} Therefore, improvement of the knowledge, about the reproductive factors associated with HPV, may help in identifying women at risk, and to develop different methods of preventive interventions.