

Original Research Article

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## Prevalence and Molecular Characterization of High-Risk Human Papilloma Viruses 16 and 18 among Women of Reproductive Age Group in a Tertiary Care Hospital

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### ABSTRACT

**Introduction:** Human Papilloma Virus (HPV) infection is the most common sexually transmitted infection in the world. People having Human Immunodeficiency Virus (HIV) are prone to develop squamous intraepithelial lesions that result in having six times higher incidence and persistence of HPV lesions while comparing with the general population. Against this backdrop, this study aims to detect the molecular characterization of HR-HPV16 and 18 isolates and their prevalence among women of reproductive age group. **Materials and Methods:** 100 samples of endocervical tissues of which 50 samples from HIV negative and 50 samples from HIV positive women were collected with two cervical swabs during gynecological examination. The tip of the swabs containing cellular materials was placed into a labeled screw-capped tube containing 3 ml of viral transport medium and they were transported to the State level Virus Research and Diagnostic Laboratory (VRDL) on the same day and stored at -80°C in a deep freezer until analyzed. Samples were subjected to DNA extraction and were subjected to PCR analysis for detection of HPV 16 and 18. **Results:** The overall prevalence of HPV 16/18 was 10% detected among HIV positive women and there were no cases among HIV negative women. Out of the 50 HIV positive women on ART, the prevalence of HPV 18 was 18% and HPV 16 was 2%. The mean age was 37.5 years. Both the mean age at first sexual intercourse and age at marriage was 17.8 years; mean age at first childbirth was 18.6 years. The prevalence of HPV 18 was 14% and HPV 16 was 2% seen among women with HIV who had no abortion. The prevalence of HPV 18 was 12% and HPV 16 was 2% observed among women who did not use contraceptives in addition, HPV 18 prevalence was 14% each and HPV 16 was 2% each seen among women who had history of STI and those who had more than one sexual partner respectively. The mean CD4 count was 327 cells/mm<sup>3</sup>. **Conclusion:** The prevalence of HR-HPV among women and its association with various diseases including cervical cancer is one of the most challenging problems in the present world. Further, the study fosters the development of overall strategies for enhancing the health status of women in general and the study area in particular by encouraging changes in the sexual behavior and lifestyle of women.

#### Keywords

Sexual activity, smoking, use of oral contraceptives, Human Papilloma Virus (HPV)

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## **Introduction**

Human Papilloma Virus (HPV) infection is the most common sexually transmitted infection in the world. (Palefsky *et al.*, 2016) HPV is an epitheliotropic virus belonging to the Papillomaviridae family which is small, non-enveloped with 50 nm diameter icosahedral capsid enclosing a genome of 8 kb double-stranded DNA. (Nejo *et al.*, 2018)

According to the International Agency for Research on Cancer (IARC), HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 66 are generally stated as “High-Risk HPVs” or HR-HPV which are associated with the development of precancerous lesion and cancers. (Sichero *et al.*, 2020) HPV requires the association of other co-factors such as the number of sexual partners, early sexual activity, smoking, use of oral contraceptives, history of sexually transmitted diseases like HIV.

These co-factors might influence the development, maintenance, and progression of intraepithelial lesions to invasive cancers. (Tran *et al.*, 2015) Immunosuppression resultant of HIV upsurges the risk of developing squamous intraepithelial lesions and might have six times higher incidence and persistence of HPV lesions when compared with the general population. (Teixeira *et al.*, 2018)

Cervical cancer is the fourth most common cancer occurring in low- and middle-income countries among women, with an estimated number of 604,127 new cases and 341,831 deaths in 2020. (Globocan, 2020)

The International Papillomavirus Society (IPVS) has declared 4th March as International HPV day in 2018 and in 2021, to commemorate International Human Papillomavirus Awareness Day (IHAD) the IPVS hosted a great high-level panel event on that day, entitled: Accelerating progress against HPV-related in the era of COVID-19 and the theme is “HPV: A Virus We All Can Beat”. (Brandt, Heather M *et al.*, 2019)

The main aim and objectives of this study to detect the Molecular characterization of Human Papilloma Virus isolates for 16 and 18 years; and also to detect the prevalence of Human Papilloma Viruses 16 and 18 among women of reproductive age group.

## **Materials and Methods**

### **Study Location**

A prospective study was carried out in the Department of Microbiology, Guntur Medical College, Guntur in association with Gynecology OPD, ART Center and DVL OPD, Government General Hospital, Guntur, Andhra Pradesh.

### **Study Period**

The study was undertaken for a period of one and a half years from June 2020 to November 2021.

### **Sample Size**

100 samples were taken from women of reproductive age group attending Gynecology OPD, ART Center and DVL OPD, Government General Hospital, Guntur, Andhra Pradesh.

### **Study Group**

50 HIV positive women of reproductive age group visiting ART Center and DVL OPD and 50 HIV negative women of reproductive age group visiting OPD of Gynecology OPD, Government General Hospital, Guntur, Andhra Pradesh were considered.

### **Inclusion Criteria**

Women of reproductive age group between 16-45 years attending OPD of Gynecology, DVL and ART Center, Government General Hospital, Guntur, Andhra Pradesh, who could communicate effectively and agree by signing/ providing thumbing impression in the consent form.

### **Exclusion Criteria**

Patients not willing to participate in the study

The age group below 16 years and above 45 years

Pregnant women

Menstruating women

Pap smear with abnormal cervical cytology

Hysterectomized women

### **Ethical Issues**

Institutional Ethical Committee (IEC) approval was obtained before carrying out the study.

The patients were explained about the study procedure. After their acceptance, the informed consent form was given in vernacular/local language (Telugu), and they were asked to sign or put thumb impression. A structured questionnaire was used in a face-to-face interview to record the socio-demographic details, sexual information, and medical history.

Socio-demographic details, such as age, residence, education and socio-economic status, were obtained. Sexual information such as age at marriage, age at first intercourse, age at first childbirth, number of sexual partners, contraceptive use and abortion history were obtained. In addition, medical information such as the history of HIV and STI history was obtained.

### **Study Procedure**

After explaining the procedure, the patients were asked to lie in lithotomy position in a closed room, and under aseptic precaution, a bi-valved speculum was introduced into the cervix in order to visualize it. After the cervix was exposed, the sample was obtained by inserting two cervical swabs 1-1.5 cm into the endocervical canal and gently rotating 3-5 full turns in a counter-clockwise direction. The tip of the swabs containing cellular materials was placed into a labeled screw-capped tube containing 3 ml of viral transport medium and they were transported to

the State level Virus Research and Diagnostic Laboratory (VRDL), Guntur Medical College, Guntur, Andhra Pradesh on the same day and stored at -80°C in a deep freezer until analyzed. Samples were subjected to DNA extraction and were subjected to PCR analysis for detection of HPV 16 and 18 using HPV 16 and 18 detection kits purchased from Bio-Medica Corporation.

### **Statistical Analysis**

The statistical analyses were performed using Microsoft Excel Data Analysis Software. In this connection, various factors of the study relating to HPV 16/18 infections were analyzed by regression model using univariate analysis. In addition, all the P values reported were made based on two-tailed tests with a significance level of 0.05. (Nejo *et al.*, 2018; Misbah Noor *et al.*, 2018; Yang, Jing *et al.*, 2020)

### **Results and Discussion**

The present study included 100 reproductive age group women of which 50 were HIV positive on ART and the rest 50 HIV negative. (Figure 1) The majority of patients ranged between the age group 36-45 years of which 54% were HIV positive and 38% were HIV negative. There was a rural predominance among 86% of HIV positive and 76% of HIV negative women. The majority of patients belonged to lower socio-economic group of which 82% were HIV positive and 52% HIV negative. The educational level was illiterate in 56% of HIV positive and 40% of HIV negative women. In addition, women less than 18 years of age having sexual intercourse as well as married had participated. 63% of HIV positive women who had first child birth were at the age of 19-20 years, whereas it was 20-25 years in 50% of HIV negative women. The majority of women had no abortion and did not use contraceptives. 36% of HIV positive women had history of STI; 64% and 42% of them had 1 sexual partner and had CD4 count ranging between 201-350 cells/mm<sup>3</sup> respectively. (Table 1) The overall prevalence of HPV 16/18 was 10%

detected among HIV positive women and there were no cases among HIV negative women. (Figure 2) Out of the 50 HIV positive women on ART, the prevalence of HPV 18 was 18% and HPV 16 was 2% (Figure 3).

The mean age was 37.5 years. Prevalence of HPV 18 was 18% and HPV 16 was 2% observed among HIV positive women from rural and lower socio-economic status. In addition, 8% and 2% of illiterate women with HIV had prevalence of HPV 18 and HPV 16 respectively. Both the mean age at first sexual intercourse and age at marriage was 17.8 years; mean age at first childbirth was 18.6 years. The prevalence of HPV 18 was 14% and HPV 16 was 2% seen among women with HIV who had no abortion. The prevalence of HPV 18 was 12% and HPV 16 was 2% observed among women who did not use contraceptives in addition, HPV 18 prevalence was 14% each and HPV 16 was 2% each seen among women who had history of STI and those who had more than one sexual partner respectively. The mean CD4 count was 327 cells/mm<sup>3</sup> (Table 2).

Infections with high-risk HR-HPV types 16 and 18 in immune-compromised, specifically HIV, can persist and lead to warts, precancerous lesions, or cancers. That is why chronic infections with HR-HPV types are deep-rooted in almost all cervical cancer cases. (Temsgen *et al.*, 2021) Available reports in connection with this study show a wide variation in the prevalence of HPV infection and genotypes distribution owing to diversified socio-economic and cultural conditions. (Senapati *et al.*, 2017) Some of the reports compared with the present study are as follows:

Since there was zero prevalence found among HIV negative women, the study deals with 50 HIV positive women only. The prevalence of HPV 18 was 18% and HPV 16 was 2% observed among HIV positive women are nearly correlating with the study by Pérez-Quintanilla, *et al.*, (2020) from Scotland (HPV 18: 20%) and and Yawo Tufa Nyasenu *et al.*, (2019) from Lome Togo (HPV 16: 1.3%) respectively. As per their studies, high prevalence of

HPV 18 was due to immunological vulnerability, change in sexual behavior and condom usage, number of sexual partners led to low prevalence of HPV 16 respectively.

The mean age was 37.5 years with a statistically significant p value of 0.0156 and 95% CI of 0.24-0.46 which is concordant with the study by Teixeira, *et al.*, (2018) from Manaus Amazonas (37.5 years) and as per his study, the peak of HR-HPV prevalence was not observed among young women. According to Mchome *et al.*, from Denmark, the HPV prevalence was higher among HIV-positive women in all age groups except among the young women less than 29 years.

Rural women of 90% (significant p value=0.00039) and urban women of 10% were reported which are nearly correlating with the study conducted by Senapati *et al.*, (2017) from Odisha (rural: 89%; urban: 11%) and as per his study sexual conception and behavior of women are conservative and men possessing multiple sexual partners are major source of HPV infection in rural women. Low-income earning women of 90% (significant p value = 0.00039) and middle-income earning women of 10% were reported which are nearly correlating with Kapoor *et al.*, (2020) from New Delhi. (Lower: 89.1%; middle: 11.9%). According to Nejo *et al.*, from Nigeria, unemployed women are low-income earners and this could increase their level of poverty as well as high-risk sexual practices resulting in acquiring STIs like HPV. In the present study, illiterate women of 50% and literate women of 50% were observed with a statistically significant p value of 0.0267 and 95% CI of 0.04-0.29 which are nearly correlating with Monterio *et al.*, (2021) from Brazil. (Illiterate-53.3%; literate-46.7%) According to Chakravarty *et al.*, (2016) from Varanasi, illiteracy, and poverty, rural women lack knowledge on sexual health and sexual practices which make them more vulnerable to unsafe sex leading to a higher risk of acquiring HPV infection.

The mean age at first sexual intercourse was 17.8 years with a statistically significant p value of 0.027 and 95% CI of 0.06-0.42 is nearly correlating with

Ginindza *et al.*, from Swaziland (17.9 years). According to Yang *et al.*, from China, the age at first sexual intercourse and number of births were risk factors. The possible reason might be that long lengths of exposure to risk factors for women with earlier sexual behavior and a greater number of births led to more serious cervical injury. The mean age at marriage and mean age at first childbirth was 17.8 years (p value of 0.0275 and 95% CI of 0.06-0.42) and 18.6 years (p value of 0.0158 and 95% CI of 0.13-0.45) are nearly correlating with Senapathi *et al.*, (2017) from Odisha (18.3 years) and Reichheld *et al.*, (2020) from Odisha (19.1 years) respectively. According to Niyazi *et al.*, (2016) from China, early marriage might be a risk factor for HR-HPV infection. Girls may be forced to marry at a young age due to poverty which might be associated with an increased risk of HR-HPV infection.

80% of women with no history of abortion and 20% with history of abortion which are statistically significant (p value of 0.001 and 95% CI of 0.24-0.13) are nearly correlating with the study conducted by Mendoza *et al.*, from Peru (71.2% vs 28.8%). 70% of women who do not use contraceptives (Significant p value= 0.0001) and the rest 30% use contraceptive which are nearly correlating with Anthony Uchenna Emeribe *et al.*, (2021) from Nigeria (78.6% vs 21.4%).

According to Travassos *et al.*, (2017) from Brazil, prolonged exposure to exogenous and endogenous hormones, such as combined oral contraceptives, is related to cervical carcinogenesis. Chronic inflammation is promoted by hormonal action in the context of prolonged use of oral contraceptives which allows exposure of the transformation zone of the ectocervix for a longer period of time; which is associated with a higher risk of acquiring HPV infection. 80% of women with a history of STI (significant p value=0.0002) whereas 20% of women with no history of STI which are nearly

correlating with Jary *et al.*, (2021) from France (88.6% vs 11.4%). According to Temesgen *et al.*, (2021) from Ethiopia, women with STI history have an altered cervical epithelium due to local inflammatory response which makes them more vulnerable to HPV persistence.

The mean number of sexual partners was one which is concordant with the study conducted by Ginindza *et al.*, from Swaziland (1), and S. Joshi *et al.*, from Pune (1). In addition, a significant p value of 0.00023 among >1 sexual partner is obtained.

According to Mchome *et al.*, (2021) from Denmark, women with the greater number of partners ( $\geq 9$ ) had more than three times higher risk of acquisition of HPV infection than women with  $\leq 1$  partner. The mean CD4 count of about 327 cells/ mm<sup>3</sup> which is statistically significant (p value of 0.019 and 95% CI of 0.08-0.34) is nearly correlating with the study conducted by Ouladlarsen *et al.*, (2018) from Morocco (330.1 cells/ mm<sup>3</sup>). According to Tartaglia *et al.*, (2017) from Italy, CD4 counts lower than 500 cells/ mm<sup>3</sup> were associated with higher HPV infections thereby reflecting the inability of HIV-positive women's immune systems to respond to opportunist infection.

The prevalence of HR-HPV among women and its association with various diseases including cervical cancer is one of the most challenging problems in the present world. In India, cervical cancer is reported to be the second commonest among women. As a result, India has been developing effective strategies for the prevention of HR-HPV since the early 1990s. Against this background, the present study is carried out to detect the prevalence of HR-HPV 16/18 and associated factors among the reproductive age group of women in Guntur, Andhra Pradesh. The study finds that HIV-positive women have more HPV prevalence than HIV- negative women.

**Table.1** Socio-demographic, Behavioral, Reproductive and Lifestyle variables of the study population according to HIV status

Variable	HIV positive women (n=50)	HIV negative women (n=50)
<b>Age in y</b>		
16-25	7 (14%)	13 (26%)
26-35	16 (32%)	18 (36%)
36-45	27 (54%)	19 (38%)
<b>Residen</b>		
Urban	7 (14%)	12 (24%)
Rural	43 (86%)	38 (76%)
<b>Socio-economic status</b>		
Lower Upper	41 (82%)	26 (52%)
	9 (18%)	24 (48%)
<b>Education</b>		
Illiterate Primary Secondary Tertiary	28 (56%)	20 (40%)
	15 (30%)	18 (36%)
	6 (12%)	10 (20%)
	1 (2%)	2 (4%)
<b>Age at 1st sexual intercourse in years</b>		
≤ 18	28 (56%)	24 (48%)
19-20	19 (38%)	13 (26%)
20-25	2 (4%)	11 (22%)
>25	1 (2%)	2 (4%)
<b>Age at marriage</b>		
≤ 18	28 (56%)	24 (48%)
19-20	19 (38%)	13 (26%)
20-25	2 (4%)	11 (22%)
>25	1 (2%)	2 (4%)
<b>Age at 1st childbirth (n= 46)</b>		
≤ 18	13 (28%)	6 (12%)
19-20	29 (63%)	4 (8%)
20-25	3 (7%)	25 (50%)
>25	1 (2%)	15 (30%)
<b>No: of abortions</b>		
0	32 (64%)	40 (80%)
1	8 (16%)	8 (16%)
2	7 (14%)	2 (4%)
3	3 (6%)	00
<b>Use of contraceptives</b>		
Yes No	11 (22%)	8 (16%)
	39 (78%)	42 (84%)

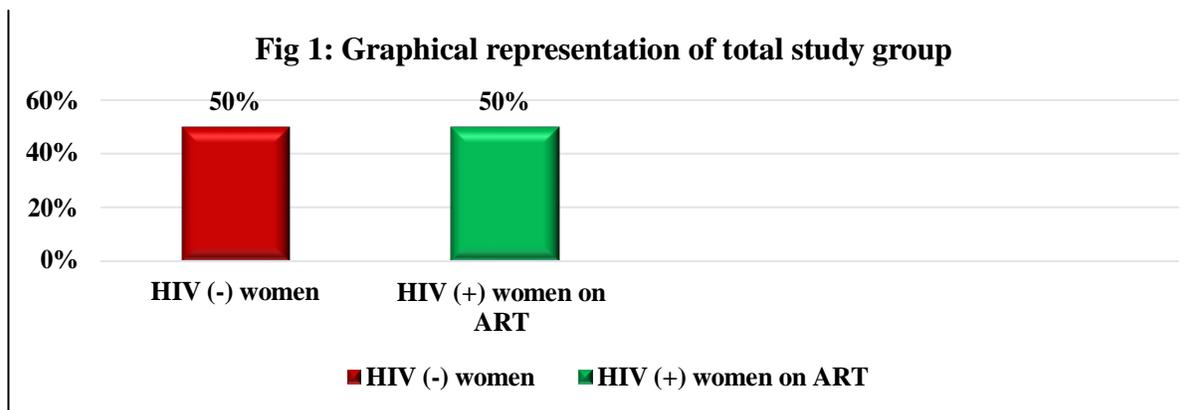
<b>STI history</b> Yes No	18 (36%) 32 (64%)	<b>00</b> <b>50 (100%)</b>
<b>No: of sexual partners</b> 1 >1	32 (64%) 18 (36%)	<b>48 (96%)</b> <b>2 (4%)</b>
<b>CD4 count in cells/mm3</b>		
<200	13 (26%)	-
201-350	21 (42%)	-
351-500	14 (28%)	-
>500	<b>2 (4%)</b>	-

**Table.2** Prevalence of HPV 16 and 18 according to Socio-demographic, Behavioral, Reproductive and Lifestyle variables in HIV positive women on ART

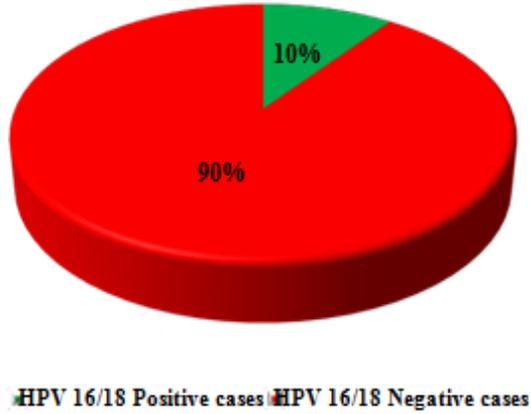
Variable	HIV positive women (n=50)	HPV 16	HPV 18	95% Confidence Interval (95% CI)	p value
<b>Age in years</b>					
16-25	7 (14%)	00	00	0.24-0.46	<b>0.0156</b>
26-35	16 (32%)	1 (2%)	2 (4%)		
36-45	27 (54%)	00	7 (14%)		
<b>Residence</b>					
Urban	7 (14%)	00	1 (2%)	-	<b>0.00039</b>
Rural	43 (86%)	1 (2%)	8 (16%)		
<b>Socio-economic status</b>					
Lower	41 (82%)	1 (2%)	8 (16%)	-	<b>0.00039</b>
Upper	9 (18%)	00	1 (2%)		
<b>Education</b>					
Illiterate	28 (56%)	1 (2%)	4 (8%)	0.04-0.29	<b>0.0267</b>
Primary	15 (30%)	00	3 (6%)		
Secondary	6 (12%)	00	2 (4%)		
Tertiary	1 (2%)	00	00		
		00			
<b>Age at 1st sexual intercourse in years</b>					
≤ 18	28 (56%)	1 (2%)	6 (12%)	0.06-0.42	<b>0.027</b>
19-20	19 (38%)	00	3 (6%)		
20-25	2 (4%)	00	00		
>25	1 (2%)	00	00		

<b>Age at marriage</b>					
≤ 18	28 (56%)	1 (2%)	6 (12%)	0.06-0.42	<b>0.0275</b>
19-20	19 (38%)	00	3 (6%)		
20-25	2 (4%)	00	00		
>25	1 (2%)	00	00		
<b>Age at 1st childbirth</b> (n= 46)					
≤ 18	13 (28%)	00	2 (4.3%)	0.13-0.45	<b>0.0158</b>
19-20	29 (63%)	1 (2.2%)	7 (15.2%)		
20-25	3 (7%)	00	00		
>25	1 (2%)	00	00		
<b>No: of abortions</b>					
0	32 (64%)	1 (2%)	7 (14%)	0.24-0.31	<b>0.001</b>
1	8 (16%)	00	1 (2%)		
2	7 (14%)	00	1 (2%)		
3	3 (6%)	00	00		
<b>Use of contraceptives</b>					
Yes No	11 (22%) 39 (78%)	00 1 (2%)	3 (6%) 6 (12%)	-	<b>0.0001</b>
<b>STI history</b>					
Yes No	18 (36%) 32 (64%)	1 (2%) 00	7 (14%) 2 (4%)	-	<b>0.0002</b>
<b>No: of sexual partners</b>					
1	32 (64%)	00	2 (4%)	-	<b>0.00023</b>
>1	18 (36%)	1 (2%)	7 (14%)		
<b>CD4 count in cells/mm3</b>					
<200	13 (26%)	00	3 (6%)	0.08-0.34	<b>0.019</b>
201-350	21 (42%)	1 (2%)	3 (6%)		
351-500	14 (28%)	00	3 (6%)		
>500	2 (4%)	00	00		

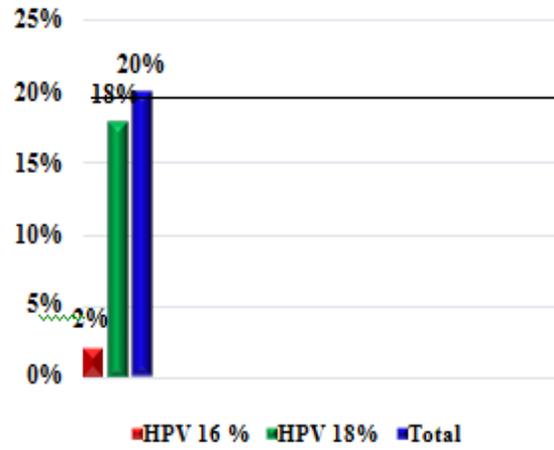
**Fig.1** Graphical representation of total study group



**Fig 2: Graphical representation of overall prevalence of HPV 16/18 among the study group**



**Fig 3: Graphical representation of prevalence of HPV 16 and 18 among HIV Positive women on ART**



According to the study, early detection and vaccination are very important for controlling the HR-HPV implicated complications leading to co-morbidity among the study groups in the future.

Further, the study provides essential information about various demographic factors associated with HR-HPV and genotypes, which will contribute to policy development and planning of prevention strategies, specifically for cervical cancer.

It supports, therefore, the guidelines of WHO for comprehensive screening incorporating HR-HPV testing with the help of PCR and strengthening of the existing conventional screening programme.

More importantly, it also provides epidemiological knowledge for the dissemination of information among women about HR-HPV, cervical cancer, and the benefits of HPV vaccinations pertinent to successful control and management.

Further, the study fosters the development of overall strategies for enhancing the health status of women in general and the study area in particular by encouraging changes in the sexual behavior and lifestyle of women.

### **Strengths of the Study**

Good laboratory practices that include: effective sample collection, transport, storage, DNA extraction, master mix preparation, and PCR analysis, are adopted in the study.

PCR available in the VRDL lab is utilized for sample analysis.

The entire work has been monitored by the guide regularly stage by stage.

The study has utilized a nucleic acid extraction kit and HPV 16/ 18 detection kit purchased from Bio-Medica Corporation, Hyderabad.

### **Limitations of the study**

The following points about the limitations of the study have to be noted.

Size of the sample is restricted to 50 reproductive women among HIV negative;

Due to zero prevalence observed among HIV-negative women, a comparative study between HIV-

positive and HIV-negative women could not be carried out.

Among HR-HPV types, only 16 and 18 genotypes were included in the study.

Follow-up of the patients was not done.

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