" DETECTION OF HIGH RISK HUMAN PAPILLOMA VIRUS IN CERVICAL TISSUE, URINE AND PLASMA IN SQUAMOUS CELL CARCINOMA OF UTERINE CERVIX BY REAL TIME POLYMERASE CHAIN REACTION – A CROSS SECTIONAL STUDY"

BY

DR. BHADRA.A.R,MBBS

DISSERTATION SUBMITTED TO
SRI DEVARAJ URS ACADEMY OF HIGHER EDUCATION & RESEARCH
TAMAKA, KOLAR, KARNATAKA
IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF MEDICINE

IN

PATHOLOGY

UNDER THE GUIDANCE OF

Dr. KALYANI R, MD, PhD, FAMS, FICP
PROFESSOR AND FORMER HOD

DEPARTMENT OF PATHOLOGY
SRI DEVARAJ URS MEDICAL COLLEGE, KOLAR

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Date: Signature of the Candidate

Place: Kolar **Dr.BHADRA.A.R**

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LIST OF ABBREVATIONS

SERIAL NO.	ABBREVIATION	EXPANSION
1.	SCC	Squamous Cell Carcinoma
2.	HPV	Human Papilloma Virus
3.	HR- HPV	High Risk Human Papilloma Virus
4.	PCR	Polymerase Chain Reaction
5.	qPCR	Quantitative Polymerase Chain Reaction
6.	RT-PCR	Real time Polymerase Chain Reaction
7.	CIN	Cervical intraepithelial Neoplasia
8.	SIL	Squamous intraepithelial lesion
9.	LSIL	Low grade squamous intraepithelial lesion
10.	HSIL	High grade squamous intraepithelial lesion
11.	SCJ	Squamocolumnar junction
12.	DNA	Deoxy ribo nucleic acid
13.	ctHPVDNA	Circulating tumor Human Papilloma Virus Deoxy ribo nucleic acid
14.	cHPV-DNA	Circulating Human Papilloma Virus Deoxy ribo nucleic acid
15.	RB	Retinoblastoma
16.	OPSCC	Oropharyngeal squamous cell carcinoma
17.	ELISA	Enzyme linked immunosorbent assay
18.	VLP	Virus Like Prticles
19.	IPC	Internal Positive Control

20.	FAM	Fluorescein amidite
21.	Cy5	Cyanine 5 fluorescent dye
22.	VIC	Vic-2'-chloro-7'-phenyl-1,4-dichloro-6-carboxyfluorescein
23.	WD	Well Differentiated
24.	MD	Moderately Differentiated
25.	PD	Poorly Differentiated
26.	AIS	Adenocarcinoma in situ
27.	FIGO	Federation of International Gynecologists and Obstetricians
28.	OBG	Obstetrics and Gynaecology
29.	MRI	Magnetic Resonance Imaging
30.	H&E	Hematoxylin and Eosin
31.	Ct value	Cycle threshold value
32.	PPV	Positive predictive value
33.	NPV	Negative predictive value
34.	LR	Likelihood Ratio
35.	UPG	Ulceroproliferative growth
36.	WHO	World Health Organisation

ABSTRACT

BACKGROUND

Cervical cancer ranks as the third most common cancer affecting females worldwide. Human papillomavirus (HPV) has been detected in approximately 95% of cervical cancer cases, underscoring its role as a primary cause of the disease. Recent studies have shown that HPV detection in urine samples is a highly effective non-invasive method for early screening of cervical cancer, particularly among young women. Urine sampling offers a convenient and non-invasive collection method, making it a preferred technique among women. This approach also enables the simultaneous testing of primary high-risk human papillomavirus (HR-HPV) and biomarkers within the same sample. The use of urine samples for HPV detection can help mitigate issues related to loss to follow-up and non-adherence to screening protocols. Furthermore, urine testing provides a viable alternative for cervical HPV detection, especially in subgroups where traditional methods may be challenging.

AIM OF THE STUDY

To detect High Risk HPV in cervical tissues, urine and plasma in cases Squamous Cell Carcinoma of Cervix

METHODS

This study was done in Departments of OBG, Pathology and Microbiology, SDUMC, Tamaka, Kolar over a period of 18 months from May 2023 to October 2024. The study comprised 75 newly diagnosed cases of primary squamous cell carcinoma (SCC) of the cervix, confirmed by cervical biopsy. For each case, cervical tissue, urine samples, and blood samples were collected. DNA extraction was performed using the Truenat instrument, and Real-Time Polymerase Chain Reaction (RT-PCR) was employed to detect various high-risk

Human Papillomavirus (HPV) types. Statistical analysis was performed using 'SPSS' software. Descriptive statistics were used to summarize demographic and clinical variables. ROC curve and likelihood ratio were also calculated

RESULTS

Our study revealed that the peak incidence of cervical cancer occurred in the 50-59 year age group, with a significant proportion (72%) of cases presenting in the postmenopausal age group. Postmenopausal bleeding was the most common clinical presentation, observed in 48% of cases. Upon per speculum examination, an ulceroproliferative growth was noted in 68% of cases, indicating advanced disease at presentation. Among the 75 cervical cancer cases, 20% had lymph node involvement, 54% were FIGO Stage II, and 66.7% were well-differentiated carcinomas, with high-risk HPV DNA detected in 77% of cervical tissue samples, 55% of urine samples, and 11% of blood samples. Urine testing showed 69% sensitivity, 94.1% specificity, 97.6% positive predictive value and 47.1% negative predictive value, making it a reliable diagnostic method, whereas plasma testing had limited sensitivity of 13.8% despite high specificity (100%) , high positive predictive value (100%) and 25.4 % negative predictive value.

CONCLUSION

This study underscores the potential of urine-based high-risk human papillomavirus (HR-HPV) detection as a non-invasive screening method for cervical cancer, offering a promising avenue for future research, development, and innovation in cervical cancer prevention, early detection, and treatment strategies. Ultimately, this approach may contribute significantly to achieving the World Health Organization's (WHO) goal of screening 70% of eligible women

by 2030, thereby reducing the global burden of cervical cancer.

KEYWORDS: Squamous cell carcinoma , High risk human papilloma virus

INTRODUCTION

INTRODUCTION

Cervical cancer is the 3rdmost common cancer in females in the world. Approximately 5,29,000 cases were newly diagnosed with cervical cancer and 2,75,100 died from the disease annually.[1] In India, cervical cancer is the 2nd most common cancer in women. 9.4% of all cancers and 18.3% of newly diagnosed cases in 2020 were of cervical cancer in women.[2] The incidence rate of cervical cancer in the Cancer registry in Bengaluru is 15.3.[3] The prevalence of Cervical Cancer in Kolar was reported as 17.55% of total female cancers and is the 2nd most common site of cancer in females. Majority of the cases were Squamous cell carcinoma (SCC) of cervix.[4]

Human papillomavirus (HPV) has been identified in approximately 95% of cervical cancers.[5] The most common oncogenic subtypes of HPV are HPV16 and HPV18 and which produce E6 and E7 oncoproteins . Currently, HPV associated cancers are identified using testing techniques as cytological examinations Papanicolau test (Pap smear), PCR (polymerase chain reaction), viral nucleic acid detection assays and tissue biopsy tests [6]

HPV detection in urine samples has been reported as the most effective non-invasive methods for early screening of cervical cancer in young women as it is easily and non invasively collected. Therefore it is a highly preferred technique among women and offers the ability to test both primary high risk human papillomavirus and biomarkers in the same sample. This use of urine sample confers opportunities to reduce loss to follow up and non-adherence to screening subject. Moreover, as cervical HPV detection is considered difficult in particular subgroups, urine testing should be regarded an acceptable alternative.[7,8]

As far as our knowledge goes, there have not been many studies done in India regarding

isolation of HR-HPV in cervical tissue, urine and plasma (triple samples) in SCC of Cervix.

There is not much literature or data suggesting detection of HPV from blood samples in India.

AIMS
&
OBJECTIVES

AIM AND OBJECTIVES OF THE STUDY

AIM:

To detect HR-HPV in cervical tissues, urine and plasma in SCC of Cervix cases.

OBJECTIVES

1. Detection of HR- HPV in cervical tissues , urine samples and in plasma in SCC of Cervix
2. To determine the association of HR-HPV in tissue with urine and plasma (triple samples) in SCC of Cervix

REVIEW OF LITERATURE

REVIEW OF LITERATURE

GROSS ANATOMY

The uterus is anatomically divided into three distinct regions: the corpus, isthmus, and cervix. The cervix, the most inferior portion of the uterus, protrudes into the upper vagina. In adult nulligravid women, the cervix measures approximately 2.5-3.0 cm in length. The cervix is composed of two main parts: the endocervix and ectocervix. The endocervix, located at the upper portion of the cervix, transitions from the lower uterine segment or isthmus of the uterus. In contrast, the ectocervix is the outer portion of the cervix that protrudes into the vaginal canal.[9]

HISTOLOGY

The cervix is a complex structure comprising a mixture of fibrous, muscular, and elastic tissue, lined by both columnar and squamous epithelium. Fibrous connective tissue predominates, providing structural support. The mature ectocervix is characterized by stratified squamous epithelium, which is organized into three distinct zones:

1. The basal/parabasal or germinal cell layer, responsible for continuous epithelial renewal.

2. The midzone or stratum spinosum, the dominant portion of the epithelium.

3. The superficial zone, containing the most mature cell population.. (Fig 1A)

The endocervical epithelium consist of a single layer of mucin-secreting, columnar epithelium which lines both the surface of the endocervical canal and the underlying glandular structures. (Fig 1B)[9]

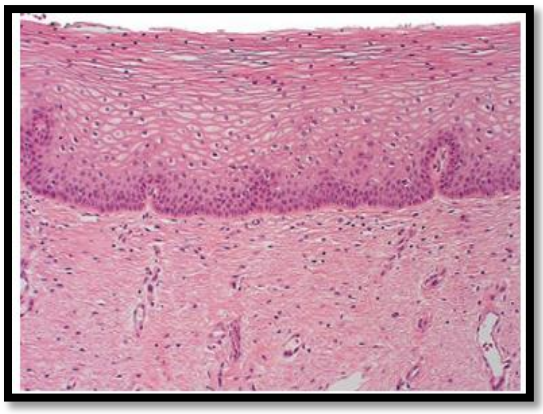


Figure 1A: Histological representation of (H&E , 200 x) Ectocervical lining [9]

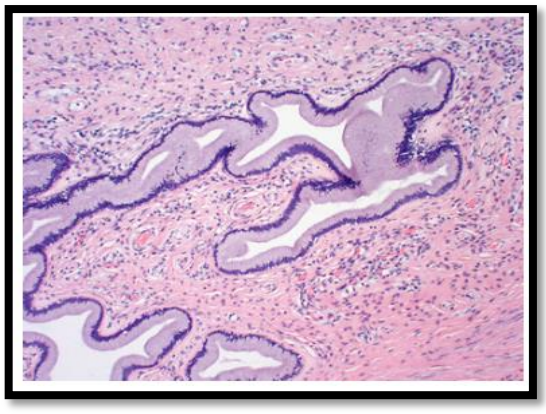


Figure 1B: Histological representation of (H&E , 200 x) Endocervical lining[9]

TRANSFORMATION ZONE

The squamocolumnar junction (SCJ) of the cervix marks the anatomical boundary between the stratified squamous epithelium of the ectocervix and the mucin-secreting columnar epithelium of the endocervix. The region where these two epithelial types coexist is specifically referred to as the "transformation zone" (TZ), a critical area of interest in cervical cancer screening and prevention

The distinctive epithelial environment of the cervix, particularly the transformation zone, renders it highly vulnerable to human papillomavirus (HPV) infection, the primary cause of cervical cancer. Immature squamous cells within this zone are especially susceptible to HPV infection, and consequently, this is where the majority of cervical precursor lesions and cervical cancers originate, highlighting the importance of targeted screening and prevention strategies.[9,10]

EMBRYOLOGY AND DEVELOPMENT

The cervix, like the uterus and vagina, develops from the paramesonephric ducts. At birth, the cervix is predominantly fibrous and makes up most of the uterus, whereas the

uterine cervix is muscular. The cervix has two types of epithelial linings: the ectocervix (covered in squamous epithelium) and the endocervix (lined with columnar epithelium). The junction where these two linings meet, known as the squamocolumnar junction, varies in location from person to person. The squamocolumnar junction migrates into the endocervical canal as age of women increases. This leads to the replacement of the endocervical epithelium with ectocervical epithelium, resulting in squamous metaplasia.[9,10]

IMMUNITY IN CERVIX

The cervix possesses a robust cellular immune system, featuring dendritic cells and lymphocytes residing within the epithelial and subepithelial layers. This specialized mucosal immunity serves as a vital defense mechanism, protecting the host against viral and bacterial infections.[9]

BENIGN DISEASES OF CERVIX

Benign cervical pathology encompasses a range of non-cancerous conditions including cysts, benign tumors, and inflammatory disorders.

INFLAMMATORY

Cervicitis: Cervicitis can manifest as either an acute or chronic condition. Acute cervicitis is marked by the presence of neutrophils within the epithelium and stroma, accompanied by swelling (edema) and increased blood flow (vascular congestion). In contrast, chronic cervicitis is characterized by a persistent inflammatory response, featuring an influx of lymphocytes, plasma cells, and histiocytes, along with the formation of granulation tissue and scarring (stromal fibrosis).

Noninfectious cervicitis: Chemical irritation, such as douching, or physical trauma from inserting objects like tampons, pessaries, or contraceptive devices can cause cervical irritation.

Infectious cervicitis: The cervix is normally inhabited by lactobacilli, which maintain a low pH environment that inhibits the growth of other microorganisms. However, various pathogens can disrupt this balance and infect the cervix, including:

Bacterial organisms: Chlamydia trachomatis, Neisseria gonorrhoeae, Mycoplasma hominis, Group B Streptococcus, Ureaplasma ureolyticum, Gardnerella vaginalis, Actinomyces israelii, Mycobacterium tuberculosis, Treponema pallidum

Fungal organisms: Candida, Aspergillus

Viral organisms: Herpes simplex virus, Human papillomavirus (HPV)

Protozoa and parasites: Trichomonas vaginalis, Ameba Schistosomes[9,10]

HUMAN PAPILLOMA VIRUS

Papillomaviruses belong to the family Papillomaviridae. These viruses are double-stranded DNA viruses characterized by a genome of approximately 8,000 base pairs, a non-enveloped virion measuring 45-55 nm in diameter, and an icosahedral capsid composed of 72 capsomers. Papillomaviruses are ubiquitous in nature. Human papillomaviruses (HPVs) are classified within the genus Alpha papillomavirus. Notably, the A9 clade, with HPV 16 as its prototypic virus, and the A7 clade, comprising HPV 18 and 45, are of particular significance due to their association with human disease. To be classified as a distinct human papillomavirus (HPV) genotype, a minimum of 10%

divergence in the base-pair sequence of the highly conserved L1 region (major capsid protein) is required compared to other genotypes. Despite their structural similarities, different HPV types exhibit notable specificity regarding the anatomical location of the epithelia they infect and the types of lesions they induce at the site of infection, highlighting the complex relationships between HPV genotypes, tissue tropism, and disease outcomes.[9,10]

The incidence of cervical cancer caused by HPV16 and HPV18 varies significantly across different regions. In East Asia, the incidence is lower approximately 68%, while in Japan it is around 60%. 82% of instances of cervical cancer in the US and Europe are caused by HPV 16 and HPV 18. While a startling 87.6% of cervical cancer cases in India are caused by HPV16 and HPV18.[11]Interestingly, a study in Bihar, India found that HPV16 was the most common genotype, detected in 76.5% of HPV-positive samples, followed by HPV18 at 10.2%. These findings emphasize the need for targeted screening and prevention strategies to combat cervical cancer in different parts of the world.

These geographical differences highlight the importance of considering regional HPV prevalence when selecting the most appropriate HPV test for primary screening. [11,12]

The most prevalent HPV types associated with cervical cancer were HPV 16, 18, 31, 33, 35, 45, 52, and 58, collectively accounting for 91% of HPV-positive cases. HPV 16 was the most common genotype, detected in 61% of cervical cancers, with a higher prevalence in squamous cell carcinomas (62%) than in adenocarcinomas (50%).In contrast, HPV 18 and 45 were more frequently found in adenocarcinomas (32% and 12%, respectively) than in squamous cell carcinomas (8% and 5%, respectively). Notably, HPV 16, 18, and 45 combined accounted for 94% of cervical adenocarcinomas. Other notable genotypes, HPV 31, 33, and 52, were each detected in 3-4% of cervical cancers.The HPV, an

oncogenic virus, is the most important pathogen causing cervix infections. The most significant contributing factor to the development of cervical cancer is unquestionably high-risk HPVs. [9,10]

HPV-16 and 18 demonstrate the strongest carcinogenic potency, accounting for approximately 70% of cancers, with HPV16 the major causal agent of squamous cell carcinoma and HPV-18 contributing approximately equally to adenocarcinoma. [9,10]

High-risk HPVs are also implicated in squamous cell carcinomas arising at many other sites, including the vagina, vulva, penis, anus, tonsil, and other oropharyngeal locations.

The carcinogenic potential of HPV is attributed to its E6 and E7 proteins, which disrupt the function of pivotal tumor suppressor proteins, p53 and RB, respectively. HPV infects immature squamous cells, but viral replication occurs in maturing squamous cells. Normally, these mature cells are arrested in the G1 phase of the cell cycle. However, when infected with HPV, they continue to progress through the cell cycle, allowing the virus to exploit the host cell's DNA synthesis machinery to replicate its own genome.

High-risk Human Papillomavirus (HPV) subtypes encode E6 and E7 proteins, which disrupt normal cell cycle regulation and DNA repair mechanisms, leading to cancer development. The E7 protein binds and degrades retinoblastoma protein (RB), promoting cell cycle progression, and inhibits cyclin-dependent kinase inhibitors p21 and p27. Meanwhile, the E6 protein binds and degrades tumor suppressor protein p53, impairing DNA repair, and upregulates telomerase, leading to cellular immortalization. In contrast, low-risk HPV subtypes have altered E6 and E7 protein functions, with reduced affinity for RB and p53, and instead dysregulate growth and survival through the Notch signaling pathway. [9,10]

Cervical carcinogenesis is explained in 3 steps

1. First step: Initial infection by a HR-HPV

2. Persistence of HPV infection leading to progression to a precursor lesion

3. Invasion –Cancer

To initiate infection, HPV must access the basal lamina and reach basal or primitive "basal-like" cells in the immature squamous epithelium, likely through minor epithelial defects or micro traumas. Establishing a transforming infection that can progress to high-grade squamous intraepithelial lesions (HSIL) and invasive cervical cancer is thought to require HPV access to specialized cells at the squamocolumnar junction, which possess distinct morphological and gene expression characteristics.

HPV's affinity for basal cells may be due to specific receptors present on these cells. Infection requires the basal cells to be actively dividing. Once infected, HPV restricts the basal cells' ability to differentiate, primarily through the E6 protein. Meanwhile, the E7 protein promotes cellular proliferation in the lower epithelial layers. This combination of delayed differentiation and sustained proliferation enables the virus to persist and expand the infected basal cell population, as non-infected cells differentiate and are replaced.

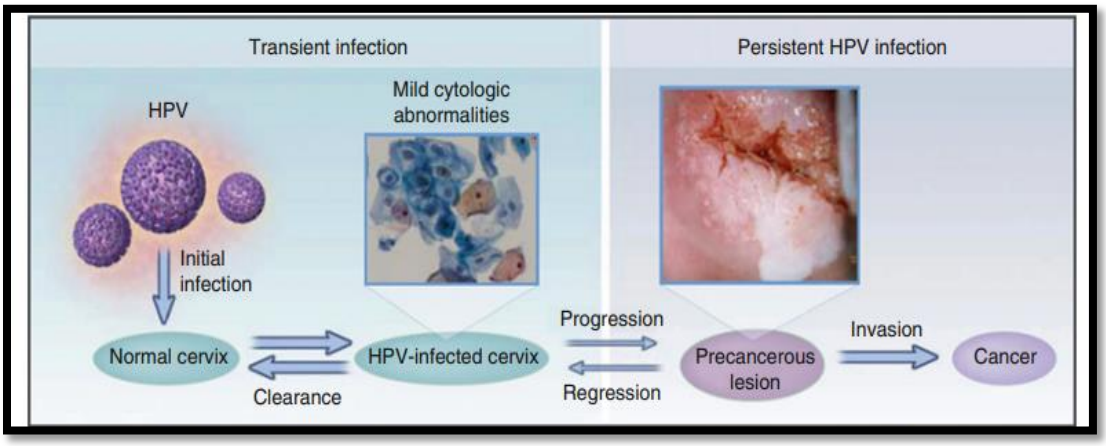


Figure 2: These are the three steps in cervical carcinogenesis [13]

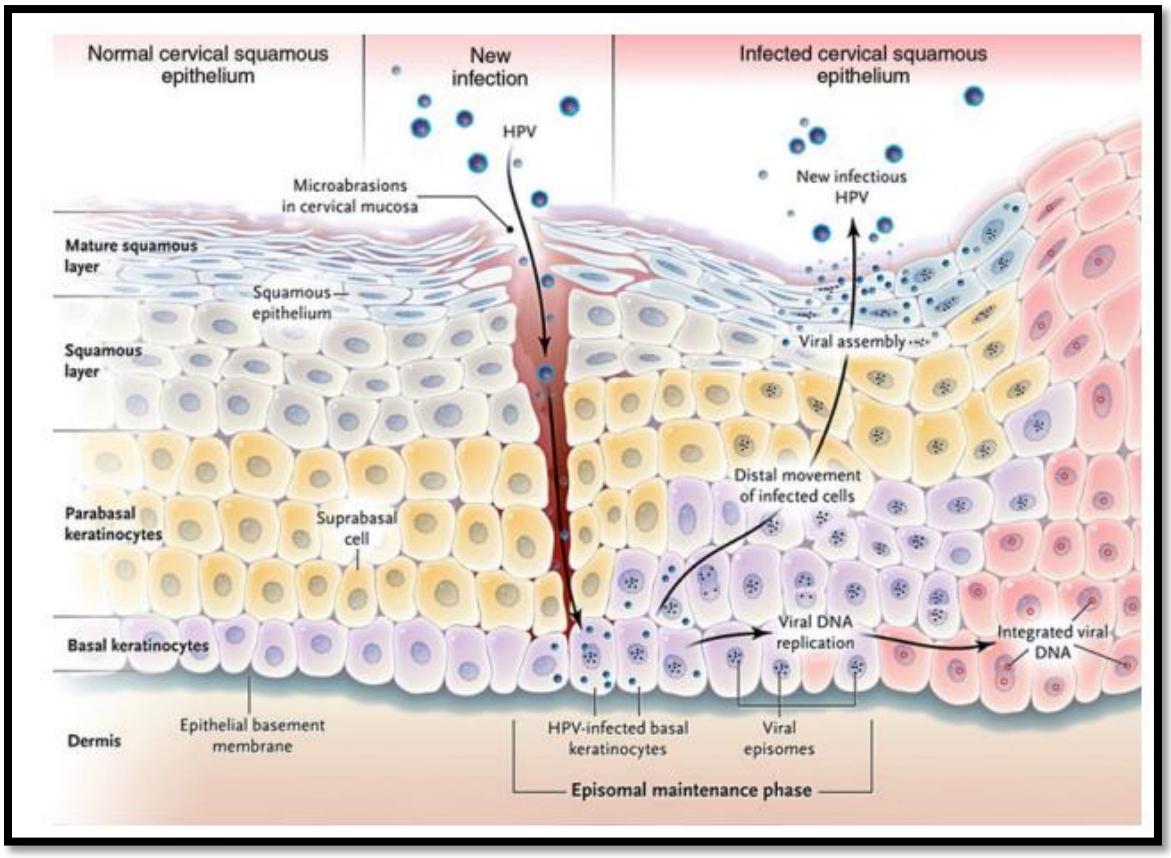


Figure 3: Pictorial representation of cervical carcinogenesis[13]

CYSTS

The most common cyst seen in cervix is the Nabothian cyst. These develop in the transformation zone. Other cysts seen in cervix can be tunnel clusters or inclusion cysts.

TABLE 1: WHO CLASSIFICATION OF UTERINE CERVICAL TUMOURS :(2020)[13]

I) SQUAMOUS EPITHELIAL TUMORS:	**A) MIMICS OF SQUAMOUS PRECURSOR LESIONS** **Squamous metaplasia**: Endocervical epithelium of single columnar cell layer replaced by the ectocervical epithelium **Atrophy of the uterine cervix:**Estrogen deficiency causing arrested maturation of squamous cells

	<u>B) SQUAMOUS CELL TUMORS AND PRECURSORS</u> **Condylomaacuminatum** **Squamous intraepithelial lesions of the uterine cervix**: Dysplasia caused by HPV in the squamous cells of ectocervixlimited to the epithelium: **Squamous cell carcinoma, HPV associated, of the uterine cervix** **Squamous cell carcinoma, HPV independent, of the uterine cervix** **Squamous cell carcinoma, NOS of the uterine cervix**
II) GLANDULAR TUMORS AND PRECURSORS	**A) BENIGN GLANDULAR LESIONS** **Endocervical polyp:** Exophytic growth covered by columnar endocervical cells with fibrovascularstroma **Mullerian papilloma of the uterine cervix:** Benign tumor of mullerian epithelium **Nabothian cyst:** Cyst filled with mucus and lined by endocervical columnar cells **Tunnel clusters:**Endocervical epithelium lined glands closely arranged in the cervical wall **Microglandular hyperplasia:**Hyperplastic lesion of small glands covered by mucinous epithelium that are closely packed **Lobular endocervical glandular hyperplasia**: Glands lined by endocervical columnar cells increased in a lobular pattern **Diffuse laminar endocervical hyperplasia**: Diffuse increase of irregular endocervical epithelium lined glands in a laminar pattern **Mesonephric remnants and hyperplasia**: Proliferation of the remnants of mesonephric duct **Arias Stella reaction of the uterine cervix**: Pregnancy and progestin associated changes in the columnar cells of endocervical gland **Endocervicosis:**Glands lined by mucinous columnar cells at the outer cervical wall

	Tuboendometrioid metaplasia: Change of endocervical glands to tubal type or endometrioid type, or both **Ectopic prostate tissue:** Epithelial cells in cervix that look like prostatic epithelium
	B) Adenocarcinomas **Adenocarcinoma in situ, HPV associated, of the uterine cervix** **Adenocarcinoma, HPV associated, of the uterine cervix** **Adenocarcinoma in situ, HPV independent, of the uterine cervix** **Adenocarcinoma, HPV independent, gastric type, of the uterine cervix** **Adenocarcinoma, HPV independent, clear cell type, of the uterine cervix** **Adenocarcinoma, HPV independent, mesonephric type, of the uterine cervix** **Other adenocarcinomas of the uterine cervix**
III) OTHER EPITHELIAL TUMORS:	Carcinosarcoma of the uterine cervix Adenosquamous and mucoepidermoid carcinomas of the uterine cervix Adenoid basal carcinoma of the uterine cervix Carcinoma of the uterine cervix, unclassifiable
IV)MIXED EPITHELIAL AND MESENCHYMAL TUMORS:	Adenomyoma of the uterine cervix Adenosarcoma of the uterine cervix
V) GERM CELL TUMORS:	Germ cell tumors of the uterine cervix

PRECANCEROUS LESIONS OF SQUAMOUS EPITHELIAL TUMORS– CERVIX

Cervical intraepithelial neoplasia (CIN), as the WHO refers to it, is one of the precursor lesions of the cervix. Squamous intraepithelial lesions (SIL) are referred to by Bethesda as Low Grade (LSIL) and High Grade (HSIL). Previously known as mild dysplasia, CIN1 is equivalent of LSIL. Although there are relatively slight alterations in the epithelium at the basal level, these lesions indicate a productive HPV infection with significant viral replication. 90% of LSIL patients revert within a year, while 10% of cases proceed to HSIL.CIN 2 and 3, formerly known as moderate and severe dysplasia [9,10]

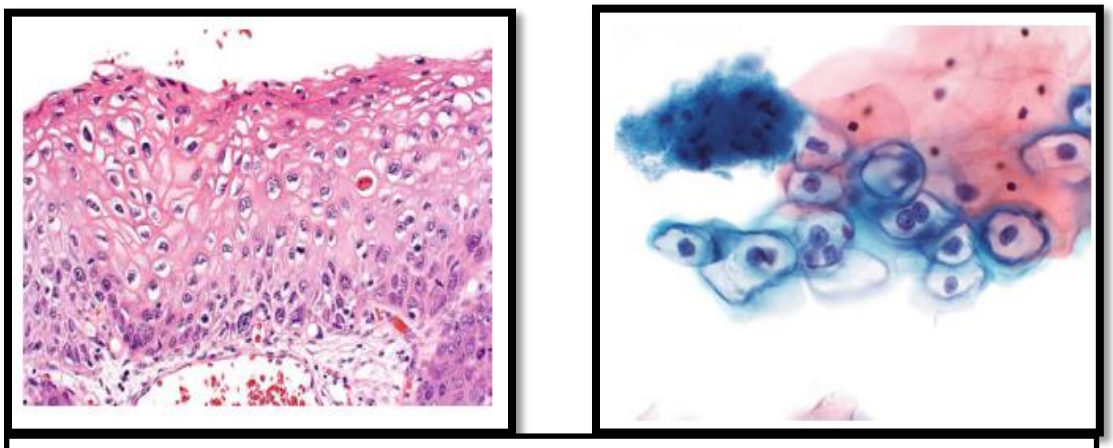


Figure 4: Pictorial representation of LSIL (H&E , 200 x) [13]

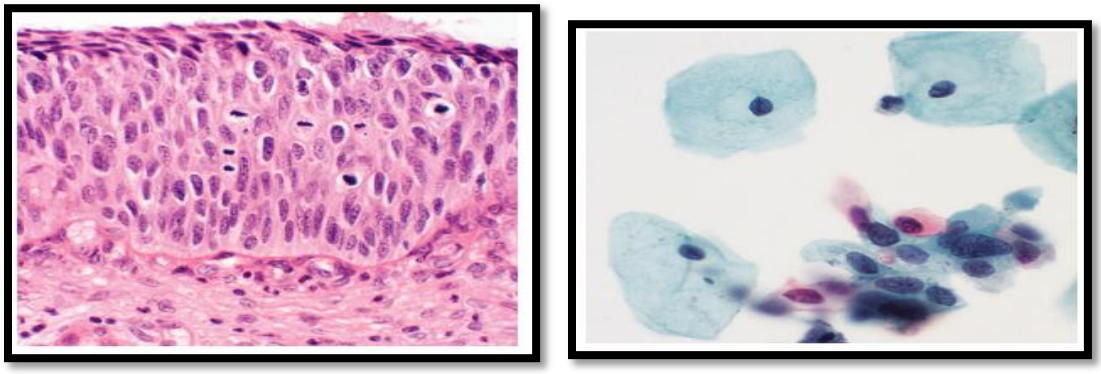


Figure 5: Pictorial representation of HSIL (H&E , 200 x) [13]

MALIGNANT TUMORS OF CERVIX:

Cervical cancer can be classified into several histologic subtypes, with the following distribution:

- Squamous cell carcinoma (80%): The most common type, originating from the squamous epithelium.
- Adenocarcinoma (15%): The second most common type, developing from glandular cells and often preceded by adenocarcinoma in situ.
- Rare subtypes (5%): Adenosquamous and neuroendocrine carcinomas.

Notably, all these subtypes are associated with HR-HPV infections.[13]

RISK FACTORS

HPV is the primary cause of cervical cancer, making risky sexual behaviors, such as having multiple partners and initiating sex at a young age, the most significant risk factors for the disease. Additional factors that increase cervical cancer risk include:

- Prolonged use of oral contraceptives
- Smoking, which is particularly linked to SCC
- Immunosuppression, which impairs the immune system's response to HPV infection

The connection between HIV and cervical neoplasia can be attributed to immunosuppression, highlighting the importance of a functional immune system in preventing HPV-related diseases.[9,10]

PRECANCEROUS LESIONS OF GLANDULAR TUMORS –CERVIX

Adenocarcinoma in situ (AIS), a precursor to adenocarcinoma, is less common than squamous cell carcinoma (SCC) precursors (SILs). AIS occurs in the endocervical glands, where the epithelial lining becomes dysplastic, showing hyperchromasia and

pseudostratification, but without invasion. Most AIS cases are associated with HR-HPV infection, with rare instances occurring without HPV. AIS is more challenging to detect through cervical screening compared to SIL.[13]

INVASIVE SQUAMOUS CELL CARCINOMA- CERVIX

An invasive epithelial tumour composed of squamous cells of varying degrees of differentiation.[13]

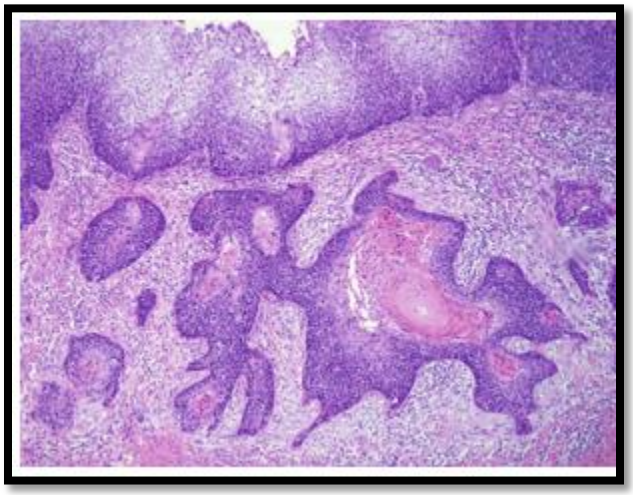


Figure 6: Pictorial representation of SCC (H&E , 200 x) [13]

They can be classified on their histological patterns as follows-

1. Squamous cell carcinoma, NOS
2. Keratinizing SCC
3. Non-keratinizing SCC
4. Papillary SCC
5. Basaloid SCC
6. Warty SCC
7. Verrucous SCC
8. Squamotransitional SCC
9. Lymphoepithelioma-like SCC

Prognostic factors with maximum implication in SCC cervix are the lymph node status and FIGO staging.

Depth of stromal invasion is a major factor in determining the outcome of patients with respect to risk of lymph node metastasis, risk of recurrence, and death from disease.[9,10]

ADENOCARCINOMA- CERVIX

An invasive epithelial tumour showing glandular differentiation. It can be both HPV dependent and HPV independent histologic classification of these tumors is based on the predominant cell type. The most common cervical adenocarcinoma type is the endocervical adenocarcinoma. The precursor lesion is adenocarcinoma in situ(AIS), endocervical type. HPV is detected in approximately 90% of cases.

ADENOSQUAMOUS CARCINOMA

Adenosquamous carcinoma is a rare, malignant epithelial tumor characterized by a combination of adenocarcinoma and squamous cell carcinoma components. These tumors typically arise from precursor lesions, including SIL and AIS, and are frequently associated with high-risk HPV types, primarily HPV-18 and HPV-16.

CARCINOSARCOMA

Carcinosarcomas, also known as malignant mixed Müllerian tumors, are rare tumors composed of both epithelial and mesenchymal components. The epithelial component typically resembles aggressive endometrioid or serous carcinoma, while the mesenchymal component can manifest as various sarcomas, including uterine stromal sarcoma, leiomyosarcoma, or heterologous elements like rhabdomyosarcoma or chondrosarcoma.

ADENOMYOMA

Adenomyoma of the cervix is a rare, benign tumor consisting of a mixture of epithelial and mesenchymal elements, characterized by endocervical-type glands embedded in a myomatous (smooth muscle-like) stroma.

GERM CELL TUMOURS OF CERVIX

Primary germ cell tumors of the cervix, including mature teratomas, yolk sac tumors, and non-gestational choriocarcinomas, are rare and histologically resemble their ovarian counterparts. A diagnosis of primary cervical germ cell tumor requires exclusion of metastasis from another site.

TABLE 2: FIGO STAGING 2018[13,14]

STAGE	DESCRIPTION
I	Carcinoma is strictly confined to the cervix (extension to uterine corpus should be disregarded)
IA	Invasive carcinoma that can be diagnosed only by microscopy with a maximum depth of invasion 3 and 5 mm in depth
IA1	Measured stromal invasion 3 mm in depth
IA2	Measured stromal invasion >3 and 5 mm in depth
IB	Invasive carcinoma with measured deepest invasion >5 mm; lesion limited to the cervix uteri with size measured by maximum tumor diameter
IB1	Invasive carcinoma >5 mm depth of stromal invasion and 2 cm in greatest dimension
IB2	Invasive carcinoma >2 and 4 cm in greatest dimension
IB3	Invasive carcinoma >4 cm in greatest dimension
II	The carcinoma invades beyond the uterus, but has not extended into the lower third of the vagina or to the pelvic wall
IIA	Involvement limited to the upper two-thirds of the vagina without parametrial involvement

IIA1	Invasive carcinoma 4 cm in greatest dimension
IIA2	Invasive carcinoma >4 cm in greatest dimension
IIB	With parametrial involvement but not up to the pelvic wall
III	The carcinoma involves the lower third of the vagina and/or extends to the pelvic wall and/or causes hydronephrosis or nonfunctioning kidney and/or involves pelvic and/or para-aortic lymph nodes
IIIA	The carcinoma involves the lower third of the vagina, with no extension to the pelvic wall
IIIB	Extension to the pelvic wall and/or hydronephrosis or nonfunctioning kidney (unless known to be due to another cause)
IIIC	Involvement of pelvic and/or para-aortic lymph nodes (including micrometastases), irrespective or tumor size and extent
IIIC1	Pelvic lymph node metastasis only
IIIC2	Para-aortic lymph node metastasis
IV	The carcinoma has extended beyond the true pelvis or has involved (biopsy proven) the mucosa of the bladder or rectum.
IVA	Spread to adjacent pelvic organs
IVB	Spread to distant organs

MRI IN CERVICAL CANCER

The latest FIGO staging system allows for accurate locoregional staging of cervical carcinoma using MRI, which provides detailed information on tumor size and extent of disease. This information has significant therapeutic implications, guiding surgical planning in early stages (I and IIA) and helping define radiation fields in advanced stages (IIB, III, and IV) where chemoradiation is the primary treatment, making MRI a crucial tool in cervical cancer staging and treatment planning.[14]

VARIOUS METHODS OF DETECTING HUMAN PAPILLOMA VIRUS IN BIOSAMPLES

HPV detection employs a range of methods, including immunocytochemistry, immunohistochemistry, electron microscopy, and Western blots for protein detection, as well as Southern blot, in situ hybridization, and dot blot for genome detection. Signal amplification techniques, such as Hybrid Capture, and target amplification methods, including PCR and Real-Time PCR, offer high sensitivity and specificity. Furthermore, serological tests like ELISA, utilizing peptides, virus-like particles (VLP), or fused E6/E7 proteins, can detect anti-HPV antibodies, providing valuable diagnostic information.[15]

POLYMERASE CHAIN REACTION

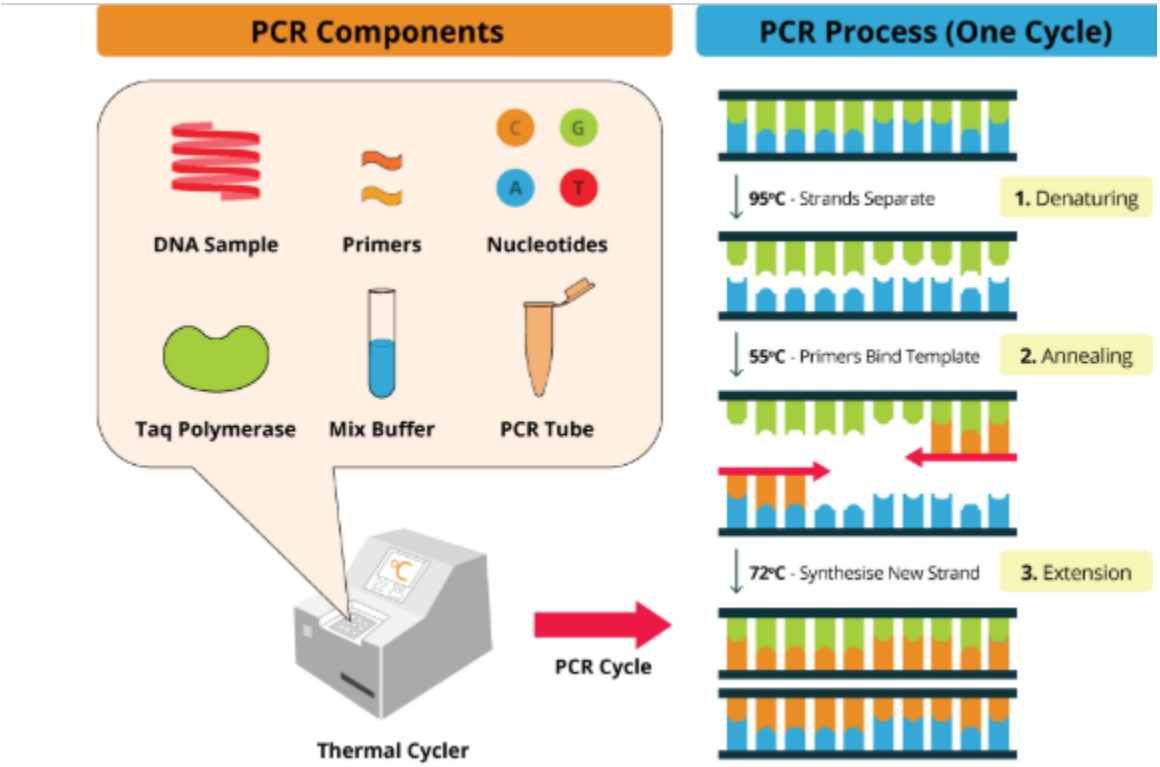


Figure 7: Pictorial representation of PCR [6]

Real-Time Polymerase Chain Reaction (RT-PCR or qPCR) is a laboratory technique that facilitates the exponential amplification of specific DNA sequences. This process involves the use of primers, DNA polymerase, and repeated thermal cycles. Simultaneously, the target sequence is detected through fluorescence, enabling real-time monitoring and eliminating the need for post-amplification analysis.

The diagnosis of HPV-associated cancers requires rapid, inexpensive, and easy-to-use pretesting assays to identify high-risk populations for further examination, particularly in resource-limited settings. Molecular biology techniques, such as PCR amplification, play a critical role in early detection and virus typing, with DNA isolation being a fundamental prerequisite. Moreover, there is increasing interest in utilizing first-void urine as a liquid biopsy for high-risk HPV DNA testing, presenting a promising approach for non-invasive screening and potentially enhancing diagnostic capabilities.

A recent meta-analysis reported that urine-based HPV testing demonstrated 87% sensitivity and 94% specificity when compared to cervical sample results. Furthermore, studies have investigated the genetic variability of HPV genotypes 31, 33, and 58, focusing on the L1 gene, E6, and E7 regions, to identify nucleotide changes associated with cervical cancer. Notably, a study in Iran reported on the genetic diversity and amino acid changes in the L1 gene of HPV 58, 33, and 31 in women with normal cytology, providing new insights into circulating HPV strains in this population.[6,7,16]

One study published in Clinical Cancer Research investigated the use of circulating tumor HPV DNA (ctHPVDNA) as a biomarker for HPV-associated oropharyngeal squamous cell carcinoma (OPSCC). The researchers developed a highly sensitive and specific digital PCR assay to quantify ctHPVDNA in plasma. They found that baseline

plasma ctHPVDNA levels had high specificity and sensitivity for detecting newly diagnosed HPV-associated OPSCC.[17]

Recent studies published in Cancers journals found that circulating HPV DNA (cHPV-DNA) can be detected in the blood of patients with cervical cancer and can be used to detect cHPV-DNA in plasma samples from patients with CIN and early-stage invasive cervical cancer. The results showed that patient with early-stage invasive cancer had detectable levels of cHPV-DNA.[18,19]

MATERIALS
&
METHODS

MATERIALS AND METHODS

STUDY DESIGN – Cross sectional observational study.

STUDY SETTING– Department of Obstetrics and Gynecology, Department of Pathology and Department of Microbiology SDUMC, Tamaka, Kolar.

SOURCE OF DATA- Newly diagnosed cases of Primary Squamous Cell Carcinoma (SCC) of Cervix specimens was collected from Department of Obstetrics and Gynecology and the study was done at Pathology and Microbiology sections in CDLS (Central Diagnostic Laboratory Services) at R.L. Jalappa Hospital and Research Center attached to Sri Devaraj Urs Medical College, Tamaka, Kolar.

DURATION OF STUDY– Cases were collected for a period of 18 months from May 2023 to October 2024

Inclusion Criteria: All fresh cases of primary SCC of cervix diagnosed by cervical Biopsy.

Exclusion Criteria: Post-Chemotherapy, Post-Radiotherapy cases, recurrent cases, secondary metastasis in cervix or any other cancer in the patient.

SAMPLE SIZE: The Estimated Sample Size is **75**

Sample size for present cross sectional study was estimated based on the sensitivity and specificity for detecting any HPV in urine (87% and 94% respectively with reference to Cervical samples), as reported in study [7], considering an absolute error of 5% with 95% confidence interval, the estimated sample size for the study is 75.

$$n = \frac{DEFF*Np(1-p)}{\{ (d^2/Z^2_{1-\alpha/2}*N-1+p*(1-p)\}}$$

- DEFF: Design effect
- N : Hypothesized % frequency of outcome factor in the population (p)
- d : Confidence limits as % of 100 (absolute +/-%)
- Z: Standard Normal Variate at 95 % .

METHODS:

- All cases of primary SCC of Cervix, newly diagnosed by cervical Biopsy were included.
- Following informed consent from the patients, parameters like Age, Parity and Clinical Features were collected by interacting with the patient and from the case files.
- Cervical tissue (fresh tissue without fixative), urine samples (early morning sample or at least 2 hours gap from last urination) and blood samples (collected in EDTA vacutainer) were collected. Blood samples were centrifuged at 1500 rpm for 5 minutes and plasma was separated. All these samples were stored in -20 degree Celsius and separately processed in Truenat instrument.
- Truenat instrument works on the principle of real time polymerase chain reaction.
- Truelab workstation comprises a sample processing device (Trueprep Auto) and a real time quantitative micro PCR analyser.
- Trueprep Auto is fully automated and uses a disposable fluidic cartridge to extract and enrich total DNA from specimen within 20 minutes.
- Nucleic acid based molecular tests have so far been restricted to centralized reference laboratories as they require skilled manpower and elaborate infrastructure.
- The Truenat instrument is simple, rapid and user friendly and offers result even at resource limited settings.

METHODS:

- For tissue biopsy

1. 100 mg approx. of the tissue biopsy sample was taken and homogenize with micro pestle. 500 microliter of lysis buffer was added and mixed well.

2. Incubated for 5 mins at room temperature and was transferred into the cartridge sample chamber.

3. The device was switched ON. The cartridge was placed in the device . At the end of about 20 minutes door was ejected automatically with beep sound.

4. 150 micro liter of the elute was pipetted to a collection chamber and stored it in the storage vial provided in the catridge pouch. The elute sample was labelled and stored at 4 degree Celsius.

- **For plasma**

1. 500 microliter of lysis buffer was added to plasma and mixed well.

2. Incubated for 5 mins at room temperature and then transferred to the catridge sample chamber.The device was switched ON. The cartridge was placed in the device . At the end of about 20 minutes door was ejected automatically with beep sound.

3. 150 microliters of the eluate were carefully pipetted out from the collection chamber.The pipetted eluate was transferred into the storage vial provided in the cartridge pouch.The storage vial was clearly labeled with the sample identification and was stored at 4 degrees Celsius

- **For urine sample**

1. 10 mL of first flow urine in a urine collection cup was collected.

2. 0.5mL was transferred from the cup to the lysis buffer tube and mixed well after tightly closing the cap. Urine collection cup was disposed.

3. The entire content from the Lysis Buffer tube containing specimen was transferred to the cartridge sample chamber.

4. The cartridge was placed in the Trueprep Auto and extraction done.

5. 150 microliter of the elute was pipetted out and stored in the storage vial provided in the cartridge pouch. The elute sample was store at 4 degree Celsius.

6. Discarded the cartridge as bio waste.

Truemix™ HPV-HR Genotyping (16/18/33/35, 31/39/45, 51/52/56/58, 59/66/68) is a lyophilized, ready-to-use, open format, multiplex Real Time Polymerase Chain Reaction (PCR) test for the semi-quantitative detection of high-risk Human Papillomavirus (HPV) types 16/18/33/35, 31/39/45, 51/52/56/58 and 59/66/68 in female cervical specimens collected by a clinician.

Nucleic acid (DNA) testing is a highly sensitive and specific method for determining the presence of infection with high-risk HPV types in cervical specimen. The DNA from the patient sample is first extracted using any commercial DNA extraction kits and runs on standard open platform real time PCR systems.

PRINCIPLE OF THE TEST

Truemix™ HPV-HR Genotyping (16/18/33/35, 31/39/45, 51/52/56/58, 59/66/68) is a

freeze dried, ready-to-use open format test, works on the principle of Real Time Polymerase Chain Reaction based on Taqman chemistry. The test targets L1 and E7 genes and also includes an internal control in one of the tubes, to verify the validity of sample collection, extraction and PCR. The Real time detection of the process is enabled using target specific fluorescent hydrolytic probes. The test consists of two processes in a single tube test:

- PCR amplification of targets and Internal Control
- Simultaneous, real time detection by dual labeled fluorescent probes.

Target sequence for this assay is L1 and E7 genes.

CONTENTS OF THE Truemix™ HPV-HR Genotyping (16/18/33/35, 31/39/45, 51/52/56/58, 59/66/68) KIT

A. Truemix™ Pouch containing

1. Strip of 8 dried down PCR mix
2. Desiccants

B. Accessory Pack containing

1. Reconstitution Buffer
2. Positive Control (Dried Down)
3. Negative Control

C. Strip of 8 PCR Flat Caps

D. Package Insert

TEST PROCEDURE

A. Sample Preparation

DNAs are extracted from the samples collected in viral lysis medium using Trueprep

AUTO . Prior verification of the suitability of the prep kit used for this application was done.

B. Positive Control Preparation

A known positive control was supplied along with the Truemix™ HPV-HR Genotyping (16/18/33/35, 31/39/45, 51/52/56/58, 59/66/68) contents. The dried down positive control in the micro tube was reconstituted. 50 µl of negative control was pipetted from the negative control vial into the micro tube of positive control using a fresh DNase/RNase free filter barrier micropipette tip. The micro tube was mixed gently for a few minutes. The reconstituted positive control was ready . The same volume as of the target, 5µL was added to all the tubes of Truemix™ HPV-HR Genotyping (16/18/33/35, 31/39/45, 51/52/56/58, 59/66/68) (Combination A to D). The negative control was also a ready to use solution.

C. Master Mix Setup

The kit provided PCR master mix as a ready-to-use, freeze dried format. Before adding sample (DNA), 5 µL of provided reconstitution buffer was added to each tube. Reconstitution buffer contains 6mM MgCl2

Table 3 : Showing Master Mix Setup

Per Tube	Volume (µL)
Reconstitution buffer	5
Elute/Positive Control/Negative Control	5
Total	10

D. Reaction Setup:

1.The tube strip was placed with dry reagents on a cold rack. [Pre-cool the rack by storing it in freezer compartment].

2. Further steps were done as quickly as possible. Temperature was kept between 0-10°C, till the tubes were placed in PCR instrument.

3. 5 µL of the reconstitution buffer was pipetted out to each PCR tube.

4. 5 µL of elute pipetted to each PCR tube.

5. The tube was spun down for approximately 30 seconds, to bring down the liquids.

SETTING UP THE REAL-TIME PCR INSTRUMENT

Table 4: Depicting the Plate settings:

Settings	
Reaction Volume	10µL
Ramp Rate	Default

Table 5 : Depicting the Fluorescence detectors (Dyes):

Description	**Gene Target**	**Reporter channel**
Target	HPV 16/31/51/68	Cy5 / equivalent
Target	HPV 33/39/52/59	FAM / equivalent
Target	HPV 18/45/58/66	Texas Red / equivalent
Target	HPV 35/56	VIC / equivalent
Internal Control	IPC	VIC / equivalent

Table 6: Demonstrating the Tube or Combination (Dyes):

Tube or Combination	Reporter channel			
	FAM	Cy5	VIC	Texas Red
Tube 1 or Combination A	HPV 33	HPV 16	HPV 35	HPV 18
Tube 2 or Combination B	HPV 39	HPV 31	IPC	HPV 45
Tube 3 or Combination C	HPV 52	HPV 51	HPV 56	HPV 58
Tube 4 or Combination D	HPV 59	HPV 68	-	HPV 66

Table 7 : Showing the Temperature profile and data acquisition:

	Stage	Cycle Repeats	Data Acquisition	Temperature [°C]	Time [seconds]
Initial Denaturation	Hold	1	-	95	60
Amplification	Cycling	40	-	95	10
			Yes	60	34

A sample is Positive if any of the HPV target from the Truemix™ HPV-HR Genotyping (16/18/33/35, 31/39/45, 51/52/56/58, 59/66/68) kit shows amplification .

In case of a negative HPV sample, only the IPC will show amplification.

If both the IPC and HPV targets in the Truemix™ HPV-HR Genotyping (16/18/33/35, 31/39/45, 51/52/56/58, 59/66/68) kit fails to amplify then the result is invalid.

Table 8 : Showing Result Interpretation

Detection Channel					Result Interpretation
Target				IPC	
HPV 33/39/52/59 (FAM)	HPV 16/31/51/68 (Cy5)	HPV 18/45/58/66 (Texas Red)	HPV 35/56 (VIC)	VIC	
+	+	+	+	+/-	Respective HPV Genotypes Positive
-	-	-	-	+	Respective HPV Genotypes Negative
+	+/-	+/-	+/-	+/-	Respective HPV Genotypes Positive
+/-	+	+/-	+/-	+/-	Respective HPV Genotypes Positive
+/-	+/-	+	+/-	+/-	Respective HPV Genotypes Positive
+/-	+/-	+/-	+	+/-	Respective HPV Genotypes Positive
-	-	-	-	-	Invalid (Collect new swab and repeat)

STATISTICAL ANALYSIS

Statistical analysis was performed using ‘SPSS’ 24 software. Descriptive statistics, including mean, standard deviation, and percentages, were used to summarize demographic and clinical variables. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated for different diagnostic tests using the area under the receiver operating characteristics (ROC) curve. Confidence intervals were computed for these diagnostic measures. Optimal cutoff values were determined based on the highest sum of sensitivity and specificity using ROC curve analysis. Likelihood ratio was also calculated for the same.

RESULTS

RESULTS

HPV analysis was performed on tissue, urine and blood samples of 75 freshly diagnosed SCC cervix cases.

The demographic and clinical data of these patients were tabulated and analyzed.

PATIENT DEMOGRAPHIC DATA

AGE

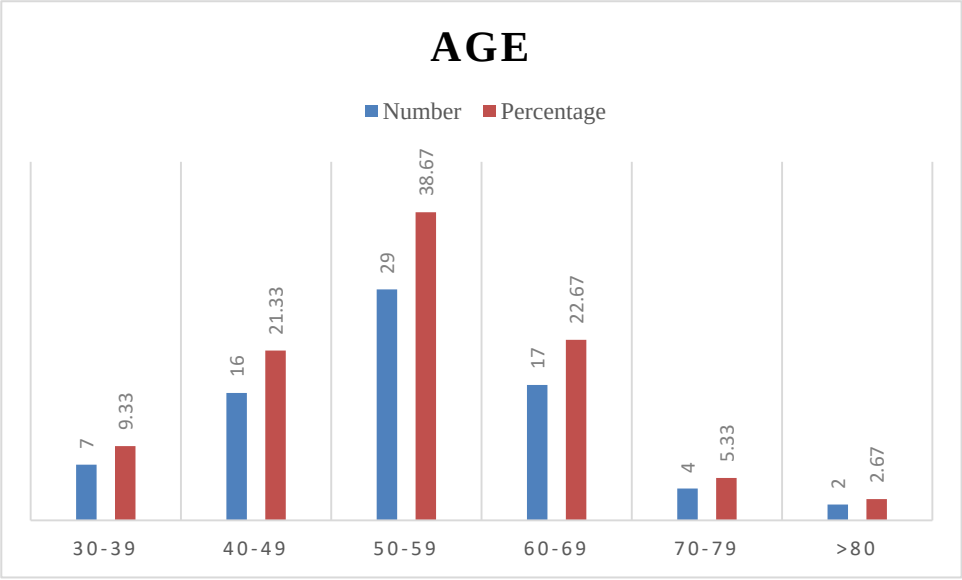
The mean age of patients diagnosed with SCC cervix is 53.92 ±10.86.

Table 9: Data showing various age groups of patients with SCC cervix

Age Group	Number	Percentage
30-39	7	9.33 %
40-49	16	21.33 %
50-59	29	38.67 %
60-69	17	22.67 %
70-79	4	5.33 %
>80	2	2.67 %

Our study population exhibited a wide age distribution of cervical cancer diagnosis, from 33 to 87 years, with a peak incidence between 50-59 years and a gradual decrease at older and younger ages.

Figure 8: Distribution of cervical cancer cases in various age groups



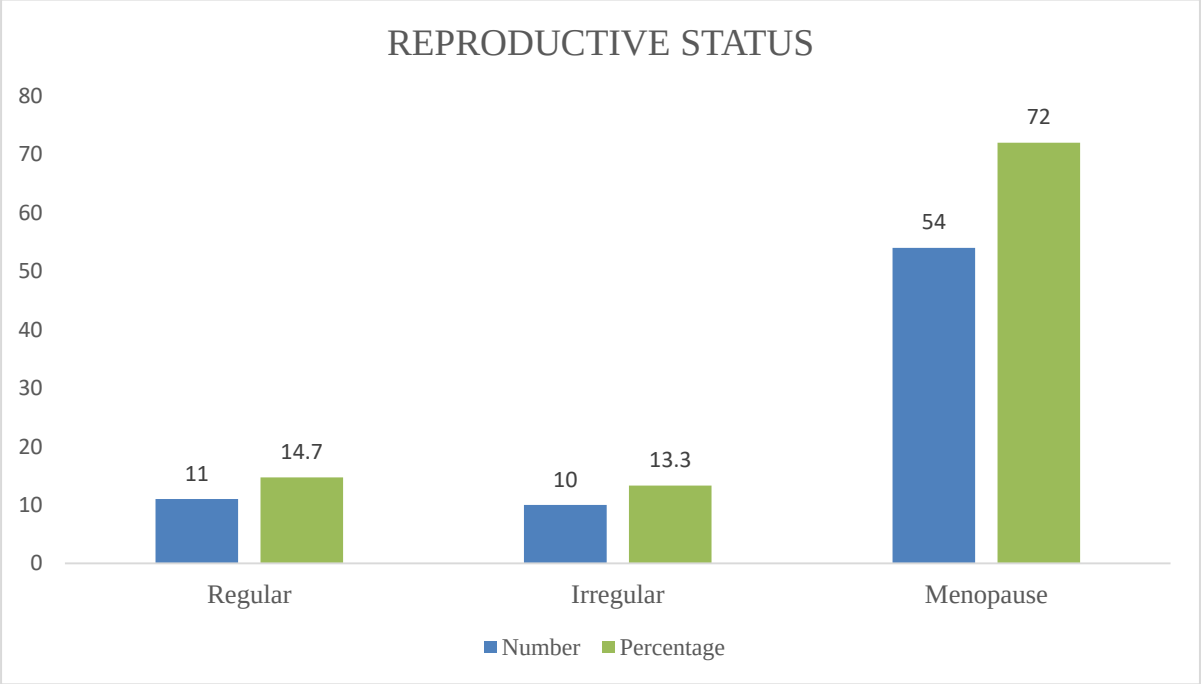
REPRODUCTIVE STATUS

Table 10 : Reproductive status of the SCC cases

Menstrual Cycle status	Number	Percentage
Regular	11	14.7 %
Irregular	10	13.3 %
Menopause	54	72 %
TOTAL	75	100

A significant proportion (72%) of SCC cervix patients were postmenopausal, and only 14.7% had regular menstrual cycles.

Figure 9 : Demonstrating the Reproductive status of SCC cervix cases



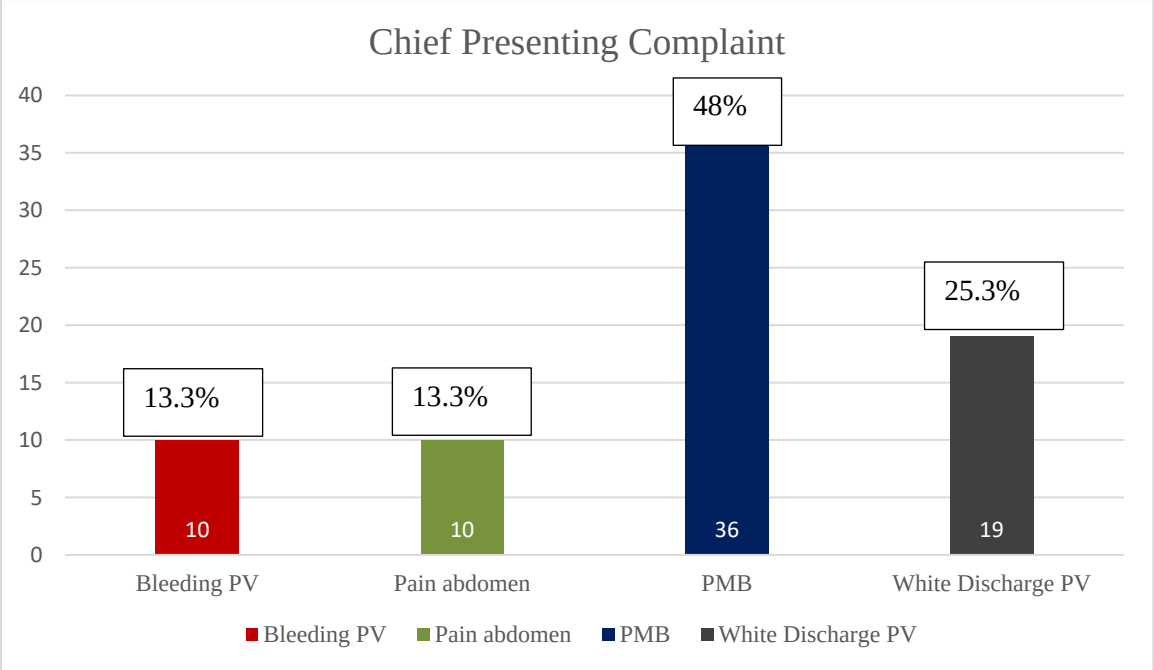
CHIEF COMPLAINTS

Table 11 : Chief presenting complaints of patients with SCC cervix

Chief Complaint	Number	Percentage
Bleeding PV	10	13.3 %
Pain abdomen	10	13.3 %
Postmenopausal bleeding (PMB)	36	48.0 %
White Discharge PV	19	25.3 %
Total	75	100

The symptom profile of patients with SCC cervix revealed postmenopausal bleeding as the most common symptom (48%), followed by abnormal vaginal discharge (25.3%), and bleeding per vaginum and abdominal pain, which occurred with equal frequency (13.3%).

Figure 10: Depicting chief complaints of SCC cases



CLINICAL FINDING

Table 12: Showing various clinical findings of patients with SCC cervix

FINDING	Frequency	Percent
Cervical erosion	9	12.0 %
Hypertrophied Cervix	15	20.0 %
Ulceroproliferative growth	51	68.0 %
Total	**75**	**100.0**

Figure 11 : Microphotograph depicting ulceroproliferative growth in cervix

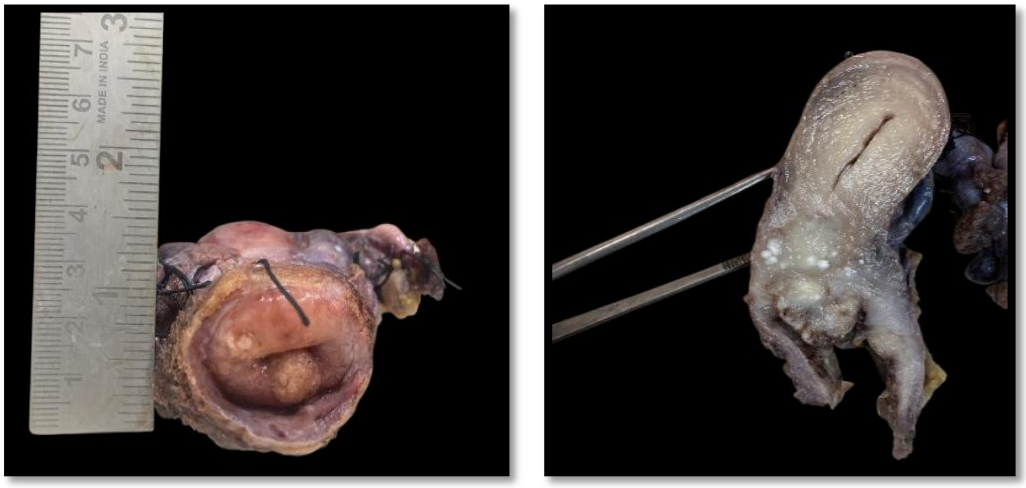
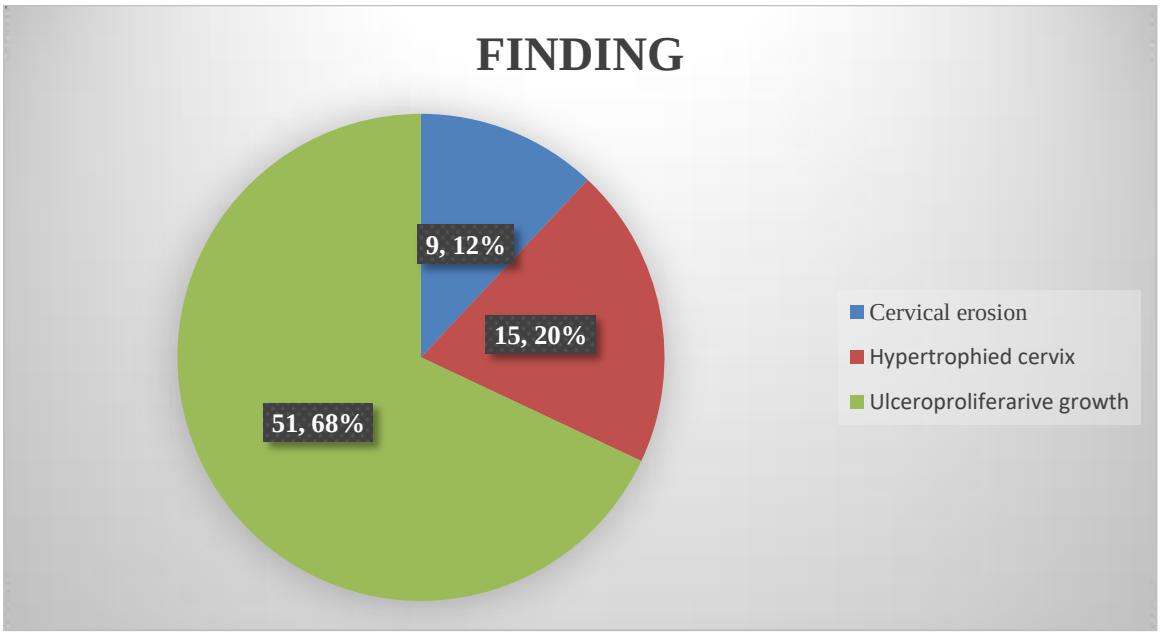


Figure 12 : Depicting the clinical findings of the patients



Majority of cases showed an ulceroproliferative growth (68%).

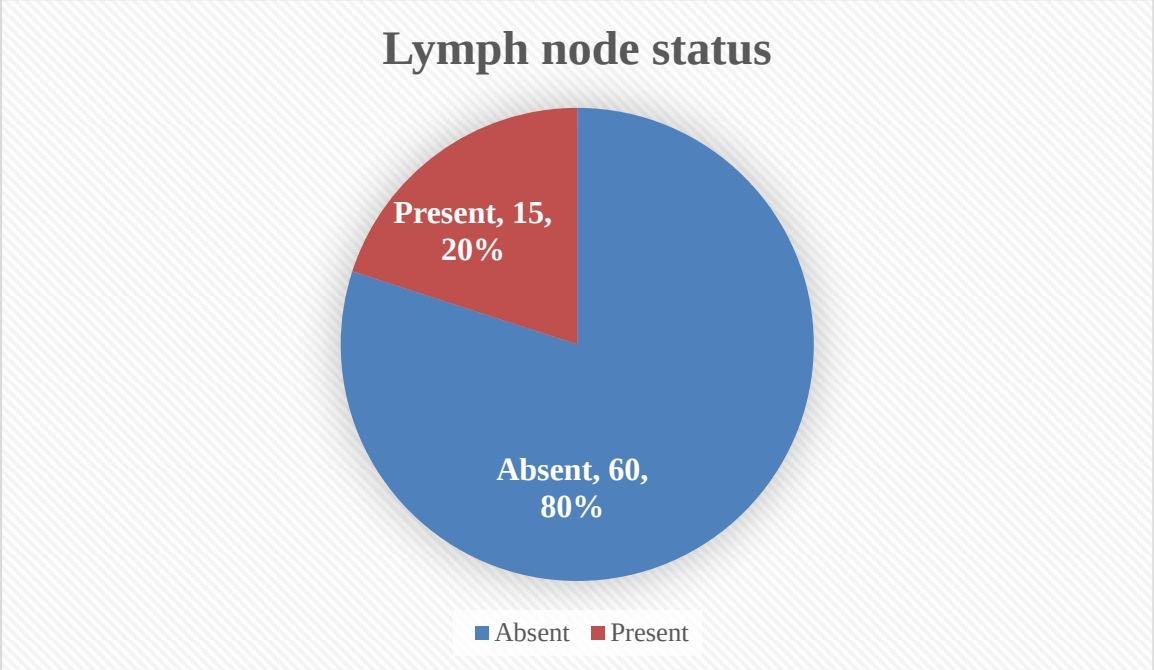
LYMPH NODE STATUS

Table 13 : Showing lymph node status of patient with SCC cervix

LN Status	Number	Percentage (%)
Absent	60	80.0 %
Present	15	20.0 %
Total	75	100

Radiological investigations showed that 15 patients, representing 20% of our study population, had lymph node involvement.

Figure 13 : Demonstrating the lymph node status of the patients with SCC cervix



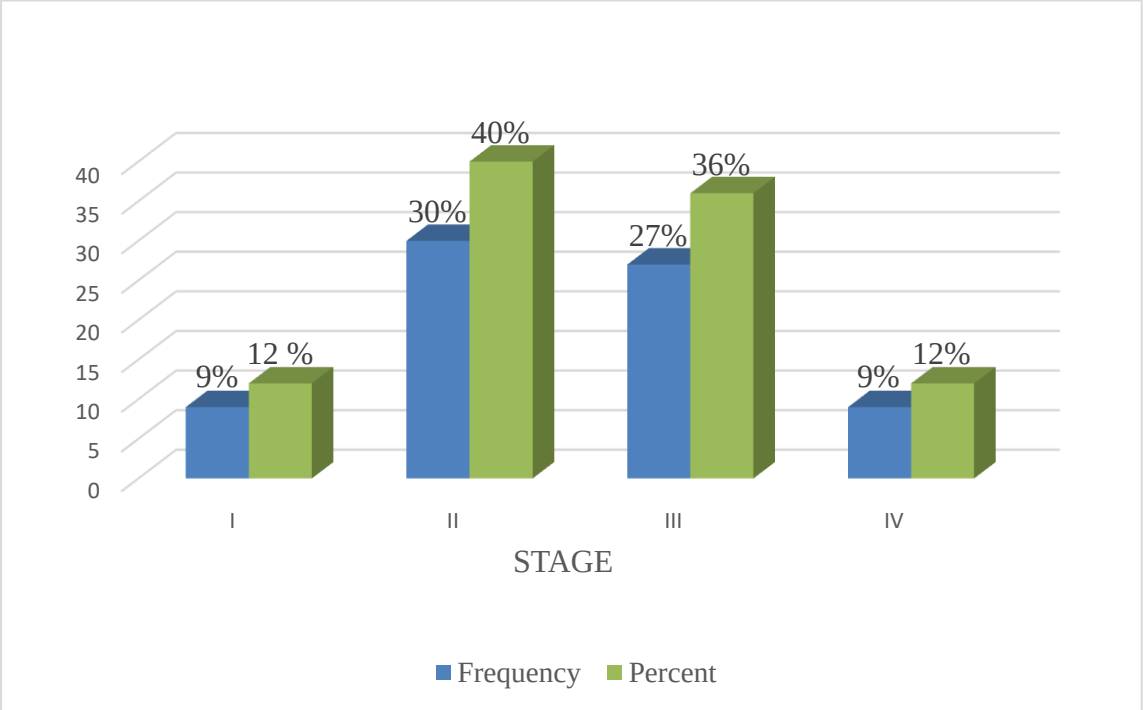
STAGE

Table 14: Demonstrating the stages of the tumor at the time of diagnosis

STAGE	Frequency	Percent
I	9	12.0 %
II	30	40.0 %
III	27	36.0 %
IV	9	12.0 %
Total	**75**	**100.0**

The majority of patients (76%) were diagnosed with SCC cervix at advanced stages, with 40% at stage II and 36% at stage III, while 12% were diagnosed at both early and metastatic stages.

Figure 14 : Stage of the disease when presented with SCC cervix



GRADE

Table 15 : Pathological grading of the cervical carcinoma

HPE	Number	Percentage
WD SCC	50	66.7 %
MD SCC	23	30.7 %
PD SCC	2	2.7 %
Total	75	100

Histopathological examination (HPE) of cervical biopsies revealed varying degrees of differentiation, with the majority (66.7%) showing well-differentiated cells, followed by moderate differentiation (30.7%), and a small proportion (2.7%) showing poor differentiation

Figure 15 : Microphotograph showing various histopathological grading

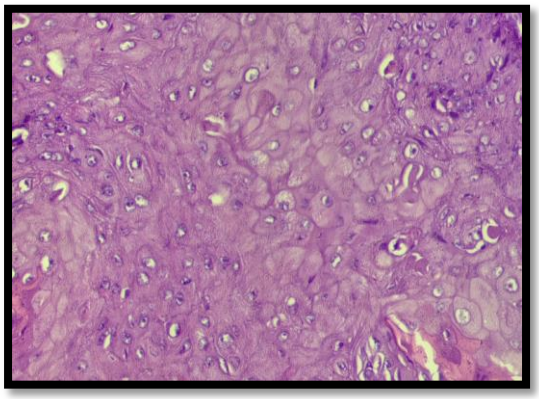


Figure 15 A: Histological representation of Well Differentiated SCC (H&E , 400x)

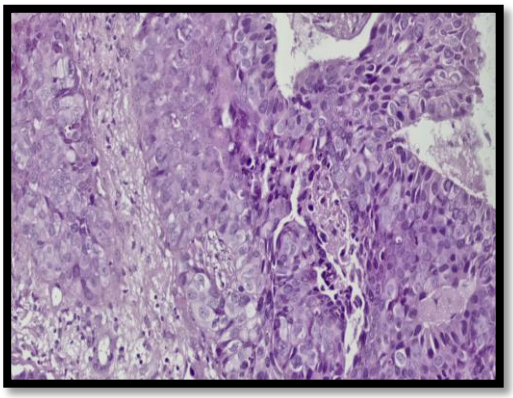


Figure 15 B: Histological representation of Moderately Differentiated SCC (H&E , 400 x)

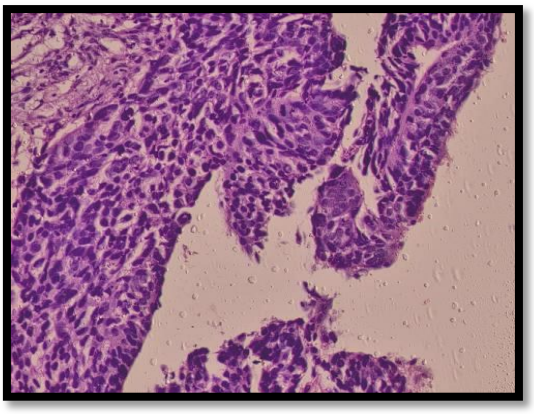
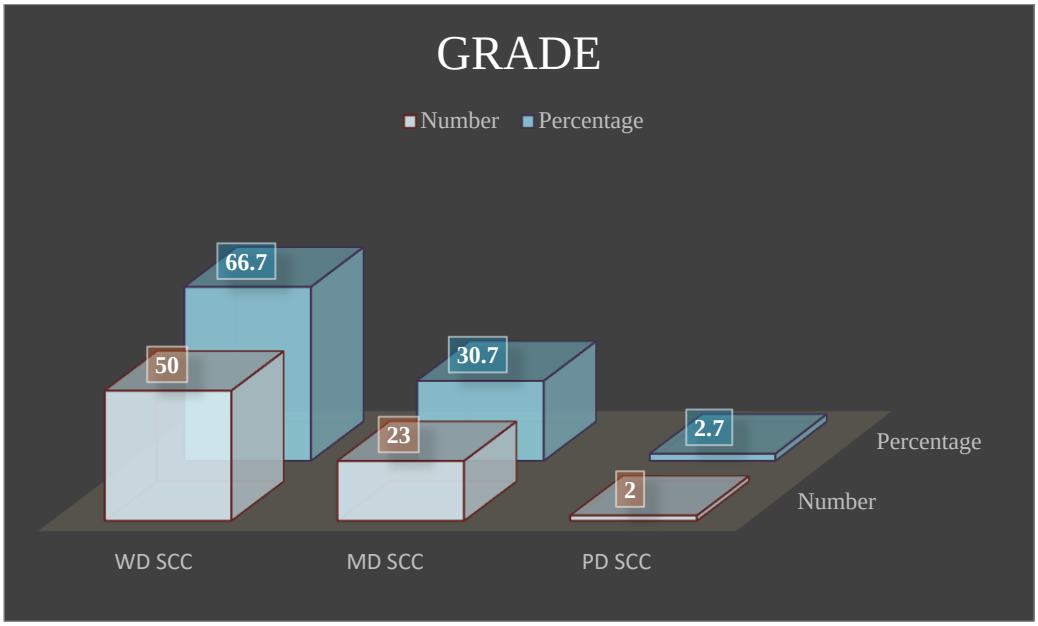


Figure 15 C: Histological representation of Poorly Differentiated SCC (H&E , 400 x)

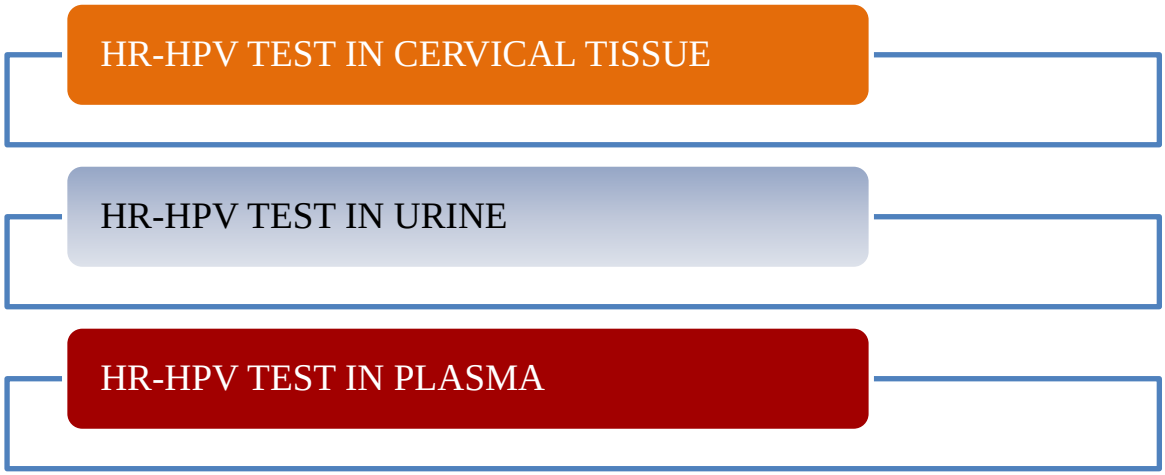
Figure 16 : Depicting the Histopathological grading of SCC cases



HR-HPV ANALYSIS

HR-HPV was analyzed in cervical tissue, urine and plasma. Among which cervical tissue is currently being used for depicting the HR-HPV status of a patient and it was used as reference standard.

HR-HPV was further analyzed in urine and plasma.

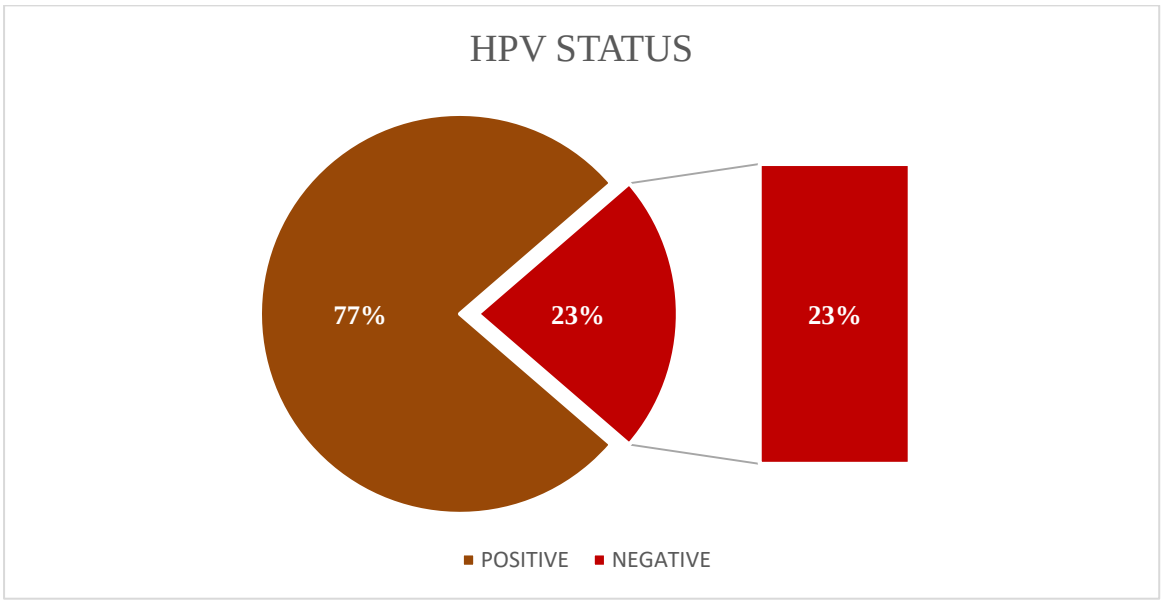


HPV STATUS (CERVICAL TISSUE)

Table 16: Depicting HPV status in cervical tissue samples

HPV	FREQUENCY	PERCENTAGE
POSITIVE	58	77 %
NEGATIVE	17	23 %
Total	**75**	**100.0**

Figure 17 : Demonstrating HPV status in cervical tissue samples



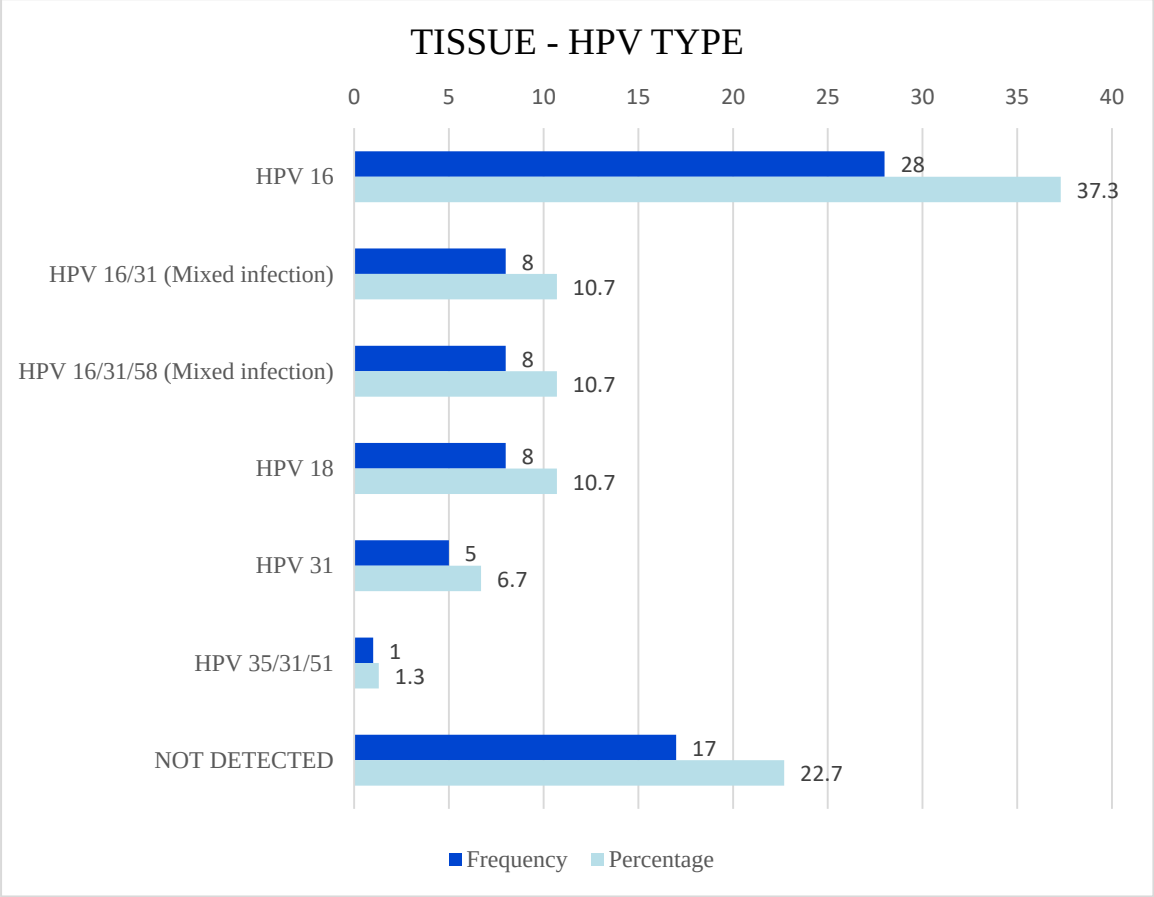
HR- HPV DNA was detected by PCR in 58 cervical tissue samples, with HPV 16 being the most common genotype, and 17 samples showing no evidence of HPV infection

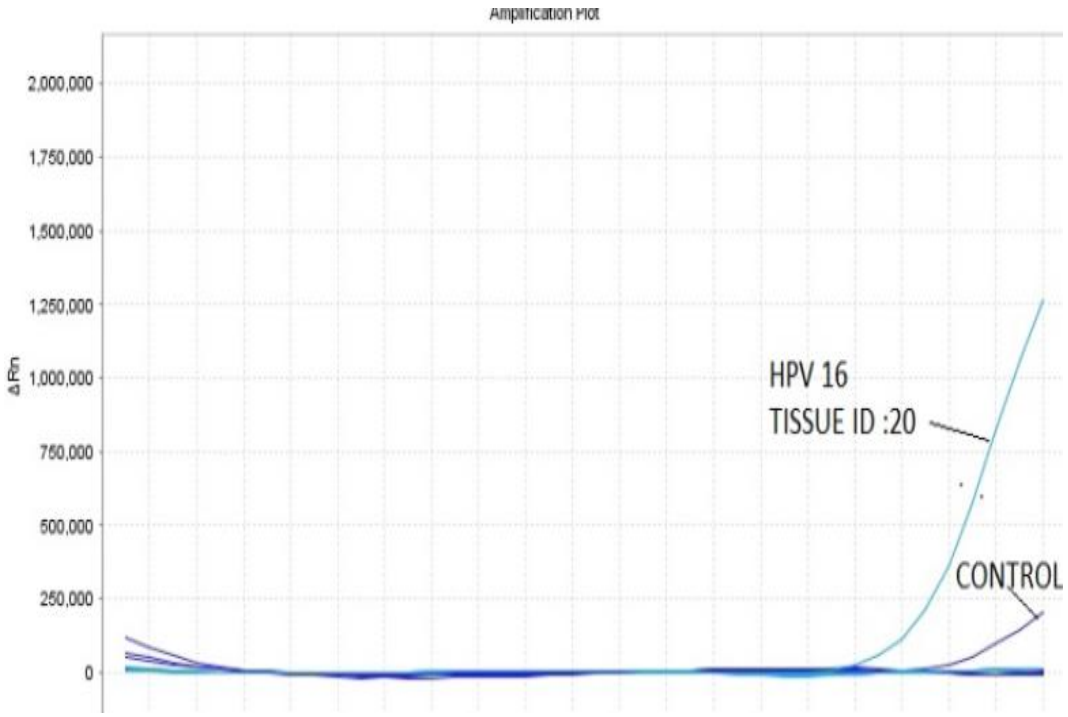
HPV TYPE

Table 17 : Showing various HR- HPV in tissue samples of SCC cases

Tissue	Frequency	Percent
HPV 16	28	37.3 %
HPV 16/31 (Mixed infection)	8	10.7 %
HPV 16/31/58 (Mixed infection)	8	10.7 %
HPV 18	8	10.7 %
HPV 31	5	6.7 %
HPV 35/31/51(Mixed infection)	1	1.3 %
NOT DETECTED	17	22.7 %
Total	75	100.0

Figure 18 : Demonstarting HR-HPV types





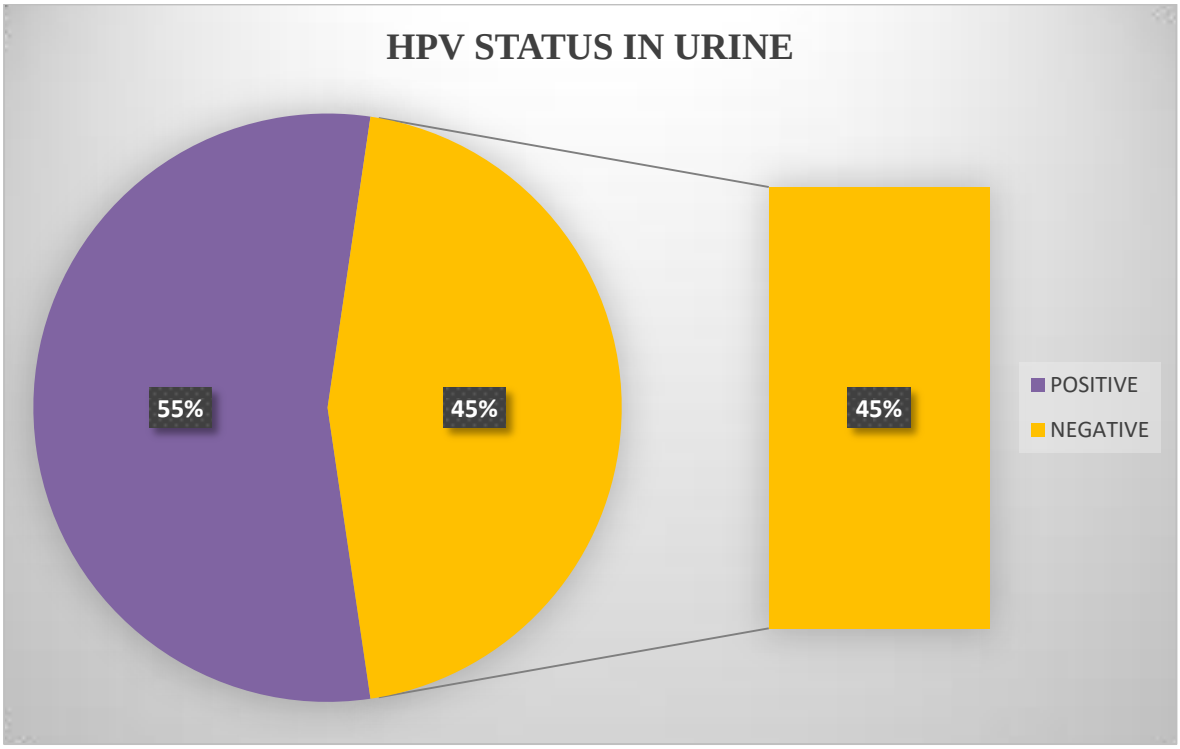
The graph shows the amplification plot of HPV 16 DNA in cervical tissue using real-time PCR. The graph shows a clear amplification curve with a Ct value of approximately 25-35. This indicates the presence of HPV 16 DNA in the sample. The negative control does not show any amplification, indicating the absence of contamination. This result supports the diagnosis of HPV-related cervical lesions.

URINE – HPV STATUS

Table 18: Demonstrating detection HPV in urine

HPV	FREQUENCY	PERCENTAGE
POSITIVE	41	55 %
NEGATIVE	34	45 %
Total	**75**	**100.0**

Figure 19 : Depicting HPV status in urine samples



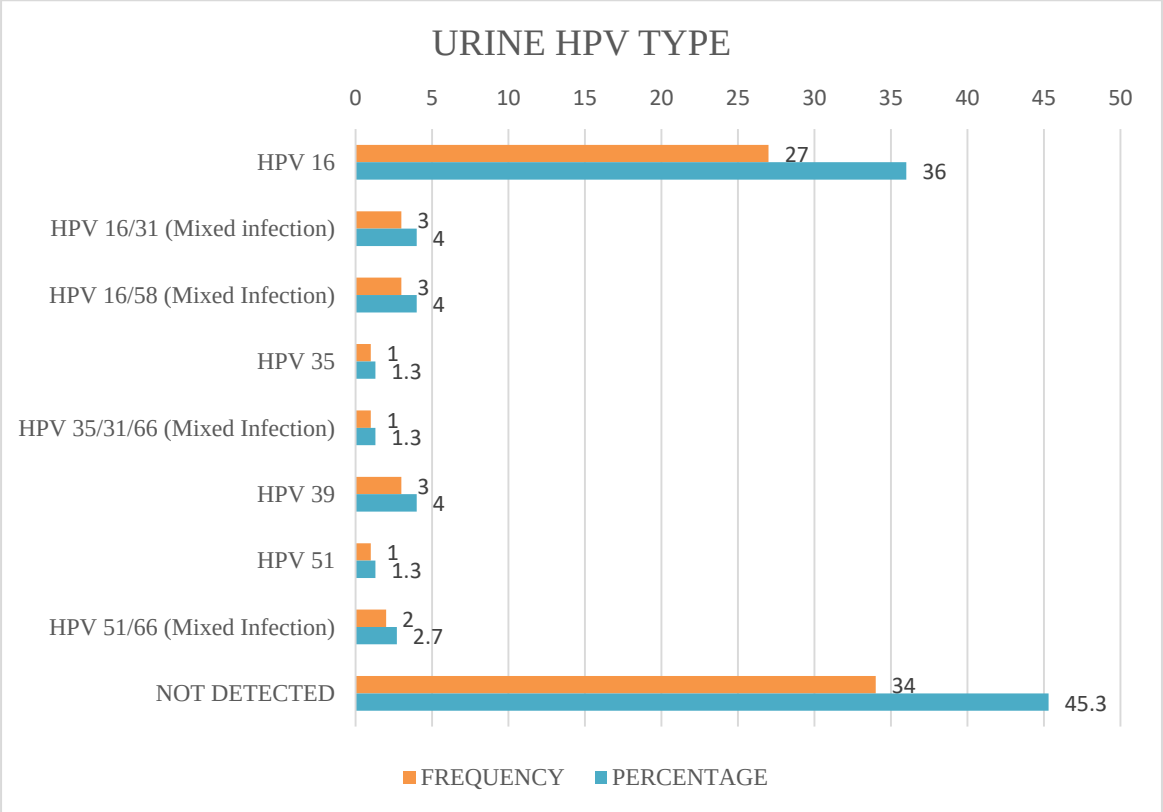
PCR analysis of urine samples revealed HPV positivity in 41 cases, predominantly HPV 16, while HPV was undetectable in 34 cases.

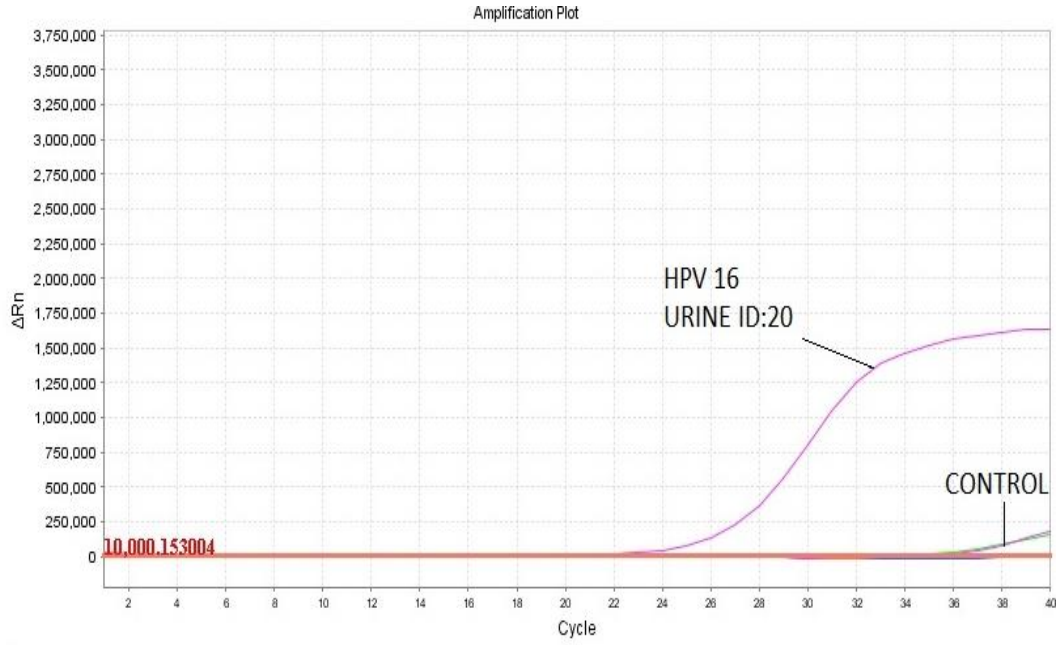
URINE –HPV TYPE

Table 19 : Demonstrating various types of HR- HPV

URINE	Frequency	Percentage
HPV 16	27	36.0 %
HPV 16/31 (Mixed infection)	3	4.0 %
HPV 16/58 (Mixed Infection)	3	4.0 %
HPV 35	1	1.3 %
HPV 35/31/66 (Mixed Infection)	1	1.3 %
HPV 39	3	4.0 %
HPV 51	1	1.3 %
HPV 51/66 (Mixed Infection)	2	2.7 %
ND	34	45.3 %
Total	75	100.0

Figure 20 : Depicting various types of HR- HPV





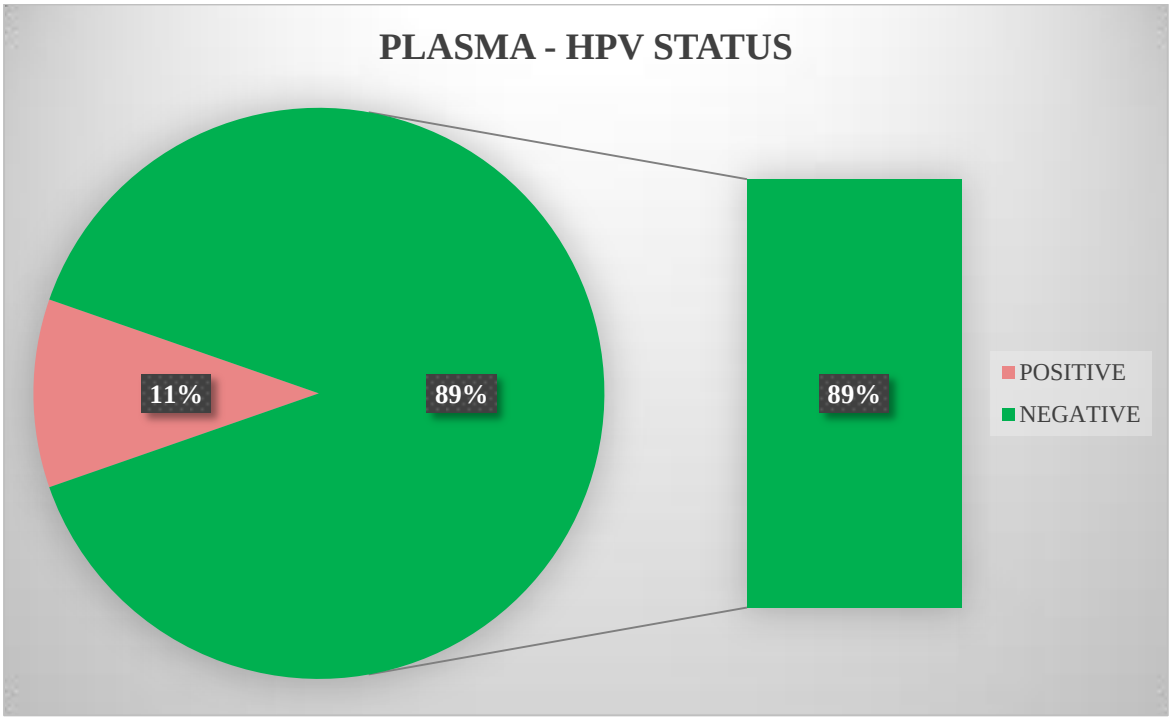
This amplification plot shows a distinct curve with a Ct value of 24-32, indicating the presence of HPV 16 DNA in the urine sample of SCC patients. The absence of amplification in the negative control confirms the specificity of the result. This finding supports the diagnosis of HPV-related cervical lesions.

PLASMA – HPV STATUS

Table 20 : Demonstrating HR- HPV detection in plasma of SCC cervix cases

HPV	FREQUENCY	PERCENTAGE
POSITIVE	8	11 %
NEGATIVE	67	89 %
Total	**75**	**100.0**

Figure 21 : Depicting detection of HPV in plasma of patients with SCC cervix



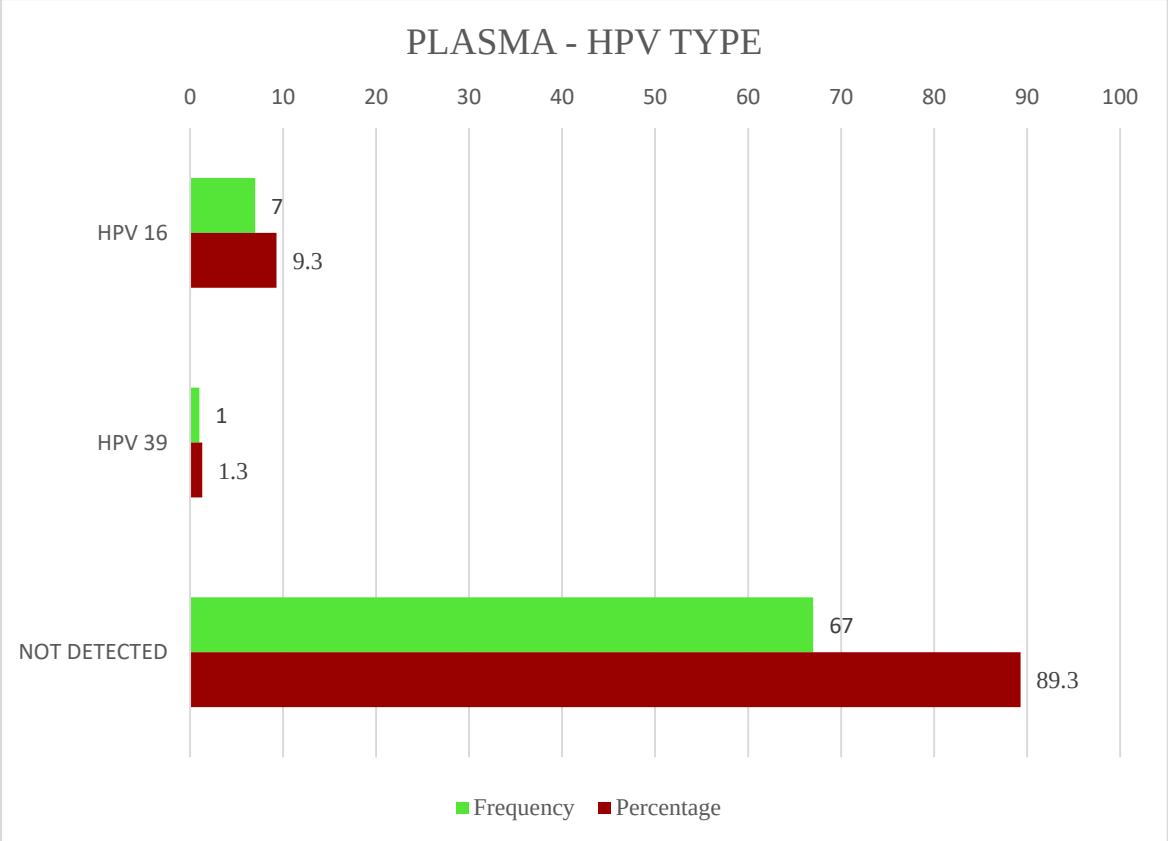
PCR-based HPV detection in blood samples yielded positive results in only 8 cases, predominantly with 7 of those 8 cases attributed to HPV 16, while HPV was undetectable in 67 cases.

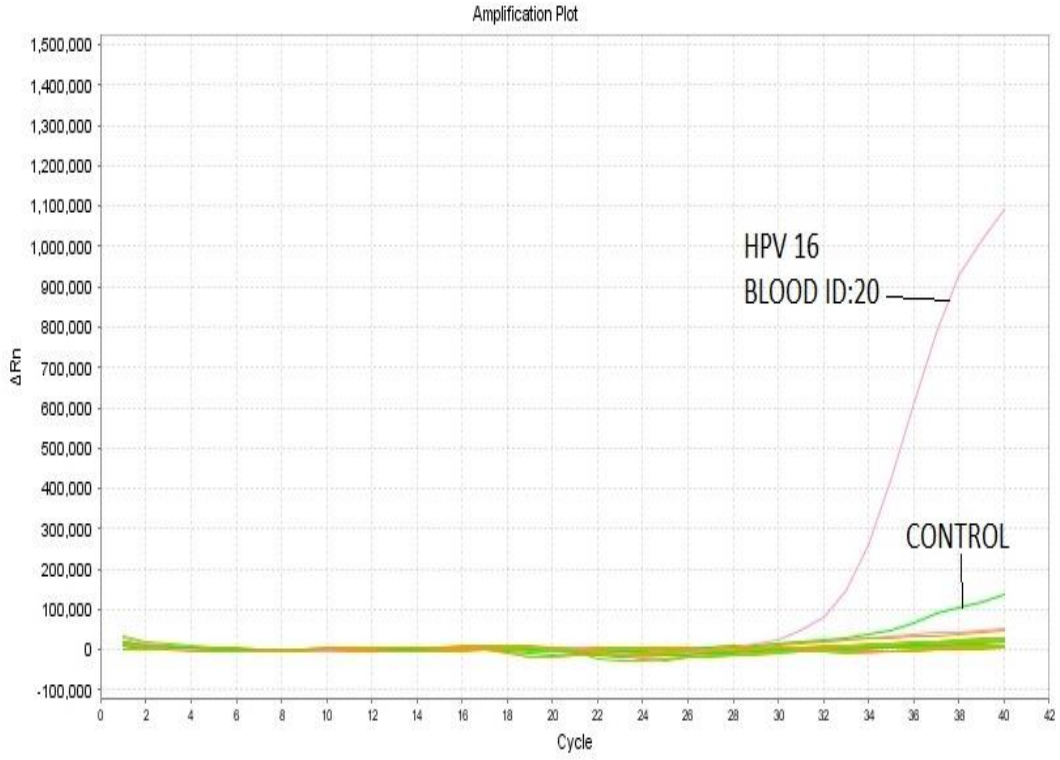
PLASMA –HPV TYPE

Table 21: Showing various types of HR- HPV in plasma

PLASMA	Frequency	Percentage
HPV 16	7	9.3 %
HPV 39	1	1.3 %
ND	67	89.3 %
Total	75	100.0

Figure 22: Demonstrating different types of HR- HPV in plasma





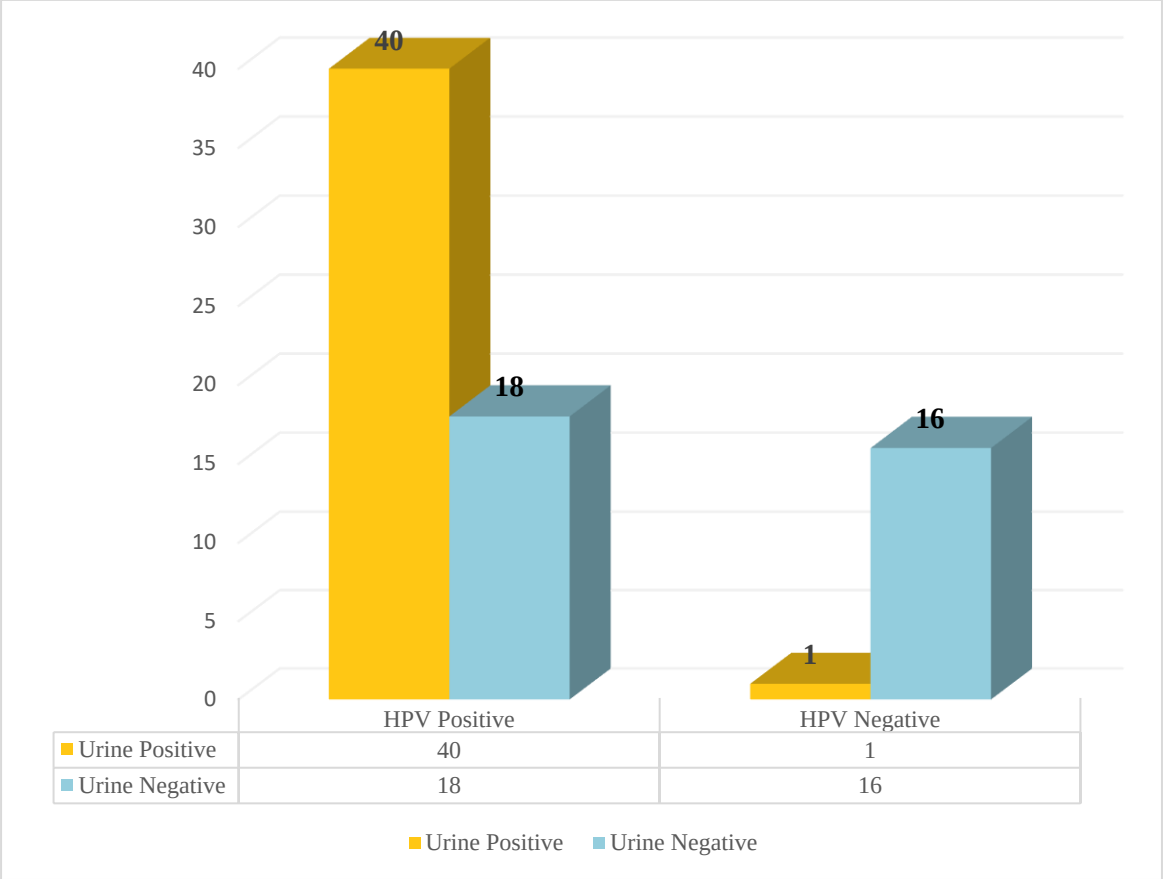
The graph shows the amplification plot of HPV 16 DNA in plasma using real-time PCR. This graph shows a clear amplification curve with a Ct value of approximately 30-35. This indicates the presence of HPV 16 DNA in the sample. The negative control does not show any amplification, indicating the absence of contamination. This result supports the diagnosis of HPV-related cervical lesions

COMPARISON OF HPV DETECTION IN URINE AND TISSUE

Table 22: Demonstrating the comparison between urine and tissue

		Tissue result		Total
Urine test		Positive	Negative	
	Positive	40	1	**41**
	Negative	18	16	**34**
Total		**58**	**17**	**75**

Figure 23: Comparing HPV detection in urine and cervical tissue



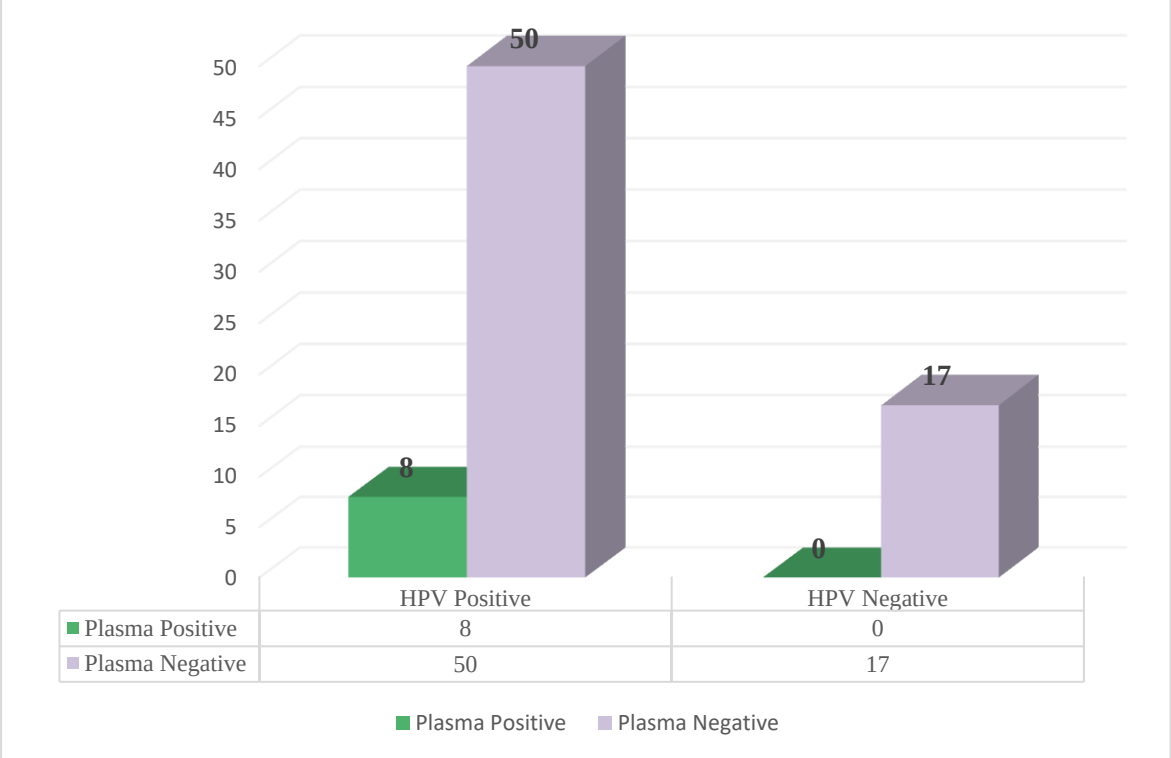
Comparing HPV status in cervical tissue (reference standard) to urine samples, we found that 40 out of 58 (69%) HPV-positive cases were also positive in urine.

COMPARISON OF HPV DETECTION IN PLASMA AND TISSUE

Table 23: Showing the comparison between plasma and tissue

		Tissue result		Total
Plasma test		Positive	Negative	
	Positive	8	0	**8**
	Negative	50	17	**67**
Total		58	17	**75**

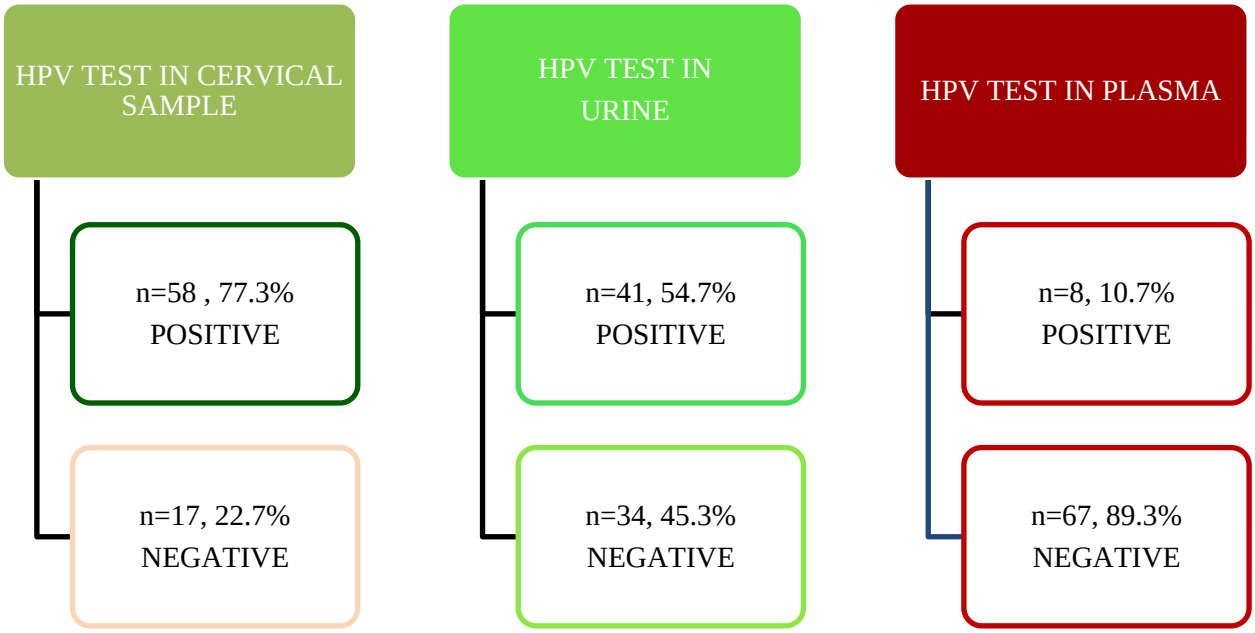
Figure 24 : Comparing the HPV in plasma and tissue



Against the reference standard of cervical tissue, plasma HPV testing demonstrated a positivity rate of only 13.8% (8/58), indicating limited diagnostic utility.

HPV TESTING RESULTS IN CERVICAL TISSUE, URINE AND PLASMA

Figure 25: Showing HPV Detection in all cervical tissue , urine and plasma samples



ACCURACY OF HPV TESTING IN URINE AND PLASMA SAMPLES

Table 24 : Demonstrating The Accuracy of HPV Testing

Parameter	Urine	Plasma
Sensitivity	69%	13.8 %
Specificity	94.1%	100 %
PPV	97.6%	100 %
NPV	47.1%	25.4 %
Diagnostic accuracy	74.6%	33.3%
LR+	11.695	Infinity
LR-	0.329	0.862

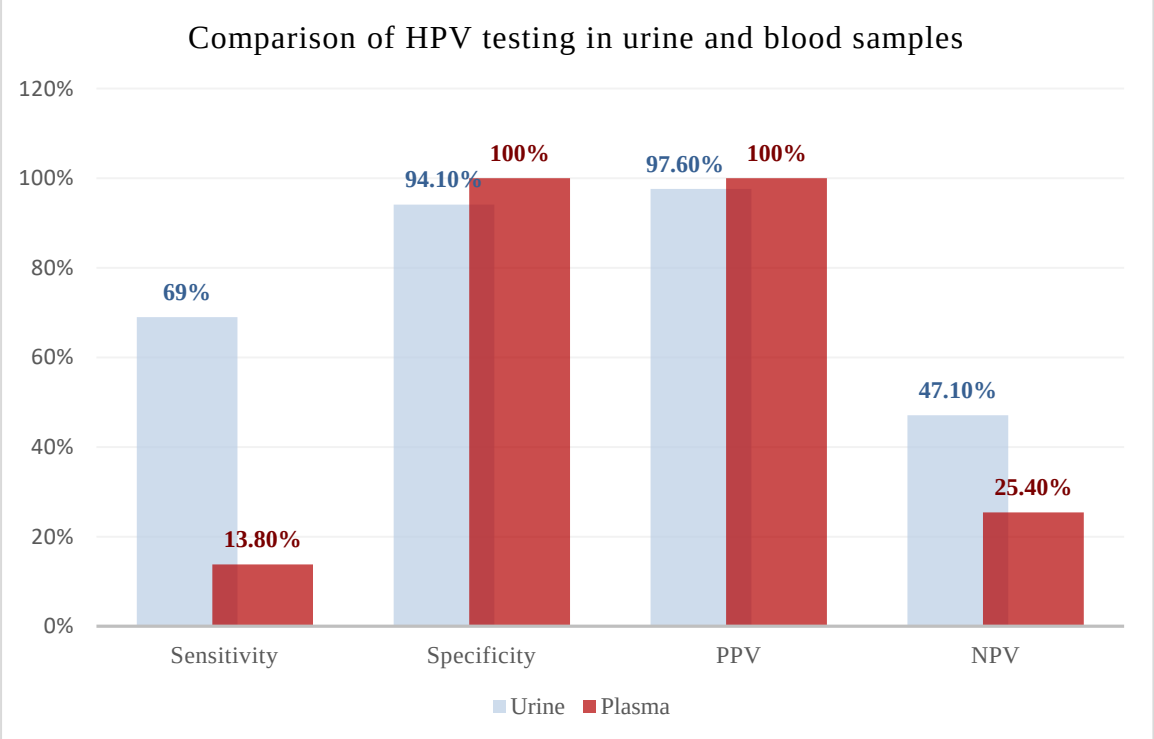
Comparative analysis of HPV testing in urine and plasma revealed significant differences in diagnostic performance. Urine testing demonstrated a sensitivity of 69% and high specificity and positive predictive value, resulting in a diagnostic accuracy of 74.6%. In

contrast, plasma testing showed a lower sensitivity (13.8%) and, despite high specificity and positive predictive value, yielded a lower diagnostic accuracy of 33.3%.

The positive likelihood ratio of both urine and plasma testing, is more than one. Henceforth, both the samples can be used to rule in the diagnosis of cervical carcinoma. Moreover, the plasma testing has a +LR of infinity, which is further from 1, hence will be a better sample for the diagnosis of carcinoma cervix.

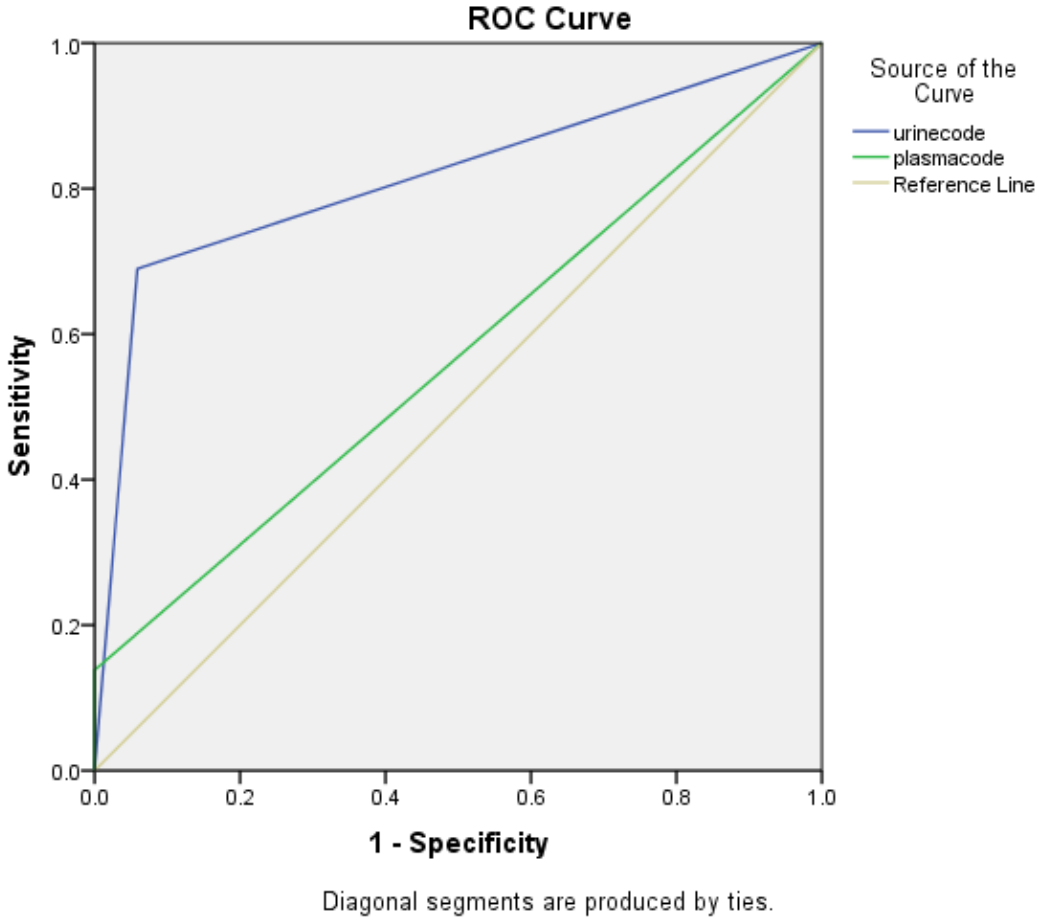
The negative likelihood ratio of both urine and plasma testing, is not less than 0.1. Hence both the samples cannot be used for ruling out cervical carcinoma.

Figure 26: Showing the comparison of HPV testing in urine and blood samples



ROC CURVE ANALYSIS

Figure 27: Showing the diagnostic performance of urine and blood samples



1. ROC Curve Overview

- The **ROC curve** evaluates the diagnostic performance of two test variables: **urine (blue curve)** and **plasma (green curve)**.
- The **diagonal line (yellow)** represents a random classifier (AUC = 0.5), meaning no diagnostic ability.

2. Area Under the Curve (AUC) Interpretation

- **AUC for urine: 0.815 (95% CI: 0.712 – 0.919, p < 0.001)**
- This indicates **good diagnostic accuracy** (AUC > 0.8).

- The **confidence interval (CI)** does not include 0.5, meaning it is statistically significant.
- **AUC for plasma: 0.569 (95% CI: 0.424 – 0.714, p = 0.39)**
- This suggests **poor discrimination ability** (AUC close to 0.5).
- The **p-value > 0.05**, meaning it is **not statistically significant**.

3. Best Cutoff Points

- **Urine:**
- The best cutoff appears to be **0.5**, with **69% sensitivity** and **5.9% false positives (1 - specificity)**.
- **Plasma:**
- The best cutoff at **0.5** provides **only 13.8% sensitivity** and **0% false positives** (meaning it rarely detects positives).

4. Final Interpretation

- **Urine is a good diagnostic test** (AUC = 0.815, significant p-value). It effectively distinguishes between positive and negative cases.
- **Plasma is a poor test** (AUC = 0.569, not significant). It does not perform much better than random chance.
- **Clinical Decision:**
- If choosing between the two, **urine is preferred** for diagnosis.
- **Plasma should not be used as a standalone diagnostic test** due to low AUC and poor sensitivity.

DISCUSSION

DISCUSSION

In the era of a global cancer pandemic, cervical cancer has the distinction of being a preventable cancer. This can be attributed to effective interventions, including vaccination against HR-HPV types and HPV-based screening. [20]

The implementation of cervical cancer screening programs, beginning with the Pap smear in the 20th century, revolutionized cervical cancer prevention leading to notable declines in mortality and incidence rates.[21] The organised screening programs in developed nations has resulted in a remarkable reduction of over 70% in cervical cancer incidence and mortality.[22] HPV testing is emerging as the preferred primary screening method due to its higher sensitivity, accuracy, and reproducibility.[23]

Despite being preventable, cervical cancer ranks fourth among cancers affecting women in terms of both incidence and mortality.[24] Alarmingly, low- and middle-income countries bear the brunt, accounting for approximately 85% of new cases and 90% of deaths.[23] Among this 21% of new cases and 23% cervical cancer deaths occurred in India, giving us a perspective on the poor screening in India.[20] A time trend analysis by Wu et al. forecasts a decline in cervical cancer incidence and mortality in England and China over the next decade, while India's rates are expected to continue rising.[25]

A recent 2023 study published that India's cervical cancer screening coverage falls woefully short of the 70% target proposed by the global cervical cancer elimination strategy, even in tertiary care centers.[26] Alarmingly, the national screening average stands at a mere 21%, with significant urban-rural disparities (10.4% vs 2.8%).[27,28] The major obstacles hindering cervical cancer screening in Indian women include lack of awareness, economic constraints, access issues, cultural and religious factors, fear, embarrassment, and distrust in healthcare systems.[28] India's patriarchal society, with its history of neglecting women's health issues, exacerbates these challenges.

To bridge this gap, it is essential to increase awareness, organize effective screening programs, and implement better strategies to combat cervical cancer in India. The pelvic examination required for Pap tests can be a significant deterrent for many Indian women, due to concerns about modesty, privacy, and discomfort. A non-invasive urine/plasma test could help alleviate these concerns and increase screening participation.

This study focuses on the potential of a less invasive test for HPV which could increase cervical cancer screening uptake among Indian women, especially considering the cultural and social barriers that contribute to low screening rates.

AGE DISTRIBUTION

In India, the peak age of cervical cancer incidence is 50-59 years, significantly higher than the 35-44 years observed in developed countries.[29] Our study corroborates this trend, with the majority (38.67%) of patients falling within the 50-59 age group. This delayed diagnosis can be attributed to inadequate screening and awareness resulting in many Indian women being diagnosed at advanced stages of the disease. It is also to be noted that the clinical significance of high-risk HPV positivity in screening increases with age. Older individuals are more likely to develop high-risk HPV-positive cervical cancer due to their higher likelihood of persistent infection.[30,31] Elderly women who have never been screened for cervical cancer may require more aggressive screening protocols to ensure timely detection and treatment.

Table 25 : Comparative analysis across studies of age of patients with SCC

STUDY	Kalyani et al[32] 2020	Guo et al[33] 2023	Ramesh et al[29] 2023	**Present study , 2025**
Place of study	Kolar	China	Chennai	Kolar
Mean age	54.3 ± 12	51.32 ± 9.72	53.93 ± 10.24	53.92 ±10.86
Range	30-80	25-72	37-74	33-87

REPRODUCTIVE STATUS

In the present study, 72% of patients with Squamous Cell Carcinoma (SCC) were post-menopausal at diagnosis. This finding is consistent with previous research from the same institute but exceeds the proportions reported in comparable studies from other regions.[32,34,35,36]

The relationship between menopause and clearance of HPV infection remains unclear due to conflicting research findings.[30,37] Hence, our study's high proportion of post-menopausal diagnoses in Kolar's rural population may be attributed to delayed medical consultations, likely stemming from limited awareness and inadequate screening programs in the region.

Table 26 : Comparative analysis across studies of reproductive status of women with SCC

Study	Berraho et al[35] 2017	Kalyani et al[32] 2020	Chen et al[34] 2020	Nasreen et al[36] 2023	Present study, **2025**
Place of Study	Morocco	Kolar, Southern India	China	Kashmir, Northern India	Kolar, Southern India
Post-Menopausal	46.5%	74.7 %	56.7%	67.4%	72 %

CHIEF COMPLAINTS

The current study found that clinically, postmenopausal bleeding emerged as the most common symptom (48%), followed by white discharge per vaginum (25.3%). These findings align with established literature that identifies bleeding and white discharge as the most common symptoms.[32,38,39]

Table 27 : Comparative analysis across studies of presenting symptom of women with SCC

	Bleeding per vagina	Vaginal discharge
Shruthi PS et al (2014)[39]	63.3%	60.3%
Kalyani R et al (2020)[32]	80%	68%
Shaffi AF et al (2024)38	79%	54.2%
Present study, 2025	48%	25.3%

CLINICAL FINDING

A notable 68% of females in our study presented with ulceroproliferative growth upon initial hospital visit, again underscoring the tendency of rural populations to delay seeking medical attention until symptoms have progressed, resulting in late-stage diagnoses. This finding is consistent with research from low-resource settings, where limited autonomy, financial dependence on men, and cervical cancer stigma contribute to delayed healthcare-seeking behaviours and advanced disease stages at diagnosis.[38]

Table 28 : Comparative analysis across studies of ulceroproliferative growth (UPG) finding in women with SCC

STUDY	Misra JS et al[40] 2006	Kalyani R et al[32] 2020	PRESENT STUDY, 2025
UPG	51.3%	65.3%	68%

LYMPH NODE INVOLVEMENT

Lymph node involvement is a critical factor in upstaging SCC cervix, as it is incorporated into the FIGO staging system. Consistent with previous studies, our study revealed that 20% of patients had lymph node involvement at diagnosis. This finding is concerning, as lymph node involvement is a proven independent predictor of poorer prognosis and lower overall survival rates.[41,42]

Table 29: Comparative analysis across studies of lymph node involvement in women with SCC

Various Studies	Ruengkhachorn I et al (2015)[43]	Jiang et al (2019)[44]	Bizzarri et al 2023[45]	Deng et al 2023[46]	Rao et al (2023)[47]	**Present study, 2025**
ABSENT	90%	79.1%	80.6%	81.27%	78%	80%
PRESENT	10%	20.9%	19.4%	18.65%	22%	20%

STAGE OF DISEASE

The stage of disease at diagnosis significantly influences overall survival and plays a crucial role in guiding treatment decisions.[33,48] Our study population primarily consisted of patients with stage II (40%) and stage III (36%) cervical cancer, which is consistent with findings from similar demographic groups.[32,38,39,49,50] This finding aligns with the overall trend in the current study, where patients often presented with more advanced stages of cervical cancer.

Interestingly, global disparities in stage of the disease have been noted, with developing countries typically diagnosing cervical cancer at more advanced stages (II and III), whereas developed countries like the US often diagnose it at stage I.[51]

Table 30 : Comparative analysis across studies of Stage of tumor in women with SCC

Stage	Kalyani R et al (2020)[32]	Lin MY et al (2023)[50]	Shaffi AF et al (2024)[38]	Fang et al (2024)[49]	Present study, 2025
I	8%	22%	18.2%	16%	12%
II	32%	23%	26.3%	44%	40%
III	40%	53%	44.6%	36%	36%
IV	20%	2%	10.8%	4%	12%

GRADE

Histological grading of our study population revealed that majority (66.7%) were well-differentiated tumors. However, a comparison with other studies showed variability in tumor grade proportions, likely due to interobserver variability among pathologists and lack of standardized grading criteria.

Table 31 : Comparative analysis across studies of tumor grade in women with SCC

	Shruthi et al (2014)[39]	Zheng et al (2016)[52]	Huang et al (2019)[53]	Kalyani R et al (2020)[32]	Wu et al (2024)[54]	**PRESENT STUDY 2025**
Well-differentiated	18%	16.8%	11%	61.8%	17.3%	66.7 %
Moderately differentiated	55.9%	52.2%	43%	23.5%	69.8%	30.7 %
Poorly differentiated	26.1%	31%	46%	14.7%	12.9%	2.7 %

HPV IN CERVICAL CANCER

Cervical cancer is primarily caused by persistent HPV infection, which is the most prevalent risk factor. [55] With over 200 identified HPV types, 15 are linked to cervical cancer, and these high-risk types are implicated in nearly all (99.7%) cases of cervical squamous cell carcinoma.[56] Predominantly, HPV genotypes 16 and 18 are responsible for more than 70% of all cervical cancer cases.[57]

The present study demonstrated a positivity rate of 77%, with HPV 16 being the most frequently detected genotype (37.3%). Our findings are consistent with recent studies which have consistently shown that HPV is a major contributor to cervical cancer and identified HPV 16 as the predominant oncogenic strain implicated in the development of SCC cervix.

Table 32 : Comparative analysis across studies showing HPV status of women with SCC cervix in tissue sample

HPV STATUS	Kuhn et al (2015)[58]	Vijayaraghavan et al (2020)[59]	Oliveia et al (2020)[60]	Helder et al (2022)[61]	Gupta et al (2022)[62]	Herbst et al (2024)[63]	**Present study 2025**
Method of detection	Hybrid capture I Assay	PCR	PCR	PCR	PCR	PCR	PCR
HPV Positive	83.3%	64%	66.1%	92%	81%	65%	77%
HPV Negative	16.7%	36%	33.9	8%	19%	35%	23%

HPV GENOTYPE

Globally, HPV 16 is the most common type and HPV 18 is the second most frequent carcinogenic strain in cervical cancer, although regional variations exist. Our study's

findings mirror the pattern, with HPV 16 being the most prevalent type. This aligns with worldwide data emphasizing HPV 16's dominant role among high-risk genotypes. Despite HPV genotype not impacting clinical outcomes or prognosis, the prevalence of high-risk types, particularly HPV 16, highlights the importance of targeting these strains in vaccine development.[64]

Table 33 : Comparative analysis across studies showing HPV types in women with SCC cervix

STUDY	REGION	HPV16	HPV18
Zampronha et al(2013)[65]	Brazil	62.7	30.2
Anderson et al(2016)[66]	Ireland	37.4	5.1
Vijayaraghavan et al(2020) [59]	India	46.6	16.2
Gupta et al (2022)[62]	India	60.4	6.25
Herbst et al(2024) [63]	Germany	35	5
Present study, 2025	India	37.3	10.7

CORRELATION OF HPV AND CLINICOPATHOLOGIC PARAMETERS

A statistically significant association (p=0.016) was observed between HPV positivity and tumor stage. However, no significant correlations were found with other parameters, such as lymph node involvement, chief complaints, or tumor grade. This suggests that while HPV is a pivotal etiological factor, its influence may be mediated by a complex interplay of host factors, immune responses, and environmental influences.

HPV Independent SCC tends to exhibit aggressive clinical behavior, leading to a poorer prognosis compared to HPV-associated tumors. Moreover, conventional screening methods, like HPV testing, may overlook these tumors, leading to delayed detection and treatment, even in regions with established screening programs.[67]

HPV TESTING IN URINE

Urine contains exfoliated cervical cells that can be analyzed for HPV-DNA using various DNA amplification and hybridization techniques.[68] Notably, the first void sample contains a significantly higher concentration of epithelial cells and HPV DNA compared to later fractions.[69]

Our study found a 55% HPV positivity rate in urine samples, which is lower than the 77% positivity rate detected in cervical tissue samples. This discrepancy is consistent with other studies, which have reported moderate under detection of HPV DNA in urine compared to cervical tissue.[59,60] However, a study by Tshomo et al. achieved better HPV detection in urine than in cervical samples, attributing this to optimized urine sampling protocols.[70]

Table 34: Comparison of HPV detection in urine and cervical tissue samples

STUDY	HPV POSITIVITY IN URINE **n (%)**	HPV POSITIVITY IN CERVICAL TISSUE SAMPLES **n (%)**
Nilyanimit et al (2017)[71]	53 (32.3%)	65 (39.6%)
Sargebt et al (2019)[72]	48 (61%)	56 (71%)
Tranberg et al (2020)[73]	27 (18%)	36 (24%)
Oliveira et al (2020)[60]	62 (50%)	82 (66.1%)
Helder et al (2022)[61]	94 (83%)	104 (92%)
Song et al (2025)[74]	362(79%)	383(83.6%)
PRESENT STUDY, 2025	41 (55%)	58 (77%)

The most common HPV genotype detected in urine was HPV 16, present in 36% of cases. This finding shows a strong analytical agreement between urine and tissue samples, both in detecting presence and identifying specific genotypes of HPV. This concordance aligns with previous research suggesting that urine samples are representative of the cellular composition at the cervix, supporting the utility of urine-based HPV testing.[70]

HPV IN PLASMA

There has been evidence that tumor DNA can be found in the circulation of patients with cervical cancer with a varied detection rate of HPV DNA found in serum or plasma samples. The detection of HPV has ranged from 7% to 45% in plasma samples of cervical cancer patients.[75] Notably, our study revealed a low screening rate of 11% (8/75) among which a concerning majority (75%) of detected cases presented at an advanced stage of the disease (Stage III B or higher) while 25% (2/8) presented with early disease. The discrepancy in these rates may be due to differences in target materials (serum or plasma), number of HR-HPV types analysed or by technical aspects like plasma volume used for test, methods used to extract DNA, tools used to analyze DNA and primers selected.[75,76] Although plasma-based screening for HPV shows limited promise, research suggests that plasma HPV detection rates increase with advancing disease stages. In fact, HPV detection in plasma has been found to serve as an indicator of disease progression and advanced cervical cancer. Moreover, plasma HPV-DNA may prove to be a valuable biomarker for monitoring therapeutic response and tracking disease progression. To fully elucidate its clinical utility, future studies are needed to investigate the role of plasma HPV-DNA detection as a marker for tumor burden and metastasis in cervical cancer management.[77,78]

Table 35 : Comparative analysis between HPV positivity in plasma and cervical tissues samples

STUDY	HPV POSITIVITY IN PLASMA **n (%)**	HPV POSITIVITY IN CERVICAL TISSUE SAMPLES **n (%)**
Cocuzza et al [76] (2017)	41 (34.2%)	53 (44.2%)
PRESENT STUDY, 2025	8 (11%)	58 (77%)

DIAGNOSTIC UTILITY OF NON-INVASIVE SAMPLES

The study evaluated urine and plasma as alternative less invasive samples for HPV detection, revealing notable differences in diagnostic accuracy.

Urine testing demonstrated a sensitivity of 69% and specificity of 94.1%, with a positive predictive value (PPV) of 97.6% and negative predictive value (NPV) of 47.1%. The diagnostic accuracy of 74.6%, along with a high area under the curve (AUC) of 0.815, positions urine as a promising non-invasive sample for HPV testing. Its utility is particularly relevant in resource-limited settings, where access to cervical tissue may be challenging.

Table 36 : Sensitivity of HPV testing across different sample types in various studies

STUDY	Physician collected cervical tissue samples	URINE (%)	PLASMA (%)
Hsu et al (2003)[79]	-	-	45.2%
Wei YC et al (2007)[80]	-	-	64.7%
Jaberipour M et al (2011)[81]	-	-	23.5%
Pathak et al. (2014)[8]	-	87%	-
Sahasrabuddhe et al. (2014)[82]	96.2%	80.8%	-
Nilyanimit et al (2017)[71]	-	56.5%	-
Tranberg et al (2020)[73]	-	63.9%	-
Cho et al. 2020[83]	93.13%	73.28%	-
Ørnskov et al. 2021[84]	97%	95%	-
Song et al (2025)[74]	96.7%	85.2%	-
PRESENT STUDY, 2025	**Comparator test n=75**	**69%**	**13.8%**

Table 37 : Specificity of HPV testing across different sample types in various studies

STUDY	Physician collected cervical tissue samples	URINE (%)	PLASMA (%)
Hsu et al (2003)[79]	-		88.6%
Wei YC et al (2007)[80]	-		100%
Jaberipour M et al (2007)[81]	-		90.91%
Sahasrabuddhe et al. (2014)[82]	35.6%	42.2%	-
Nilyanimit et al (2017)[71]	-	70.6%	-
Tranberg et al (2020)[73]	-	96.5%	-
Song et al (2025)[74]	20.2%	23.2%	-
PRESENT STUDY, 2025	**Comparator test n=75**	**94.1%**	**100%**

On the other hand, plasma testing showed limited efficacy, with a sensitivity of 13.8% and diagnostic accuracy of 33.3% in the present study. Despite its high specificity and PPV, plasma's poor sensitivity limits its standalone diagnostic utility as a screening test. This finding is in line with previous research indicating that circulating HPV DNA is often below detectable levels in plasma, particularly in early-stage disease.[75]

IMPLICATIONS FOR SCREENING AND DIAGNOSIS

The findings of this study carry important implications for cervical cancer prevention and management. HPV-based screening has higher sensitivity compared to conventional

cytological screening, allowing for longer intervals between screenings.[85] Indian women, particularly in rural areas, prefer self-collection of samples for screening.[86] This was further highlighted when an Indian survey revealed that 60% of women preferred self-collection over physician-collected samples, and 65% favored urine over vaginal self-sampling.[87]

Self-sampling for HPV screening is gaining traction, offering benefits such as reduced hospital visits, preserved modesty, and increased accessibility. Both cervicovaginal and urine samples are being investigated for integration into screening protocols. A comparison study of HPV DNA detection in self-collected urine and vaginal samples done in India by Thomas et al showed fair agreement between urine and vaginal samples.[87] Women in the screening population demonstrated a strong preference for urine self-collection. However, to ensure safe and effective self-collection of cervicovaginal samples, education and awareness programs should address concerns regarding potential self-inflicted injury and provide clear guidance on proper technique.[69,86]

The high sensitivity and specificity of urine-based HPV testing in our study highlight its potential as a cost-effective and patient-friendly alternative to tissue-based diagnostics. Its implementation in screening programs could significantly enhance coverage, particularly in populations with limited access to healthcare facilities.

Advantages of Urine Sampling for HPV testing

- Non Invasive with easy sampling
- Easily accessible
- Home-based urine collection is well-accepted and ranked as the most preferred future screening procedure by patients[88]
- No need for hospital visits or technician-assisted sample collection

Disdvantages of Urine Sampling for HPV testing

- Distance and cost of sample transport
- Improper transport
- Issues with quality of samples, such as spillage or inadequate samples [86]

Given the low screening uptake for cervical cancer in India, introducing urinary HPV testing could be beneficial, as it is non-invasive and has favorable acceptability in the Indian population. [8,72,87,88] Furthermore, even one round of HPV screening is effective in reducing mortality due to cervical cancer.[86]

Although urinary HPV detection shows slightly lower sensitivity compared to cervical samples, this limitation can be offset by repeated screenings throughout a woman's lifetime. Improving accuracy rates of urinary HR-HPV detection can be achieved through standardized procedures for urine sample collection ,transport, modified HPV assays, self-collection devices and advent of specifically modified HPV assay for urine samples.[70,88]

IMPLICATIONS IN MANAGEMENT

Plasma HPV testing has limited diagnostic value for screening purposes as highlighted by the low sensitivity in our study. But HPV detection in both cervical and plasma samples was found to increase with the severity or stage of the disease.[76] The circulating tumor cells in plasma can indicate recurrence or progression of disease.[77,78] Notably, our study showed high specificity for HPV detection in plasma, suggesting that plasma viremia may be a useful tool for monitoring treatment response and prognosis. Further research is needed to optimize plasma-based HPV detection, potentially through advanced molecular techniques such as digital PCR or next-generation sequencing.

VACCINE MONITORING

As vaccine coverage expands nationwide, driven by growing awareness, a convenient and non-invasive method for measuring HPV antibody levels is becoming essential. Urine-based HPV detection, utilizing specialized molecular assays for anti-HPV antibody detection, is likely to emerge as a vital tool for vaccination surveillance in the near future.[70,88] Further research in this area will be essential to explore its potential and applications.

FUTURE DIRECTIONS

1. Investigating the clinical utility of urine-based testing in primary screening programs, by modifying the technique exclusively for urine samples, particularly in low-resource settings, to expand cervical cancer prevention strategies.
2. Enhancing the sensitivity of plasma-based HPV detection to enable its application in liquid biopsy.
3. Conducting molecular studies on HPV genotyping to explore its correlation with treatment response and prognosis, ultimately informing personalized approaches to cervical cancer care.
4. Exploring the use of urinary HPV detection for vaccination surveillance, offering a promising avenue for monitoring vaccine effectiveness and population immunity.

LIMITATIONS

LIMITATIONS OF THE STUDY

1. The relatively small sample size (n=75) may limit the generalizability of the results.
2. Additionally, the cross-sectional design precludes longitudinal assessment of HPV's role in disease progression. Future studies with larger, diverse cohorts and longitudinal follow-ups are warranted to validate these findings and explore causal relationships.
3. The only histological type of cervical cancer considered in this study was squamous cell carcinoma of cervix.

CONCLUSION

CONCLUSION

This study highlights the potential of urine-based high-risk human papillomavirus (HR-HPV) detection as a non-invasive screening method for cervical cancer. The non-invasive nature of urine testing can help alleviate cultural, social, and religious barriers associated with traditional Pap tests, which require a pelvic examination. This can significantly improve screening uptake among Indian women, who often face concerns regarding modesty, privacy, and discomfort.

To effectively combat cervical cancer in India, it is crucial to increase awareness, implement organized screening programs, and develop innovative strategies. The findings of this study emphasize the importance of less invasive testing methods to enhance cervical cancer screening participation, ultimately contributing to better health outcomes for women in India.

This presents a promising avenue for future research, development, and innovation in cervical cancer prevention, early detection, treatment strategies and reach the WHO goal of 70% screening of women by 2030.

SUMMARY

SUMMARY

The primary objective of the current study was to detect High Risk HPV in cervical tissues, urine samples and in plasma in Squamous Cell Carcinoma of Cervix.

For this purpose, 75 cases of primary diagnosis of SCC cervix cases were taken and their tissue , urine and plasma samples were collected from May 2023 to October 2024

Following salient features are noted:

1. The peak incidence of age of presentation in current study was 50-59 years.
2. Most cases of cervical carcinoma were noted in postmenopausal age group (72% cases)
3. Most common chief complaint noted was postmenopausal bleeding (48 % cases)
4. Most of them presented with an ulceroproliferative growth (68% cases)
5. Magnetic Resonance Imaging (MRI) assessment revealed lymph node involvement in 20% of cases .
6. The most prevalent FIGO stage among the cases were Stage II.
7. Histological grading revealed that the majority of cases (66.7%) were well-differentiated carcinomas, representing the most common histological grade.
8. High risk HPV DNA was detected by PCR in 58 (77%) cervical tissue samples, with HPV 16 (37.3%) being the most common genotype
9. PCR analysis of urine samples detected HPV positivity in 41 (55%) cases, with a predominance of HPV type 16 (36%)
10. PCR-based HPV detection in blood samples revealed positive results in 8 (11%) cases, with HPV 16 (87.5%) being the predominant subtype, accounting for 7 out of the 8 positive cases.
11. Notably, HPV 16 was consistently present in all cases of mixed HPV infections.
12. The study found that when comparing HPV status in cervical tissue (considered

the gold standard) to urine samples, there was a concordance in 69% of cases (40 out of 58). This means that in 40 cases, the HPV positivity detected in cervical tissue was also detected in the corresponding urine samples, indicating a moderate level of agreement between the two sample types.

13. The study revealed that plasma HPV testing had a relatively low positivity rate of 13.8% compared to the gold standard of cervical tissue testing. Out of 58 cases that were positive for HPV in cervical tissue, only 8 cases (13.8%) were positive in plasma, highlighting a lower detection rate for plasma HPV testing
14. HR-HPV was detected in 77% in cervical tissue sample , 55% in urine sample and 11% in blood samples
15. Urine testing exhibited a sensitivity of 69%, coupled with high specificity of 94.1% positive predictive value of 97.6% and negative predictive value (47.1%), yielding an overall diagnostic accuracy of 74.6%
16. Plasma testing showed a markedly lower sensitivity of 13.8%, despite high specificity of 100%, positive predictive value of 100% and negative predictive value (25.4 %) , its overall diagnostic accuracy was significantly lower at 33.3%
17. Urine can be used as a reliable diagnostic test as AUC was 0.815 and sensitivity was 69%
18. Due to low AUC (0.569) and poor sensitivity (13.8 %), plasma testing is not recommended as a standalone diagnostic tool for detecting HPV

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BIBLIOGRAPHY

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ANNEXURE

ANNEXURE I

INFORMED CONSENT FORM

- **STUDY TITLE:** DETECTION OF HIGH RISK HUMAN PAPILLOMA VIRUS IN CERVICAL TISSUE, URINE AND PLASMA IN SQUAMOUS CELL CARCINOMA OF UTERINE CERVIX BY REAL TIME POLYMERASE CHAIN REACTION – A CROSS SECTIONAL STUDY
- I, _____have read or have been read to me the patient information sheet and understand the purpose of the study, the procedure that will be used, the risk and benefits associated with my involvement in the study and the nature of information will be collected and disclosed during the study.
- I have had my opportunity to ask my questions regarding various aspects of the study and my questions are answered to my satisfaction.
- I, the undersigned, agree to participate in this study and authorize the collection and disclosure of my personal information for the dissertation.
- You are requested to sign / provide thumb impression only if you voluntarily agree to participate in the study. All information collected from you will be kept confidential and will not be disclosed to any outsider. Your identity will not be revealed. You will not receive any monetary benefits to participate in this research. All procedures have been read out to me in my regional language.
- This informed consent document is intended to give you a general background of study. Please read the information carefully and discuss with your family members. You can ask your queries related to study at any time during the study. If you are willing to participate in the study you will be asked to sign an informed consent form by which you are acknowledging that you wish to participate in the study and entire procedure will be explained to you by the study doctor. You are free to withdraw your consent to participate in the study any time without explanation and this will not change your future care.
- For any clarification you are free to contact the investigator.
- PRINCIPAL INVESTIGATOR: Dr. BHADRA.A.R
- PHONE NO. : 7034170171

Name and signature / thumb impression Date:

Place:

(Witness)

ಒಪ್ಪಿಗೆ ಪತ್ರ

ಅಧ್ಯಯನದ ಸ್ಥಳ : ಶ್ರೀ ದೇವರಾಜ್ ಅರಸು ವೈದ್ಯಕೀಯ ಮಹಾವಿದ್ಯಲಯ, ಟಮಕ, ಕೋಲಾರ.

ಅಧ್ಯಯನದ ಶೀರ್ಷಿಕೆ :ಗರ್ಭಕಂಠದ ಬಯಾಪ್ಸಿ, ಮೂತ್ರದಮಾದರಿಗಳೂ ಮತ್ತು ಗರ್ಭಾಶಯದ ಗರ್ಭಕಂಠದ ಸ್ಮೆಮಸ್ ಸೆಲ್ ಕಾರ್ಸಿನೋಮದಲ್ಲಿ ಹೆಚ್ಚಿನ ಅಪಾಯದ ಮಾನವ ಪ್ಯಾಪಿಲೋಮ ವೈರಸ್ ಅನ್ನು ನೈಜ ಸಮಯದಲ್ಲಿ ಪೋಲಿಮರೇಸ್ ಚೈನ್ ರಿಯಾಕ್ಷನ್ ಮೂಲಕ ಪತ್ತೆ ಮಾಡುವುದರ ಸಂಬಂಧದ ತುಲನಾತ್ಮಕ ಅಧ್ಯಯನ.

ಮುಖ್ಯ ಸಂಶೋಧಕರ ಹೆಸರು : ಡಾ||ಭವ್ಯ.ಎ.ಆರ್, ಮೊದಲನೇ ವರ್ಷದ ಸ್ನಾತಕೋತ್ತರ ವಿದ್ಯಾರ್ಥಿನಿ, ಶ್ರೀ ದೇವರಾಜ್ ಅರಸು ವೈದ್ಯಕೀಯ ಮಹಾವಿದ್ಯಾಲಯ, ಟಮಕ, ಕೋಲಾರ.

ಭಾಗವಹಿಸುವವರ ಹೆಸರು_____ ಅದ ನಾನು ಗರ್ಭಕಂಠದ ಬಯಾಪ್ಸಿ, ಮೂತ್ರದಮಾದರಿಗಳೂ ಮತ್ತು ಗರ್ಭಾಶಯದ ಗರ್ಭಕಂಠದ ಸ್ಮೆಮಸ್ ಸೆಲ್ ಕಾರ್ಸಿನೋಮದಲ್ಲಿ ಹೆಚ್ಚಿನ ಅಪಾಯದ ಮಾನವ ಪ್ಯಾಪಿಲೋಮ ವೈರಸ್ ಅನ್ನು ನೈಜ ಸಮಯದಲ್ಲಿ ಪೋಲಿಮರೇಸ್ ಚೈನ್ ರಿಯಾಕ್ಷನ್ ಮೂಲಕ ಪತ್ತೆ ಮಾಡುವುದರ ಸಂಬಂಧದ ತುಲನಾತ್ಮಕ ಅಧ್ಯಯನ.

ಈ ಅಧ್ಯಯನದ ಪೂರ್ತಿ ವಿವರವನ್ನು ಹಾಗೂ ಅದರ ಉದ್ದೇಶವನ್ನು ನನಗೆ ಅರ್ಥ ಆಗುವ ಹಾಗೆ ತಿಳಿಸಿಕೊಟ್ಟಿದ್ದಾರೆ . ಈ ಅಧ್ಯಯನದ ಬಗ್ಗೆ ಹಲವಾರು ಪ್ರಶ್ನೆ ಕೇಳಲು ಅವಕಾಶ ದೊರೆತಿದ ಹಾಗೇ ನನ್ನ ಪ್ರಶ್ನೆಗಳಿಗೆ ನನಗೆ ತೃಪ್ತಿಕರವಾದ ಉತ್ತರಗಳು ದೊರೆತಿದೆ.

ನನ್ನಿನಿಂದ ಓದಲ್ಪಟ್ಟ ಅಥವಾ ನನಗೆ ಓದಿದ ಅಧ್ಯಯನದ ಉದ್ದೇಶ ನನಗೆ ಅರ್ಥವಾಗಿದ್ದು ನನ್ನಿಂದ ಸಂಗ್ರಹಿಸಲ್ಪಟ್ಟ ಮಾಹಿತಿಯನ್ನು ಕೇವಲ ಅಧ್ಯಯನಕ್ಕಾಗಿ ಬಳಸಲಾಗುವುದು ಹಾಗೂ ಮಾಹಿತಿಯನ್ನು ಗೌಪ್ಯವಾಗಿ ಇರಿಸಲಾಗುವುದು ನನ್ನ ಗುರುತನ್ನು ಬಹಿರಂಗ ಪಡಿಸುವುದಿಲ್ಲ. ನನಗೆ ಅಧ್ಯಯನದಲ್ಲಿ ಯಾವುದೇ ವೆಚ್ಚ ತಗಲುವುದಿಲ್ಲ ಎಂಬ ಅರಿವು ನನಗೆ ಆಗಿದೆ.

ಭಾಗವಹಿಸುವವರ ಸಹಿ : _____

ಭಾಗವಹಿಸುವವರ ಹೆಸರು : _____

ಸಾಕ್ಷಿದಾರರ ಸಹಿ : _____

ಸಾಕ್ಷಿದಾರರ ಹೆಸರು : _____

ANNEXURE II

PATIENT INFORMATION SHEET

- **STUDY TITLE:**DETECTION OF HIGH RISK HPV IN CERVICAL TISSUE , URINE AND PLASMA IN SQUAMOUS CELL CARCINOMA OF UTERINE CERVIX BY REAL TIME POLYMERASE CHAIN REACTION– A CROSS SECTIONAL STUDY.
- **PLACE OF STUDY:** Department of Pathology, Sri Devaraj Urs Medical College, Kolar.

- Cervical Cancer is the 2nd most common cancer in females in Kolar. The main aim is to study the isolation of HPV in cervical tissue, urine and blood samples in Squamous Cell Carcinoma of cervical cases.
- You are requested to participate in a study conducted by the department of Pathology as a part of dissertation. This study will be done on cases of SCC of Cervix . The specimens will be collected from the department of Obstetrics and Gynaecology , SDUMC, Kolar.
- For this study no extra tissue will be collected from you. Blood and urine samples will be collected, and all will be tested for detection of human papilloma virus using polymerase chain reaction. This study is approved by the institutional ethical committee. The information collected will be used only for dissertation, presentation and publication. There is no compulsion to agree to participate.

- You are requested to sign / provide thumb impression only if you voluntarily agree to participate in the study. All information collected from you will be kept confidential and will not be disclosed to any outsider. Your identity will not be revealed. You will not receive any monetary benefits to participate in this research and no money will be incurred from you. All expenses will be met by the principal investigator.
- This informed consent document is intended to give you a general background of study. Please read the information carefully and discuss with your family members. You can ask your queries related to study at any time during the study. If you are willing to participate in the study you will be asked to sign an informed consent form by which you are acknowledging that you wish to participate in the study and entire procedure will be explained to you by the study doctor. You are free to withdraw your consent to participate in the study any time without explanation and this will not change your future care.
- For any clarification you are free to contact the investigator.
- PRINCIPAL INVESTIGATOR: Dr. BHADRA.A.R
- PHONE NO. : 7034170171

ಮಾಹಿತಿ ಪತ್ರ

ಅಧ್ಯಯನದ ಶೀರ್ಷಿಕೆ : ಗರ್ಭಕಂಠದ ಬಯೋಪ್ಸಿ, ಮೂತ್ರದಮಾದರಿಗಳು ಮತ್ತು ಗರ್ಭಾಶಯದ ಗರ್ಭಕಂಠದ ಸ್ಮೆಮಸ್ ಸೆಲ್ ಕಾರ್ಸಿನೋಮದಲ್ಲಿ ಹೆಚ್ಚಿನ ಅಪಾಯದ ಮಾನವ ಪ್ಯಾಪಿಲೋಮ ವೈರಸ್ ಅನ್ನು ನೈಜ ಸಮಯದಲ್ಲಿ ಪೋಲ್ ಯಮರೇಸ್ ಚೈನ್ ರಿಯಾಕ್ಷನ್ ಮೂಲಕ ಪತ್ತೆ ಮಾಡುವುದರ ಸಂಬಂಧದ ಕುರಿತು ಅಧ್ಯಯನ.

ಮುಖ್ಯ ಸಂಶೋಧಕರ ಹೆಸರು : ಡಾ॥ಭವ್ಯ.ಎ.ಆರ್. ಮೊದಲನೇ ವರ್ಷದ ಸ್ನಾತಕೋತ್ತರ ವಿದ್ಯಾರ್ಥಿನಿ, ಶ್ರೀ ದೇವರಾಜ್ ಅರಸು ವೈದ್ಯಕೀಯ ಮಹಾವಿದ್ಯಲಯ, ಟಮಕ, ಕೋಲಾರ.

ಅಧ್ಯಯನದ ಸ್ಥಳ : ಶ್ರೀ ದೇವರಾಜ್ ಅರಸು ವೈದ್ಯಕೀಯ ಮಹಾವಿದ್ಯಾಲಯ, ಟಮಕ, ಕೋಲಾರ.

ನಾನು, ಡಾ॥ ಭವ್ಯ ಎ.ಆರ್. ಮೊದಲನೆಯ ವರ್ಷದ ಸ್ನಾತಕೋತ್ತರ ವಿದ್ಯಾರ್ಥಿನಿಯಾಗಿ ಒದುತ್ತಿದ್ದೇನೆ. ಈ ಅಧ್ಯಯನದ ಮುಖ್ಯ ಸಂಶೋಧಕಿ ನಿಮ್ಮನ್ನು ಈ ಮೇಲ್ಕಂಡ ಅಧ್ಯಾಯನಕ್ಕೆ ಸ್ವಾಗತಿಸುತ್ತೇನೆ ಹಾಗೂ ನಾನು ಈ ಗರ್ಭಕಂಠದ ಬಯೋಪ್ಸಿ, ಮೂತ್ರದಮಾದರಿಗಳು ಮತ್ತು ಗರ್ಭಾಶಯದ ಗರ್ಭಕಂಠದ ಸ್ಮೆಮಸ್ ಸೆಲ್ ಕಾರ್ಸಿನೋಮದಲ್ಲಿ ಹೆಚ್ಚಿನ ಅಪಾಯದ ಮಾನವ ಪ್ಯಾಪಿಲೋಮ ವೈರಸ್ ಅನ್ನು ನೈಜ ಸಮಯದಲ್ಲಿ ಪೋಲಿಮರೇಸ್ ಚೈನ್ ರಿಯಾಕ್ಷನ್ ಮೂಲಕ ಪತ್ತೆ ಮಾಡುವುದರ ಸಂಬಂಧದ ಕುರಿತು ಅಧ್ಯಯನ.

ಈ ಅಧ್ಯಯನ ಮಾದರಿಯನ್ನು ರೋಗಶಾಸ್ತ್ರ ವಿಭಾಗ, ಶ್ರೀ ದೇವರಾಜ್ ಅರಸು ವೈದ್ಯಕೀಯ ಮಹಾವಿದ್ಯಲಯ, ಕೋಲಾರದಿಂದ ನಡೆಸಲಾಗುತ್ತಿದ್ದು ಈ ಅಧ್ಯಯನವನ್ನು ಸಾಂಸ್ಥಿಕ ನೈತಿಕ ಸಮಿತಿಯು ಅನುಮೋದಿಸುತ್ತದೆ. ಸಂಗ್ರಹಿಸಿದ ಮಾಹಿತಿಯನ್ನು ಪ್ರಬಂಧ ಮತ್ತು ಪ್ರಕಟಣೆಗೆ ಮಾತ್ರ ಬಳಸಲಾತ್ತದೆ. ಭಾಗವಹಿಸಲು ಒಪ್ಪಿಕೊಳ್ಳಲು ಯಾವುದೇ ಒತ್ತಾಯವಿಲ್ಲ. ನೀವು ಸ್ವಯಂಪ್ರೇರಣೆಯಿಂದ ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸಲು ಒಪ್ಪಿದರೆ ಮಾತ್ರ ಹೆಚ್ಚಿರಳಿನ ಗುರುತನ್ನು ಸಹಿ ಮಾಡಲು / ಒದಗಿಸಲು ನಿಮ್ಮನು ವಿನಂತಿಸಲಾಗಿದೆ.

ನಿಮ್ಮಿಂದ ಸಂಗ್ರಹಿಸಲಾದ ಎಲ್ಲಾ ಮಾಹಿತಿಯನ್ನು ಗೌಪ್ಯವಾಗಿ ಇರಿಸಲಾಗುತ್ತದೆ ಮತ್ತು ಯಾವುದೇ ಹೊರಗಿನವರಿಗೆ ಬಹಿರಂಗಪಡಿಸಲಾಗುವುದಿಲ್ಲ. ನಿಮ್ಮ ಗುತರುತನ್ನು ಬಹಿರಂಗಪಡಿಸಲಾಗುವುದಿಲ್ಲ. ಈ ಸಂಶೋಧನೆಯಲ್ಲಿ ಭಾಗವಹಿಸಲು ನೀವು ಯಾವುದೇ ಹಣಕಾಸಿನ ಪ್ರಯೋಜನಗಳನ್ನು ಸ್ವೀಕರಿಸುವುದಿಲ್ಲ. ಈ ತಿಳುವಳಿಕೆಯುಳ್ಳ ಸಮ್ಮತಿಯ ಆಧಾರಗಳನ್ನು ನಿಮಗೆ ಸಾಮಾನ್ಯ ಅಧ್ಯಯನದ ಹಿನ್ನೆಲೆಯನ್ನು ನೀಡಲು ಉದ್ದೇಶಿಸಲಾಗಿದೆ. ದಯವಿಟ್ಟು ಕೆಳಗಿನ ಮಾಹಿತಿಯನ್ನು ಎಚ್ಚರಿಕೆಯಿಂದ ಓದಿ ಮತ್ತು ನಿಮ್ಮ ಕುಟುಂಬದ ಸದಸ್ಯರೊಂದಿಗೆ ಚರ್ಚಿಸಿ. ಅಧ್ಯಯನದ ಸಮಯದಲ್ಲಿ ಯಾವುದೇ ಸಮಯದಲ್ಲಿ

ಅಧ್ಯಯನಕ್ಕೆ ಸಂಬಂಧಿಸಿದ ನಿಮ್ಮ ಪ್ರಶ್ನೆಗಳನ್ನು ನೀವು ಕೇಳಬಹುದು. ನೀವು ಅಧ್ಯಯನದಲ್ಲದಲ್ಲಿ ಸಿದ್ಧರಿದ್ದರೆ, ತಿಳುವಳಿಕೆಯುಳ್ಳ ಸಮ್ಮತಿಯ ನಮೂನೆಗೆ ಸಹಿ ಹಾಕಲು ನಿಮ್ಮನ್ನು ಕೇಳಲಾಗುತ್ತದೆ ಮತ್ತು ಆದರ ಮೂಲಕ ನೀವು ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸಲು ಬಯಸುತ್ತೀರಿ ಎಂದು ಒಪ್ಪಿಕೊಳ್ಳುತ್ತೀರಿ ಮತ್ತು ಸಂಪೂರ್ಣ ಕಾಯ ವಿಧಾನವನ್ನು ಅಧ್ಯಯನ ವೈದ್ಯರು ನಿಮಗೆ ವಿವರಿಸುತ್ತಾರೆ. ವಿವರಣೆಯಿಲ್ಲದೆ ಯಾವುದೇ ಸಮಯದಲ್ಲಿ ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸಲು ನಿಮ್ಮ ಸಮ್ಮತಿಯನ್ನು ಹಿಂಪಡೆಯಲು ನೀವು ಸ್ವತಂತ್ರರಾಗಿದ್ದೀರಿ ಮತ್ತು ಇದು ನಿಮ್ಮ ಭವಿಷ್ಯದ ಕಾಳಜಿಯನ್ನು ಬದಲಾಯಿಸುವುದಿಲ್ಲ.

ಯಾವುದೇ ಸ್ಪಷ್ಟೀಕರಣಕ್ಕಾಗಿ ನೀವು ತನಿಖಾಧಿಕಾರಿಯನ್ನು ಸಂಪರ್ಕಿಸಲು ಮುಕ್ತರಾಗಿದ್ದೀರಿ.

ಸಂಶೋಧಕರ ಹೆಸರು : ಡಾ॥ ಭದ್ರ.ಎ.ಆರ್., ಮೊದಲನೆಯ ವರ್ಷದ ಸ್ನಾತಕೋತ್ತರ ವೈದ್ಯಕೀಯ ವಿದ್ಯಾರ್ಥಿನಿ,
ಶ್ರೀ ದೇವರಾಜ್ ಅರಸು ವೈದ್ಯಕೀಯ ಮಹಾವಿದ್ಯಾಲಯ, ಟಮಕ, ಕೋಲಾರ.
ದೂರವಾಣಿ ಸಂಖ್ಯೆ : 7034170171

ನಿಮ್ಮ ಸಮಯ ಮತ್ತು ಪರಿಗಣನೆಗೆ ನನ್ನ ಧನ್ಯವಾದಗಳು.

ANNEXURE-III

TITLE: DETECTION OF HIGH RISK HUMAN PAPILLOMA VIRUS IN CERVICAL TISSUE, URINE AND PLASMA IN SQUAMOUS CELL CARCINOMA OF UTERINE CERVIX BY REAL TIME POLYMERASE CHAIN REACTION – A CROSS SECTIONAL STUDY

PATIENT PROFORMA

Name :
Age:
Hospital Number:

Anonymised Sample No:

Chief complaint :

History of presenting illness :

Past history :

Personal history :

Mentrual history:

Parity :

Local examination:

Biopsy Number:

Cervical biopsy:

Histopathological diagnosis :

HPV Negative -

HPV Positive (Subtype) –

Urine Sample:

HPV Negative -

HPV Positive (Subtype) –

Blood sample:

HPV Negative -

HPV Positive (Subtype) –

ANNEXURE-IV

KEYS TO MASTER CHART

PMB	Post Menopausal Bleeding
C/F	Clinical findings
Cx	Cervix
UPG	Ulceroproliferative growth
P/V	Per vagina
WD	Well-differentiated
MD	Moderately-differentiated
PD	Poorly-differentiated
HPV	Human Papilloma Virus
LN	Lymph node
HPE	Histopathological Examination
SCC	Squamous Cell Carcinoma
ND	Not Detected

MASTER CHART

SI NO	BIOPSY NO	UHID	AGE	PARITY	MENSTRUAL	CHIEF COMPLAINT	C/F	LN STATUS	HPE	TUMOUR SIZE	STAGE	TISSUE	URINE	PLASMA
1	3040/22	165520	87	P5L5	Menopause	PAIN ABDOMEN	UPG	Absent	WD SCC	6.1	III B	HPV 16	HPV 16	ND
2	3065/22	167121	42	P1L1	Menopause	WHITE DISCHARGE PV	Cx Hypertrophied	Absent	WD SCC	3.9	II B	HPV 16/31(Mixed infection)	HPV 16	ND
3	3159/22	170226	75	P3L2A1D1	Menopause	PMB	Cx erosion	Absent	WD SCC	1	II B	HPV 35/31/51	HPV 51	ND
4	3189/22	170736	50	P3L3	Menopause	WHITE DISCHARGE PV	Cx Hypertrophied	PRESENT	WD SCC	6.5	III C1	HPV 16	HPV 16	ND
5	3273/23	129918	49	P3L2	Menopause	WHITE DISCHARGE PV	UPG	PRESENT	WD SCC	6.2	IV A	HPV 16/31(Mixed infection)	HPV 16/31(Mixed infection)	ND
6	3338/22	96869	65	P7 L3	Menopause	PMB	Cx Hypertrophied	Absent	WD SCC	4.2	III B	HPV 31	ND	ND
7	3368/22	172865	50	P5 L4	Menopause	PMB	UPG	Absent	MD SCC	5.6	II B	HPV 16	HPV 16	ND
8	3463/22	180266	50	P4 L2	Menopause	PMB	UPG	Absent	WD SCC	5.2	II B	HPV 16	HPV 16	ND
9	3472/22	180761	46	P2 L2	Regular	WHITE DISCHARGE PV	UPG	PRESENT	WD SCC	5	III B	HPV 16	HPV 16/31(Mixed infection)	ND
10	16/23	183383	50	P8 L8	Menopause	WHITE DISCHARGE PV	UPG	Absent	MD SCC	3.6	II B	HPV 16	HPV 16	ND
11	83/23	185189	70	P2 L2	Menopause	PAIN ABDOMEN	UPG	Absent	MD SCC	4.6	III B	HPV 16	HPV 16/31(Mixed infection)	ND
12	280/23	191707	66	P4 L4	Menopause	PMB	UPG	Absent	MD SCC	6.2	III B	HPV 16	HPV 16	HPV 16
13	281/23	191468	38	P2 L2	Regular	WHITE DISCHARGE PV	Cx Hypertrophied	Absent	MD SCC	3.8	II B	HPV 16	ND	HPV 16
14	512/23	198168	65	P7 L3	Menopause	PMB	UPG	PRESENT	MD SCC	6.5	IV A	HPV 16	HPV 16	HPV 16
15	806/23	207520	65	P4 L4	Menopause	PMB	UPG	Absent	WD SCC	4.2	II A	HPV 16/31(Mixed infection)	HPV 16	ND
16	858/23	209378	50	P2 L2	Menopause	PMB	UPG	Absent	WD SCC	6	II B	HPV 16	HPV 16	ND
17	979/23	214070	55	P5 L4	Menopause	PAIN ABDOMEN	UPG	PRESENT	WD SCC	5.3	III C1	HPV 16/31(Mixed infection)	HPV 16	ND
18	1033/23	214073	55	P3 L3	Menopause	PMB	UPG	PRESENT	WD SCC	6.3	IV B	HPV 16	HPV 16	ND
19	1137/23	218923	60	P3L2D1	Menopause	WHITE DISCHARGE PV	UPG	Absent	MD SCC	4.6	II B	HPV 16/31/58 (Mixed infection)	HPV 16	ND
20	1176/23	220475	50	P3L3	Menopause	PMB	Cx erosion	PRESENT	WD SCC	5.1	IV B	HPV 16	HPV 16	ND
21	1260/23	222728	50	P1L1	IRREGULAR	BLEEDING PV	UPG	Absent	MD SCC	6.5	III B	HPV 16/31/58 (Mixed infection)	HPV 16	HPV 16
22	1280/23	223857	55	P6 L6	Menopause	PAIN ABDOMEN	UPG	Absent	WD SCC	3.8	III B	HPV 18	ND	ND
23	1379/23	226237	58	P6 L5	Menopause	PMB	Cx Hypertrophied	Absent	MD SCC	2.3	III B	HPV 18	ND	ND
24	1400/23	226896	48	P4 L4	Menopause	WHITE DISCHARGE PV	UPG	Absent	WD SCC	4.8	II B	HPV 16/31/58 (Mixed infection)	HPV 16	ND
25	1482/23	229778	58	P5 L5	Menopause	PMB	UPG	Absent	WD SCC	6.7	II B	HPV 16/31/58 (Mixed infection)	HPV 16/58 (Mixed Infection)	ND
26	1531/23	230573	55	P3 L3	Menopause	PAIN ABDOMEN	UPG	Absent	MD SCC	6.2	III B	HPV 16/31/58 (Mixed infection)	HPV 16	ND
27	1663/23	234902	80	P3 L3	Menopause	WHITE DISCHARGE PV	UPG	Absent	WD SCC	2.3	II B	HPV 16/31/58 (Mixed infection)	HPV 16	ND
28	1664/23	233346	44	P3 L3	IRREGULAR	BLEEDING PV	Cx Hypertrophied	Absent	MD SCC	4.2	II B	ND	ND	ND
29	1802/23	239401	60	P0	Menopause	WHITE DISCHARGE PV	UPG	PRESENT	WD SCC	5.1	IV A	ND	ND	ND
30	1803/23	239482	68	P6 L6	Menopause	PMB	UPG	Absent	MD SCC	5.5	II B	ND	ND	ND
31	1940/23	243619	70	P4 L3	Menopause	PMB	UPG	Absent	WD SCC	3.6	III B	HPV 16/31/58 (Mixed infection)	HPV 16/58 (Mixed Infection)	ND
32	2003/23	225378	34	P2 L2	IRREGULAR	BLEEDING PV	Cx Hypertrophied	Absent	WD SCC	1.4	II B	HPV 16	ND	ND
33	2148/23	251182	45	P2 L2	Regular	BLEEDING PV	UPG	Absent	WD SCC	4.2	II B	ND	ND	ND
34	2310/23	257010	45	P5 L5	Regular	WHITE DISCHARGE PV	Cx erosion	Absent	MD SCC	2.4	I B	HPV 16	HPV 51/66 (Mixed Infection)	ND
35	2439/23	245207	34	P2 L2	IRREGULAR	BLEEDING PV	Cx erosion	Absent	WD SCC	2.2	I B	HPV 16/31/58 (Mixed infection)	HPV 16/58 (Mixed Infection)	ND
36	2453/23	261277	52	P3 L3	Menopause	PMB	UPG	Absent	WD SCC	5.2	III B	HPV 18	ND	ND
37	2621/23	265437	50	P4L4	IRREGULAR	BLEEDING PV	Cx Hypertrophied	PRESENT	WD SCC	7.6	IV B	HPV 18	ND	ND
38	2712/23	268459	34	P1 L1	IRREGULAR	BLEEDING PV	UPG	Absent	MD SCC	2.1	I B	ND	ND	ND
39	2762/23	230106	45	P4 L4	Menopause	PMB	UPG	Absent	MD SCC	5.6	II B	HPV 16	HPV 16	ND
40	2841/23	260083	45	P0	Menopause	WHITE DISCHARGE PV	UPG	Absent	MD SCC	4.5	I B	ND	ND	ND
41	2861/23	272593	48	P2L0D2	Regular	BLEEDING PV	UPG	PRESENT	WD SCC	4.6	III C1	HPV 16	HPV 16	ND
42	2862/23	272737	34	P2 L2	Regular	WHITE DISCHARGE PV	UPG	Absent	WD SCC	3.2	I B	ND	HPV 35/31/66(Mixed Infection)	ND
43	3055/23	278450	60	P4L4A1D1	Menopause	WHITE DISCHARGE PV	UPG	Absent	MD SCC	3.7	II B	HPV 16/31 (Mixed infection)	ND	ND
44	583/22	121086	45	P3 L3	IRREGULAR	BLEEDING PV	Cx Hypertrophied	Absent	MD SCC	3.9	II B	HPV 16/31 (Mixed infection)	HPV 35	ND
45	3201/23	283075	50	P6 L5	Menopause	PMB	UPG	Absent	WD SCC	4.8	III B	HPV 16	HPV 16	ND
46	3211/23	281404	33	P2L2	IRREGULAR	BLEEDING PV	Cx erosion	Absent	MD SCC	3.2	II B	ND	ND	ND
47	3379/23	288253	50	P3 L3	Menopause	PMB	UPG	PRESENT	WD SCC	4.9	III C1	HPV 16	HPV 16	ND
48	3821/23	299748	58	P8 L8	Menopause	PMB	UPG	PRESENT	PD SCC	5.6	IV A	HPV 16	HPV 16	ND
49	3906/23	303436	65	P6L5	Menopause	PAIN ABDOMEN	UPG	Absent	MD SCC	3.8	III B	HPV 16	ND	ND

SI NO	BIOPSY NO	UHID	AGE	PARITY	MENSTRUAL	CHIEF COMPLAINT	C/F	LN STATUS	HPE	TUMOUR SIZE	STAGE	TISSUE	URINE	PLASMA
50	4007/23	307304	60	P9 L7	Menopause	WHITE DISCHARGE PV	UPG	Absent	WD SCC	2	I B	ND	ND	ND
51	4116/23	308557	57	P3 L3	Menopause	PMB	UPG	Absent	WD SCC	4.2	III B	HPV 31	HPV 16	HPV 16
52	4453/23	322010	67	P8 L8	Menopause	PMB	UPG	Absent	WD SCC	4.1	II	HPV 16/31(Mixed infection)	ND	ND
53	4621/23	327488	53	P2 L2	Menopause	PMB	UPG	PRESENT	WD SCC	6.6	IV A	HPV 16	ND	ND
54	4647/23	328783	58	P5 L5	Menopause	PMB	Cx Hypertrophied	Absent	WD SCC	3.2	II B	HPV 16	HPV 51/66 (Mixed Infection)	ND
55	4657/23	325319	60	P5L4D1	Menopause	PAIN ABDOMEN	UPG	PRESENT	WD SCC	3.5	III C1	HPV 16	ND	ND
56	4663/23	65951	55	P2L2	Menopause	PMB	Cx erosion	Absent	WD SCC	4.2	III B	ND	ND	ND
57	2136/24	411031	54	P3 L3	Menopause	PMB	UPG	Absent	WD SCC	4.2	III B	HPV 18	HPV 39	ND
58	3008/24	449828	48	P3 L3	IRREGULAR	WHITE DISCHARGE PV	Cx erosion	PRESENT	MD SCC	4.6	III C1	HPV 16	ND	ND
59	4113/24	486264	52	P4 L4	Menopause	PMB	UPG	Absent	WD SCC	6.5	III B	HPV 31	HPV 16	HPV 16
60	4470/24	496288	60	P4 L4	Menopause	PMB	UPG	Absent	WD SCC	3.6	II B	HPV 18	HPV 39	ND
61	4569/24	468610	56	P2 L2	Menopause	PMB	UPG	Absent	WD SCC	6.7	II B	ND	ND	ND
62	4643/24	501512	62	P4 L4	Menopause	PMB	Cx Hypertrophied	Absent	WD SCC	3.9	II B	ND	ND	ND
63	4642/24	268880	46	P2 L2	IRREGULAR	WHITE DISCHARGE PV	Cx Hypertrophied	Absent	MD SCC	3.1	I B	ND	ND	ND
64	4719/24	494887	52	P3 L3	Menopause	PMB	UPG	Absent	WD SCC	6.1	III B	HPV 16/31 (Mixed infection)	ND	ND
65	4994/24	509890	48	P3 L3	Regular	WHITE DISCHARGE PV	Cx erosion	Absent	WD SCC	4.8	II B	HPV 18	HPV 39	ND
66	5352/24	517141	66	P4 L4	Menopause	PMB	UPG	Absent	WD SCC	6.1	III B	HPV 16	ND	ND
67	3219/24	459016	61	P3 L3	Menopause	PMB	UPG	Absent	WD SCC	2.8	II B	ND	ND	ND
68	4157/24	288607	45	P3L2D1	Regular	PAIN ABDOMEN	Cx Hypertrophied	Absent	PD SCC	3.2	II B	ND	ND	ND
69	2436/24	425056	34	P3L3	Regular	PAIN ABDOMEN	Cx erosion	Absent	WD SCC	4	III C	HPV 31	HPV 16	HPV 16
70	2651/24	432208	61	P3 L3	Menopause	PMB	UPG	Absent	WD SCC	3.8	II B	ND	ND	ND
71	2652/24	434253	50	P3 L3	Regular	PAIN ABDOMEN	Cx Hypertrophied	Absent	WD SCC	3.2	II B	HPV 18	ND	HPV 39
72	2730/24	437595	74	P4L3D1	Menopause	PMB	UPG	Absent	WD SCC	6.1	III B	HPV 31	HPV 16	ND
73	4509/24	368752	54	P1L1	Menopause	PMB	UPG	Absent	MD SCC	6.2	IV B	HPV 16	ND	ND
74	2876/24	426412	56	P2L2	Menopause	PMB	UPG	Absent	WD SCC	3	I B	ND	ND	ND
75	4662/24	497319	44	P1 L1	Regular	WHITE DISCHARGE PV	Cx Hypertrophied	Absent	WD SCC	2.7	I B	HPV 16	ND	ND