Chapter 2
Review of Literature

2.1 Burden of cervical cancer

- Cervical cancer is a highly feared form of genital cancer that primarily starts in the uterine cervix. It is ironic that despite being one of the most frequently and easily screened organ systems of the female genital tract, it still accounts for a major chunk of cancers among women. This is mainly due to a lack of basic understanding of the importance of routine screening, which has been a significant contributor to the high incidence of cervical cancer among women in developing nations.

- Over the past five decades, there has been a definite change in the trend of cancer development among women in India. As a result, cervical cancer has now slipped down to the second position, with breast cancer being the only cancer more prevalent among women.

- Cervical cancer is a type of cancer that affects the cervix, which is the lower part of the uterus. It is the second most common gynecologic cancer in women, but it can be both curable and preventable when detected in its early stages. The Pap smear test is a crucial screening method for identifying cervical cancer at an early stage. However, analysing Pap smear images can be challenging due to the complex structure of the cells, as well as the presence of cell folding and overlapping. In order to overcome these challenges, it is necessary to separate individual cells from a cluster and remove the cytoplasm and nucleus for further processing. The classification of a smear image as normal or abnormal is achieved by identifying the parameters using a suitable classifier. Proper diagnosis and early detection of cervical cancer can save lives, so regular screening is highly recommended for women (Somasundaram, Gnanasaravanan, & Madian, 2020).

- Cervical cancer was the fourth most prevalent cancer in women and the seventh overall, with approximately 528,000 new cases recorded in 2012. Studies have shown that around 85% of the total cases worldwide occur in developing countries, where it accounts for 12% of all cancers diagnosed in females (GLOBOCAN 2012: Estimated Cancer Incidence, Mortality and Prevalence Worldwide in 2012, 2012).

- According to GLOBOCAN 2012, the regions with the highest estimated age-standardized rates (ASR) are Eastern Africa (42.7), Melanesia (33.3), Southern Africa

- (31.5), and Middle Africa (30.6). The lowest rates are found in Australia/New Zealand (5.5) and Western Asia (4.4). (Figure 2.1) (Abdikarim IK, 2017; GLOBOCAN 2012: Estimated Cancer Incidence, Mortality and Prevalence Worldwide in 2012, 2012).

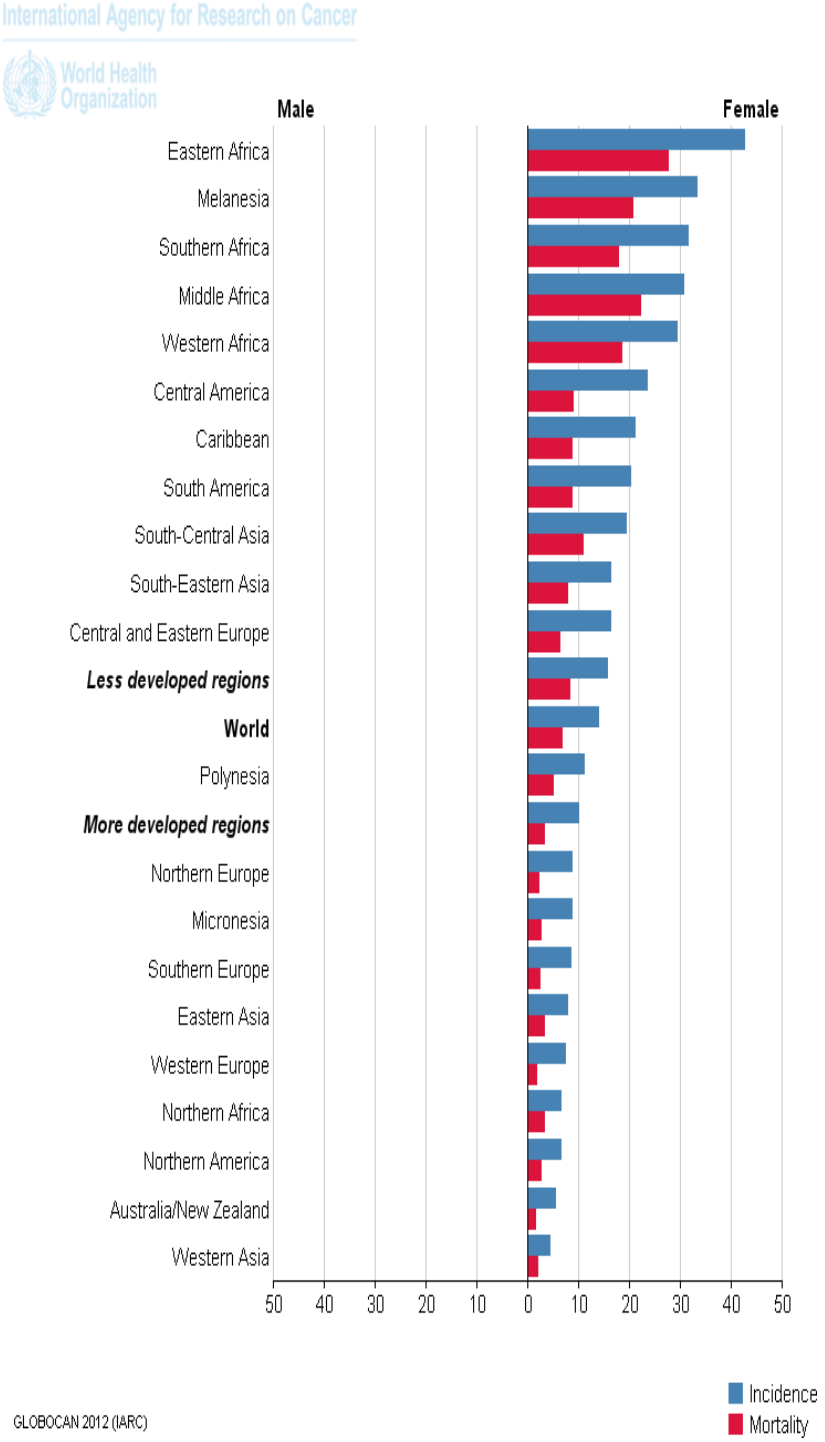

Figure 2.1: Estimated age-standardized rates per 100,000 (*GLOBOCAN 2012: Estimated Cancer Incidence, Mortality and Prevalence Worldwide in*)

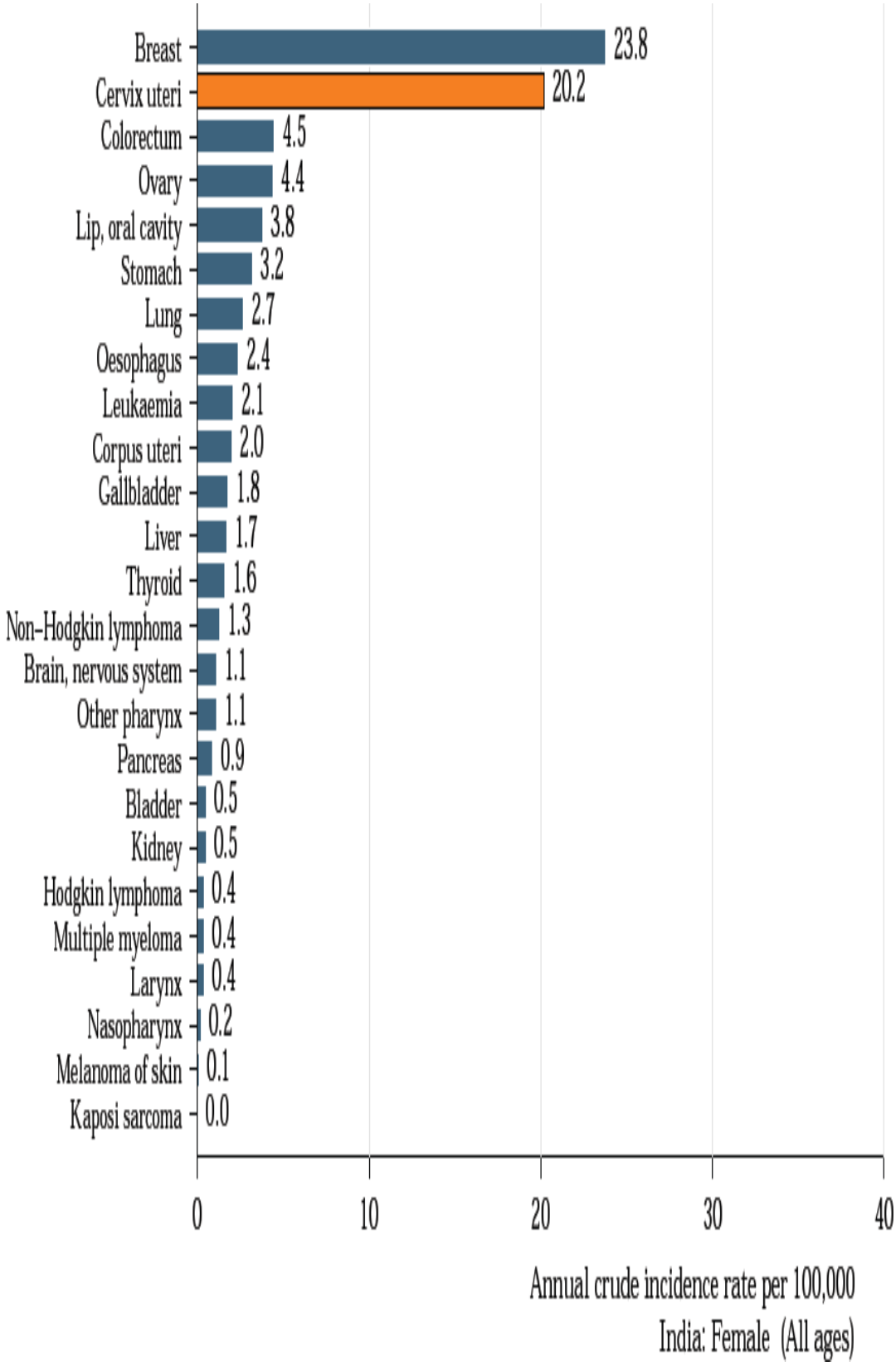

Figure 2.2: Cervical cancer in comparison to other types of cancer in women (About cervical cancer in India, 2020)

- Cervical cancer is the most common type of cancer among women in less developed regions of the world and often results in the death of young women. In 2012, an estimated 266,000 women died from cervical cancer, which accounted for 7.5% of all female cancer deaths. The majority of these deaths, about 87%, occurred in less developed regions (GLOBOCAN 2012: Estimated Cancer Incidence, Mortality and Prevalence Worldwide in 2012, 2012).
- In India, there are 432.20 million women aged 15 or older who are at risk of developing cervical cancer. Annually, approximately 1,22,844 women are diagnosed with cervical cancer, and 67,477 lose their lives to the disease. Cervical cancer is the second most common cancer among Indian women and also among women aged between 15 and 44 years. Around 7.9% of women in the general population are estimated to have cervical HPV infection at any given time, while 84.1% of invasive cervical cancers are caused by HPVs 16 or 18 (2022).
- Cervical cancer is the second most frequent cancer in women worldwide, with an estimated 570,000 new cases and 311,000 deaths from the disease occurred in 2018 (Prakash et al., 2016). Approximately 90% of death from cervical cancer occurred in low- and middle-income countries. The high mortality rate from cervical cancer globally could be reduced through a comprehensive approach that includes prevention, early diagnosis, effective screening and treatment program (M. Arbyn et al., 2020). In India the burden of cervical cancer is still high. According to the world cancer statistics,
- >80% of all the cervical cancer cases are found in low-resource countries, because of lack of awareness and difficulty in running cytology-based screening programs, more than one-fifth of all cancer deaths occur in India (Sachan, Singh, Patel, & Sachan, 2018).
- Every year, 123,907 women in India are diagnosed with cervical cancer, and 77,348 women die from the disease (Bruni L, 2023). Cervical cancer is a preventable disease due to the long preinvasive stage (Bal, Goyal, Suri, & Mohi, 2012). There is a need to spread cervical cancer screening awareness programs, and educate the women regarding the symptoms of cancer, name and detail of screening test and motivate them to visit the hospital for cancer screening so, due to widespread screening programs, has been significant in mortality from cervical cancer in developing countries.
- Cervical cancer is usually developed after prolonged phase of pre-invasive lesions in the

cervix (Sankaranarayanan, Wesley, & Cancer, 2003). Early cervical epithelial changes can be identified by a Pap smear test, which is the primary screening test for detection of precancerous cervical intraepithelial neoplasia and the early stage of invasive cervical cancer (Sachan et al., 2018). In Pap test a trained workers obtains cervical smear that is stained in a laboratory and then analyzed by trained cyto- pathologist. In India till date there are no suitable, large-scale, cost-effective population-based screening programs to detect pre-invasive stages of carcinoma cervix (Dipanwita Ghosh et al., 2019).

- During the last two decades, efforts have been made to identify high-risk cases of squamous intraepithelial lesions (SIL) through study of the study of the expression of different molecular marker in cervical carcinogenesis. The AgNOR pleomorphic counts has drawn considerable attention for its application in discriminating high-risk precancerous lesions of the cervix that most likely progress to a high grade and eventually to carcinoma of the cervix (Pinto, Crum, & Hirsch, 2010).

- If there is suspicion of cervical carcinoma due to abnormal Pap report or observation of exophytic growth, a confirmation of diagnosis is done by examining the punch biopsy specimen of uterine cervix tissue. After the diagnosis of invasive cervical carcinoma is confirmed, the next step is to stage the disease. Staging estimates the extent of the disease and is necessary before treatment of the lesion. The staging process involves physical examination, instrumentation, radiologic studies, and histopathological findings. The staging of cervical cancer is summed up in table 3 as per the criteria laid down by the Cancer Committee of the International Federation of Gynecology and Obstetrics (FIGO) (Pecorelli, 2009).

2.2 Pathogenesis of Cervical Cancer

- Understanding cell dynamics is crucial for comprehending how tumors behave. In order to predict the behavior of a particular tumor, one can measure its rate of proliferation. The cell cycle can be divided into four phases based on nuclear chromatin activity, namely S, G1, G2, and G0. In the S phase, DNA reduplication actively occurs in the cells, which is followed by a brief resting phase. The G2 phase is the second period of dormancy before active mitosis. Therefore, the amount of DNA present at the end of the S phase determines the proliferative activity, which can be detected by AgNOR (Oxford Textbook of Cancer Biology, 2019).

- Cells in the G0 phase are considered stable and inactive. However, they can be stimulated to enter the G1 phase of replication. During the replication process, in the late G1 phase, the tumour suppressor gene P53 plays a crucial role in preventing cells from continuing through the cell cycle. It's important to note that this effect is not present in mutant variants (Oxford Textbook of Cancer Biology, 2019).

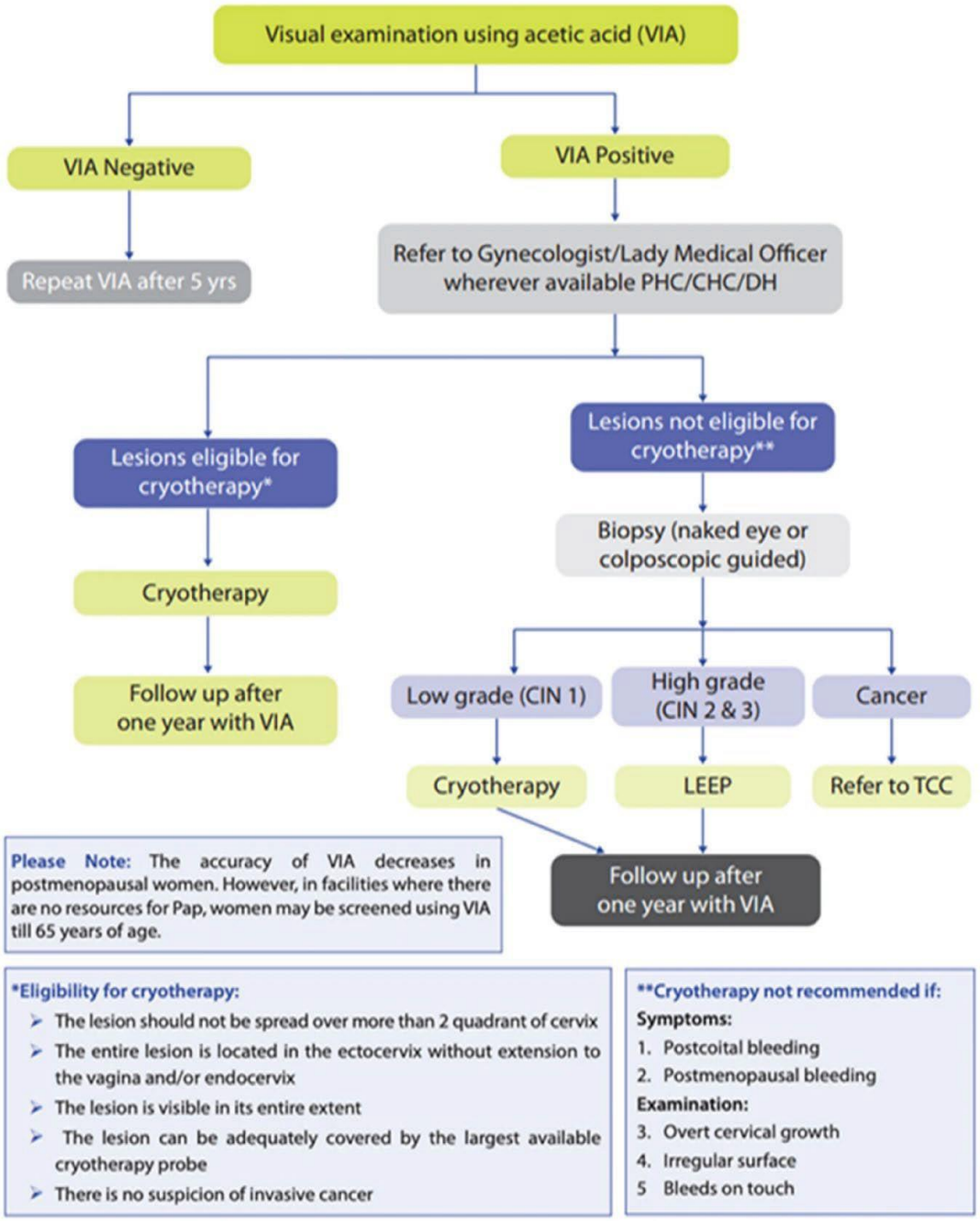

Figure 2.3: Screening and management Algorithm for Cervical cancer (Guideline from Government of India, Operational Framework for Management of Common Cancers, 2016)

- Staging requires certain physical examination, instrumentation, radiologic studies and histopathological findings. The staging of cervical cancer is summarized in table 2.1.

Primary Tumor (T)		
TNM categories	FIGO stages	Surgical Pathologic findings
TX		Primary tumor cannot be assessed.
T0		No evidence of primary tumor.
Tisb		Carcinoma *in situ* (preinvasive carcinoma).
T1	I	Cervical carcinoma confined to uterus (extension to corpus should be disregarded).
T1ac	IA	Invasive carcinoma diagnosed only by microscopy. Stromal invasion with a maximum depth of 5.0 mm measured from the base of the epithelium and a horizontal spread of ≤7.0 mm. Vascular space involvement, venous or lymphatic, does not affect classification.
T1a1	IA1	Measured stromal invasion ≤3.0 mm in depth and ≤7.0 mm in horizontal spread.
T1a2	IA2	Measured stromal invasion >3.0 mm and ≤5.0 mm with a horizontal spread of ≤7.0 mm.
T1b	IB	Clinically visible lesion confined to the cervix or microscopic lesion >T1a/IA2.
T1b1	IB1	Clinically visible lesion ≤4.0 cm in greatest dimension.
T1b2	IB2	Clinically visible lesion >4.0 cm in greatest dimension.
T2	II	Cervical carcinoma invades beyond uterus but not to pelvic wall or to lower third of vagina.
T2a	IIA	Tumor without parametrial invasion.
T2a1	IIA	Clinically visible lesion ≤4.0 cm in greatest dimension.

Primary Tumor (T)		
TNM categories	FIGO stages	Surgical Pathologic findings
T2a2	IIA2	Clinically visible lesion >4.0 cm in greatest dimension.
T2b	IIB	Tumor with parametrial invasion.
T3	III	Tumor extends to pelvic wall and/or involves lower third of vagina, and/or causes hydronephrosis or nonfunctioning kidney.
T3a	IIIA	Tumor involves lower third of vagina, no extension to pelvic wall.
T3b	IIIB	Tumor extends to pelvic wall and/or causes hydronephrosis or nonfunctioning kidney.
T4	IV	The carcinoma has extended beyond the true pelvis or has involved (biopsy proven) the mucosa of the bladder or rectum. A bullous edema, as such, does not permit a case to be allotted to stage IV.
T4a	IVA	Spread of the growth to adjacent organs (bladder, rectum, or both)
T4b	IVB	Spread to distant organs
Regional Lymph Nodes (N)		
NX	Regional lymph nodes cannot be assessed	
N0	No regional lymph node metastasis	
N1	Regional lymph node metastasis	
Distant metastases (M)		
M0	No distant metastases	
M1	Distant metastases (including peritoneal spread; involvement of supraclavicular, mediastinal and paraaortic lymph nodes; and lung, liver or bones)	

Table 2.1: TNM and FIGO Classification for Cervical Cancer (Pecorelli, 2009)

- Strips of DNA containing RNA genes can be seen inside the nucleolus while DNA

replication is actively occurring. The polymerase I enzyme assists in the transcription of these DNA fragments. In this context, ribosome factories are referred to. A small group of enzymes known as M phase stimulating factors regulate the cell cycle (Oxford Textbook of Cancer Biology, 2019).

- There are two subunit proteins in this factor. 1. The 34 Kd protein, which is the Cdc2 gene's byproduct. 2. A 45 Kd protein-containing component that builds up during the course of the cell cycle and is obliterated at the end of mitosis. "Cyclins" is the term given to these proteins. This protein is necessary to end mitosis in a cell cycle since the inactivation of these cyclins also results in the inactivation of factors that promote the M phase (Oxford Textbook of Cancer Biology, 2019).
- To carry out various cellular processes during both mitosis and interphase, a number of critical chemical reactions such as phosphorylation and dephosphorylation are needed. The P34 kinase, along with other cyclins, histones (for nuclear envelope breakdown), PP60, and nucleolin, has several well-known substrates. A significant argyrophilic protein, Nucleolin, plays a vital role in regulating the entry of cells into mitosis, along with other nuclear proteins. Therefore, Nucleolin is essential for DNA transcription (Fulcher & Sapkota, 2020). The protein plays a crucial role in triggering the cell's entry into mitosis. The 34cdc2 kinase system, responsible for regulating the cell's transition into pre-mitotic and mitotic phases, primarily utilizes this protein as its substrate. It also plays a significant role in regulating RNA splicing by influencing rDNA, as well as in the synthesis and assembly of ribosomes (Golub, Maryańska- Nadachowska, Anokhin, & Kuznetsova, 2023).
- The protein cdc2 phosphorylates nucleolin, which leads to significant changes in the shape of DNA and transcription. This process causes nucleolin to become argyrophilic. The specific location of the NOR is in the stalk region of the 18S rDNA gene cluster. However, there are some exceptions to this. The protein cdc2 phosphorylates nucleolin, which leads to significant changes in the shape of DNA and transcription. This process causes nucleolin to become argyrophilic. The specific location of the NOR is in the stalk region of the 18S rDNA gene cluster. However, there are some exceptions to this. ("Cell cycle regulators,").

2.3 Menopause and Cervical Cancer

- India accounts for about 20% of cervical cancer cases reported from the world. A local study found four types of cancer (breast, cervical, oral, and lung) account for more than 41% of the nation's cancer burden, with mouth and breast cancer being more common in men and breast and cervical cancer more common in women. When it comes to prevention, the first cancer that comes to mind is cervical cancer. This cancer is mostly diagnosed among middle-aged and menopause women (Forouzanfar et al., 2011). Despite this, there is no national programme in place that offers fundamental preventive measures like screening services (Dehghan, Isari, Abbaszadeh, & Ghonchehpour, 2022).

- Ignorance of pre- and postmenopausal symptoms can result in a significant risk factor for cancer. Due to ignorance and lack of awareness, women can misjudge the two symptoms concurrently or separately. Throughout their lifespan, women experience a variety of physiological changes, including menopause (Changkun, Bishwajit, Ji, & Tang, 2022).

- Women who are close to or have reached menopause may worry that the symptoms they are feeling are caused by menopause or another illness, such cancer. It's critical to understand that some menopause symptoms can resembles, which are cancers that originate in the female reproductive system out of which uterine cancer, ovarian cancer, and cervical cancer are the three most prevalent cancers (Surakasula, Nagarjunapu, & Raghavaiah, 2014).

- Early cervical cancer frequently has no symptoms, which emphasizes the importance of screening for cervical cancer with regular Papanicolaou (Pap) tests. It is crucial to be aware of the similarities and differences in symptoms so that worrying, or "red flag," symptoms of a potential underlying cancer are not disregarded or mistakenly identified as symptoms of menopause. Early cervical cancer most often has no symptoms. The most typical sign of cervical cancer is irregular or excessive vaginal bleeding (Cohen, Jhingran, Oaknin, & Denny, 2019).

- The signs and symptoms of cervical cancer frequently resemble those of other diseases. Abnormal vaginal bleeding, such as after sex, in between periods, or after menopause, is one of the potential warning indicators. Other red flags include irregular vaginal discharge, prolonged menstrual bleeding, and pain during sexual activity. There is a

reliable test for detecting cervical cancer and precancerous abnormalities in the cervix, which are frequently picked up early in a Pap test. This is why a post-menopausal woman should continue to have cervical cancer screenings at the intervals suggested by her doctor along with visual inspection (per speculum and per vaginal examination) (Peirson, Fitzpatrick-Lewis, Ciliska, & Warren, 2013). Many findings suggest the requirement to screen asymptomatic women for cervical cytology, and physical examination as many precursor lesions of cervical cancer may remain hidden for long time (Juhi D Mahadik, 2017).

2.4 Argyrophilic Nucleolar Organiser Region (AgNOR)

- AgNOR are DNA loops that polymerase-I uses to encode for the creation of ribosomal RNA. Since NOR are argyrophilic, they can be found using the silver impregnation method. The quantity of AgNORs in a cell nucleus is a good indicator of its proliferative and kinetic behaviour. Individual AgNORs may disperse when nucleolar disaggregation occurs in rapidly proliferating cells, such as malignant cells (Trerè, 2000).

- Here's an explanation of why there might be an increase in the number of AgNORs counted in a particular area: 1. There could be an increase in the number of AgNOR-bearing chromosomes in the karyotype. 2. There could be an increase in transcriptional activity, resulting in more argyrophilic cells becoming visible. 3. There could be an increase in the rate at which cells proliferate, leading to a significant population displaying positive. 4. Crocker et al. (1989) revealed that tumors with increased AgNOR counts in interphase nuclei are more likely caused by cellular proliferation than by N ploidy because diploid cells transiently become tetraploid in G2 phase, which leads to a temporary doubling of NOR-bearing acrocentric chromosomes. The NORs disperse and then re-aggregate just before and after mitotic division, increasing the countable AgNORs in the nucleus (Shenk & Ganem, 2016).

- NOR dots consist of discrete, spherical-shaped granules, each roughly 15 nm in diameter. The dense fibrillar component is composed of fibrils that are 3-5 nm thick and tightly packed with electrons. The fibrillar center, on the other hand, is a loose network of fibrils that have an average diameter slightly larger than the dense fibrillar component, measuring 4-8 nm. Crocker et al. (1989) developed an important and widely-used one-step colloidal silver staining process that is simple to use. The AgNOR interphase visible by light microscopy is equivalent to the fibrillar center seen in electron microscopy (Guo

& Eisenberg, 2008).

- Dr. Derenzini D'Trere discovered in 1993 that a high number of metaphasic AgNORs in hepatocytes of individuals with chronic liver disease is linked to a higher risk of hepatocellular carcinoma (Srigley & Delahunt, 2009). In 1993, Yamanoto N. conducted a study on the role of NOR proteins as markers for proliferative activity in renal cell cancer. The study found that the levels of argyrophilic nucleolar protein in a cell indicate its level of activity and rDNA transcriptional activity. As the grade of cancer advanced, the AgNOR levels in renal cell carcinomas became higher than those in normal tubules. The study also showed that infiltrating types like the sarcomatoid type have greater NOR rates. Patients with renal cell carcinoma who had counts of 4.5 or higher displayed a significantly higher (Ghazi, Arumugam, Foolchand, & Chuturgoon, 2020).
- Isaka et al. (1993) conducted a study on 96 patients with renal cell carcinomas and observed that AgNOR counts increase with upgrading. They also found that the number of AgNORs in pleomorphic, spindle cell, and Bellini duct carcinoma were higher than in common forms of cell types (Abe, Masai, Isaka, Shimazaki, & Matsuzaki, 1993). The study conducted by Shimazui T et al. in 1995 examined the prognostic significance of nucleolar organizing regions in renal cell carcinomas (Delahunt, Ribas, Nacey, & Bethwaite, 1991). In 1996, Tannafel A and colleagues investigated the usefulness of ploidy and proliferation markers in predicting renal cell carcinoma. Their findings suggest that cellular proliferation could serve as an additional indicator of biological aggression. They observed a statistical correlation between AgNOR dots and tumor grade (Tannapfel et al., 1996).
- Tomobe N et al (1997) identified AgNOR as a novel prognostic biomarker in renal cell carcinoma patients (Rabjerg, 2017). Delahuni et al. (1988) evaluated prognostic indicators for renal cell carcinoma and found only nuclear grade related to outcome (Delahunt, 1998). Consistent links to survival have been found with organising areas (PCNA and Ki-67 antigen). Statistical relevance of NOR in breast cancer was established in 1988 by Smith R. and Crocker J (Haerslev, Jacobsen, & Zedeler, 1996). In 1989, Giri et al. established a link between AgNOR and both benign and malignant breast tumors (Jakić-Razumović, Petrovecki, & Dominis, 1995).
- In 1988, J. Crocker and Paramjithnar from the Department of Histopathology at the East

Birmingham Hospital conducted a study on AgNORs in 90 paraffin sections of non-Hodgkin lymphomas. The study found a significant difference between the average number of AgNORs per nucleus in low grade lymphomas (between 1 and 1.5) and high grade lymphomas (between 4.4-6.8 per nucleus) (Crocker, Boldy, & Egan, 1989). In 1988, John Crocker, David A.R. Boldy, Mark, and others developed a standardized method for counting AgNOR. Initially, all the Silver-stained structures were countable. However, when they were formed into clusters, each structure with almost aggregated or partially disaggregated nucleoli was treated as a separate entity. Additionally, each AgNOR counted inside a nucleolus was tallied as one unit along with the smaller AgNOR visible outside the nucleus. Overall, the recommendation was to count all AgNOR dots, whether intra or extra nucleolar (Reble et al., 2014).

- In 1995, a study was conducted by D. Prathiba, Sarah Kuruvilla, and their colleagues at Sri Ramachandra Medical College and Research Centre in Chennai. The study examined the use of AgNORs in premalignant and malignant cervix lesions. The results showed that the average AgNOR count increased gradually from normal to CIN I, II, III, and invasive cancer (Prathiba & Kuruvilla, 1995). The NOR and proliferative index were studied in 1989 by Newbotd M, Rollason P, and Ward K. They found that glandular and squamous carcinomas of the cervix display a lack of polarity and have significant prognostic value (Newbold, Rollason, Luesley, & Ward, 1989).

- During a study in 1993, Lakshmi S. et al. discovered that the number of AgNOR particles increased with the severity of dysplasia in cervical lesions (Darkwah et al., 2021). The study by Agarwal J and Gupta JK in 1997 compared NORs in neoplastic and non-neoplastic cervix epithelium (Omoniyi-Esan, Osasan, & Ojo, 2006). In 1997, researchers March Calore EE and Made My. Cavaliere from Brazil found that AgNOR counting can aid in identifying and distinguishing individual cases of intraepithelial neoplasia from challenging cases of cervicitis (Uma et al., 2006). In 1997, Jyotima Agarwal, J.K. Gupta, and their colleagues from the Department of Pathology at Kamala Nehru Memorial Hospital in Allahabad discovered that the average AgNOR count is higher in cases of CIN, malignant metaplasia, chronic cervicitis, and adeno carcinomas, compared to squamous and adeno squamous carcinoma (Gangopadhyay, Chakrabarti, Ghosh, & Giri, 2011).

- Terlikowski et al. (1998) proposed that AgNOR counts might be useful in assessing cervical lesions and clarifying histological diagnosis by comparing AgNOR counts of CIN II and SIL in May 1998 (Goyal, Mohi, & Bal Singh, 2012). In 2001, a study was conducted by J.S. Misra Vinita Das, Madhulika Singh, and colleagues at the Department of O & G, King George's Medical University in Lucknow, India, to determine the effectiveness of AgNOR count as a tumor marker in cervical carcinogenesis. The study showed that AgNOR was significant in distinguishing between different types of cervical lesions, and subsequent studies linked high AgNOR levels to the development of dysplastic lesions (Mohanty, Nayak, & Padhy, 2020). In 1999, Kurian, Al Nafussi, and colleagues investigated the relationship between invasive and microinvasive cervix adenocarcinoma and cervical glandular intra epithelial neoplasia (Kurian & al-Nafussi, 1999).

- The AgNOR staining approach is time and cost efficient, and can be applied to both old and new tumor material without requiring complicated equipment or specialized fixatives. Here are the main drawbacks of the current counting processes: 1. They are typically done manually, which makes them time-consuming and laborious. 2. The main reason for inconsistent and inaccurate results is due to observer error. 3. The dots of AgNOR interphase nuclei do not always match the number of such types in the karyotype, as discovered by Underwood and Giri in 1998. 4. Overlap and coalescence may lead to inaccurate counts, as noted by Crocker et al. 5. This issue can be observed in all cultures and smear squash cytologies (Ahmed, Al-Adhraei, & Ashankyty, 2011).

- The survival curve and NOR indices showed good correlation. Patients in the low NOR index group had a 100% survival rate, whereas death was noticeably greater in those with higher NOR index. Within each cancer grade, patients with low NOR indices have a better prognosis than those with high NOR indices.

2.5 Molecular markers Ki67/p53

- Cervical carcinoma is a common cancer in women worldwide, with 528,000 new cases in 2012. 266,000 deaths from cervical cancer were documented, making up 7.5% of all female cancer-related deaths. In India, 123,000 new cases and 67,000 deaths were reported due to cervical cancer in 2012. Human papillomavirus is a major risk factor for this cancer (Kalyani, Das, Bindra Singh, & Kumar, 2010; Pimple & Mishra, 2022).

- The high-risk human papilloma virus (HPV type 16 and 18) and cervical cancer are strongly linked in up to 95% of cases, according to numerous epidemiological studies undertaken over the past 20 years. Retinoblastoma protein (Rb) and p53 transcription factor are two tumor suppressor genes that are crucial regulators that are disturbed in cervical cancer cells. The genesis of cervical cancer is influenced by the interconnected signaling pathways governed by Rb and p53 (Yuan et al., 2020).
- Ki-67 protein greatly influences cell proliferation and is used as a marker for malignancies, including cervical cancer which is most commonly caused by HPV. The pathogenesis of cervical neoplasia involves a multistep process, with high-risk type viruses (16, 18) and other carcinogens playing a role (Gupta & Mania-Pramanik, 2019; Sun & Kaufman, 2018; X. Zhang, Zeng, Cai, & Ruan, 2021).
- After a protracted period of latent growth, low and/or high-grade cervical intraepithelial neoplasia (CIN) may regress or develop into an invasive cervical cancer. The HPV genome encodes proteins with the capacity to trigger apoptosis prevention and unregulated proliferation (Klaes et al., 1999).
- The two early HPV genes, E6 and E7, are essential for the development of tumors. The crucial stage in cervical carcinogenesis is the incorporation of viral DNA into host DNA. Viral oncoproteins E6 and E7 exhibit unregulated production when the host tumor suppressor genes (p53 and pRb) are inactivated. As a result, the usual maturation sequence will be lost, resulting in a proliferative, persistent HPV infection. According to Kalof AN (2007) and Magaldi TG (2012), this will ultimately develop into high grade dysplasia and advance to aggressive cancer (Nicole S. L. Yeo-Teh, Yoshiaki Ito, & Sudhakar Jha, 2018).
- Cell proliferation is either a direct or indirect foundation for the multi-step process of carcinogenesis. The well-known cell proliferation marker Ki-67 is a nuclear proliferation related antigen (Kim SM, 2001). In the predictive assessment of cervical cancer, cell proliferation has been described as a further measure (Nicole S. L. Yeo- Teh et al., 2018).
- The goal of the current study was to understand how these cellular proteins and biomarkers relate to different types of dysplasia and cancer. Premalignant and malignant cervical lesions were more frequently detected in people aged 40 to 49 in the current investigation. According to Misra JS (2009), the most incidence of cases is recorded in

older women over the age of 40 (SIL-10.71%, carcinoma cervix-1.3%) (Rani, Narasimha, Kumar Ml, & Sr, 2014).

- According to one study's findings, women between the ages of 35 and 50 were the most frequently affected by cervix cancer (Aswathy S, 2012). Squamous cell carcinomas make up about 90% of cervical malignancies, with adenocarcinomas making up the remaining 10%. According to several research (Das BC, 2000; Misra JS, 2009), squamous cell carcinoma accounts for about 85–90% of cases and adenocarcinoma accounts for the remaining instances (Shruthi, Kalyani, Lee, & Narayanaswamy, 2014).
- In a different study, the majority of cervical carcinomas had squamous cell carcinoma as their primary histological subtype, followed by adenocarcinoma (Kalyani, Das, Bindra, & Kumar, 2010).
- The tumour suppressor gene P53 is crucial in the defense against the emergence of cancer. The genome is monitored by wild type p53. DNA Protein Kinase is required for the activation of p53 in response to DNA damage (Levine et al., 1994).
- One of the HPV E6 gene's most crucial targets is the p53 gene. There are two significant methods through which p53 contributes to cervical cancer. The first occurs when E6 interacts with E6 associated protein (E6AP), a protein ligase that binds ubiquitine. When the E6/E6AP complex attaches to the center of p53, it is quickly ubiquitinated and sent to the proteosomes. E6 protein can therefore promote the degradation of p53.
- As a result, the p53 level in cervical carcinoma cells is low. In summary, inactivation of p53 is a critical stage in the development of cervical cancer (S. Li et al., 2019).
- Second, carcinomas are only infrequently linked to p53 mutations. However, mutations can be detected in cervical tumors that are HPV positive or negative, showing that there is no relationship between HPV and p53 status. According to Tommasino M. (2003), mutated p53 implies that p53 can be functionally inactivated in cervical cancer cells either through interaction with E6 or by gene mutation (Levine et al., 1994).
- In SIL, p53 immunoreactivity first appears. In a few cells in the basal layer of LSIL, the expression of p53 first became visible. It then increased in tandem with the growth of neoplastic cells in HSIL and carcinoma in situ, and it was more widespread in invasive lesions (Jeffers, Richmond, Farquharson, & McNicol, 1994).

- Although the prevalence of p53 positive in cervical cancer varies across research, they all show that invasive SCC frequently exhibits p53 immunoreactivity. However, according to a different study, there are no statistically significant changes in the expression of p53 between normal squamous mucosa, squamous metaplasia, SCC, and CIN (Nakamura, Obata, Daikoku, & Fujiwara, 2019).
- On this subject, various conclusions have been published. In the absence of mutations, overexpression or stability of the wild type, as well as the heating impact of the microwave oven, can all be used to explain positive p53 immunoreactivity found in normal cervical epithelium (Tan GC, 2007). This may have contributed to the overexpression of p53 in our study's normal instances as well. While other studies found no significant correlation between p53 expression and HSIL when compared to normal, some did (Lawal, 2017).
- The potential causes of excessive p53 expression in SCC include mutations in the p53 gene, abnormal accumulation of nonmutant p53 protein caused by abnormal p53 homeostasis in tumor cells, conformational changes in the wild type p53 protein that cause it to switch from suppressor to promoter or mutant p53, and/or detection of normal p53 that has been abnormally stabilized or increased in quantity (Lingen et al., 2000).
- In intraepithelial lesions, the expression of p53 extends beyond basal cells, indicating lack of normal proliferative cell control and loss of epithelial maturation, particularly in HSIL (Rizzolio et al., 2012).
- Adenocarcinoma shows higher levels of p53 expression compared to squamous cell carcinoma and adenosquamous carcinoma. However, this conclusion is based on a small sample size and has limited practical significance. Malignant cases showed grade II to III staining intensity while normal and premalignant cases showed grade I intensity. A majority of CIN cases were grade I intensity negative, while 65.2% of malignant cases were grade III positive (Sankalecha, Gupta, Gaikwad, Shirole, & Kothari, 2017).
- Retinoblastoma protein (pRb), a nuclear phosphoprotein that is produced as a result of the Rbgene, is crucial for controlling the cell cycle. Therefore, a loss of normal Rb function may permit unchecked cell proliferation, which could lead to both the initiation of tumourigenesis events and the progression of malignancy (Rizzolio et al., 2012).

- Rb function can become abnormal due to Rb gene mutation or HPV-E7 binding to Rb-E2F, which removes cell cycle restriction. The tumor suppressor protein pRb interacts with HPV-16 E7 and is functionally inactivated as a result. Retinoblastoma protein instability is another effect of E7. As a result, active E2F transcription factors are released, which stimulates S-phase cell cycle entry. In the cervical cancer cell line, E7 protein is primarily located in the nucleus and to a lesser level in the cytoplasm. Ca Ski in situ and in vitro, indicating that nuclear resident E7 likely plays a significant role in the development of cervical cancer in humans (Mir et al., 2023).
- Rb mRNA and its protein product are typically absent as a result of Rb mutations. A powerful indicator of underlying Rb mutations has been the complete absence of nuclear reactivity in every part of the tumor in conjunction with a positive internal control (Noraini, Siti-Aishah, & Kwan, 2003).
- Premalignant lesions showed higher pRb expressions when compared to normal cervical epithelium (Y. T. Kim & Zhao, 2005). Rb gene down regulation may be linked in cervical carcinogenesis as pRb staining was less intense in invasive carcinoma than SIL (Salcedo et al., 2002).
- In our investigation, 90% of the basal layer of normal cervical epithelium had pRb(n) antibody, with 80% of these cells scoring +1. 95% of LSIL cases with premalignant lesions had positive immunoreactivity in the basal and parabasal layer, with the majority having a +2 score. With the majority having a +1 or +3 score, 83.5% of HSIL patients demonstrated positive in the basal and parabasal layer/full thickness. We also like to draw attention to the fact that cytoplasmic stain might be seen in instances of HSIL (27.8% of them) (Gayathri, Kalyani, Hemalatha, & Vasavi, 2012).
- The spread of gene products from the nucleus to the cytoplasm, which will absorb the brown stain, is a potential additional factor. According to studies, E7 protein was primarily present in the nucleus and only little in the cytoplasm of cervical cancer. The HPV E7 oncoprotein interacts to Rb to create a complex that may be present in the cytoplasm and picks up the stain. To support these findings, additional research has to be conducted. According to cytoplasmic immunoreactivity in mitotic cells, pRb diffuses to other cellular sites when the nuclear membrane is absent (Valdovinos-Torres et al., 2008).

- In both premalignant and malignant lesions, the staining intensity of pRb(n) varies from grade 1 to 2, whereas that of pRb(c) varies from grade 0 to 1. This variance in staining was most likely caused by the cells' asynchronous progression through the cell cycle. The expression of Ki67, a cell cycle-associated protein found in the peri- chromosomal area and used to assess the growth fraction of cells in human malignancies, is correlated with cell proliferation. During the G1, S, G2, and mitotic phases of the cell cycle, the non-histone heterodimer Ki67 nuclear antigen is found in the nuclei of the cells (Ghosh, Roy, Murmu, Mandal, & Roy, 2019).
- Although it cannot be used to forecast how they will behave, Ki-67 expression is helpful in differentiating the various degrees of dysplasia (Valdovinos-Torres et al., 2008). Invasive carcinoma (SCC & AC) was shown in one study to have a +4 score, indicating that Ki-67 expression tends to positively correlate with the histologic grade (R, Arunachalam, Amitkumar, John, & Sudalaimuthu, 2023).
- In CIN samples, Ki-67 expression is higher than in normal or metaplastic epithelium, and high-grade CIN exhibits stronger Ki-67 staining than low-grade CIN. In order to distinguish and grade CIN, Ki-67 is typically employed (Hosseini et al., 2023). In one study, Ki-67 expression was found to be higher in SCC and CIN I/II/III but to be lower in squamous metaplasia and normal squamous mucosa (Birajdar et al., 2014).
- Additionally, we discovered that all adenocarcinoma patients displayed +4 Ki-67 positivity. Normal cervical epithelium and cancer patients also showed a significant correlation (p0.001). Commenting on the results has less relevance because there were so few cases. However, there are a number of opposing views, and one study claims that it was unable to find a meaningful relationship between the mitotic cycle regulators p53, pRb, and Ki-67 with the progression of LSIL (Sarma, Das, & Sarmah, 2021).
- This study graded staining in different cervical conditions. Light staining was seen in normal cervical epithelium, moderate staining in premalignant lesions, and grades II to III in invasive carcinomas. No correlation was found between clinical stages and the indicators. A 2008 study by Khunamornpong S found no correlation between p53 expression and tumor characteristics in early-stage cervical carcinoma (Benevolo et al., 2006).
- The eight kilobase (kb) HPV genome is a double-stranded DNA molecule. All

papillomavirus varieties have the same relative arrangement of the eight to ten open reading frames (ORFs) within their genomes. One unique feature of papilloma viruses is that they're partially overlapping ORFs are placed on a single DNA strand. According to Walter and Philip (2004), the genome can be split into three regions: the early protein area (E1–E8), the late protein region (L1 and L2), and the long control region (LCR) lacking coding potential. The two most significant oncogenic proteins are E6 and E7. These proteins perform a variety of pleiotropic tasks, including transmembrane signaling, cell cycle regulation, transformed established cell lines, primary cell line immortalization, and chromosomal regulation (Dyson, Howley, Münger, & Harlow, 1989).

- According to Dyson et al. (1989) and Davies (1993), the interactions that are believed to be most significant for their transforming functions are E6 binding to the tumor suppressor gene product p53 through the cellular protein E6-AP and E7 binding to the retinoblastoma tumor suppressor gene product pRb and its related pocket proteins, p107 and p130. The initial contact stops cells from going through p53-mediated apoptosis by causing p53 to be rapidly degraded by ubiquitin-dependent proteases (Thomas, 1999). Interfering with cell cycle regulation is one of the effects of the E7-pRb interaction. The E6-p53 and E7-pRb interactions appear to work together to reduce the precision of mitosis. Furthermore, HPV E6 and E7 can cause aberrant centrosome duplication through mechanisms unrelated to p53 binding and telomerase activation, respectively (Dyson et al., 1989).

2.5.1 p53 – tumor suppressor protein

- The E6 oncoproteins of high-risk HPV interfere with the function of the cellular tumor suppressor protein p53 through the induction of increased proteasome-dependent p53 degradation. High risk HPV E6 proteins target the cellular E3 ubiquitin ligase E6-AP to p53, resulting in the transfer of ubiquitin peptides from E6-AP to p53, which marks p53 for degradation by the 26S proteasome (Yim & Park, 2007).

- Low risk and cutaneous epithelia-infecting HPV E6 proteins are unable to target the cellular p53 protein for degradation through the proteasome pathway. Although E6-induced loss of p53 is an important element of E6-induced cellular transformation, recent studies have. identified a number of additional cellular targets of E6 that may also play an important role. These included the following: proteins involved in apoptosis and

immune evasion, such as Bak, Bax, TNF receptor 1 (TNF R1), FADD and c-Myc; proteins involved with epithelial organization and differentiation, such as paxillin, E6BP/ERC-55, zyxin and fibulin-1; Proteins with a PDZ-binding motif that are involved in cell-cell adhesion, polarity, and proliferation control include hDLG, hScrib, PKN, MAGI-1, MAGI-2, MAGI-3, or MUPP1; additionally, proteins involved in DNA repair include XRCC1 and 6-O-methylguanine-DNA methyltransferase (MGMT). Figure shows 2.4 p53 and cancer progression (Yim & Park, 2007).

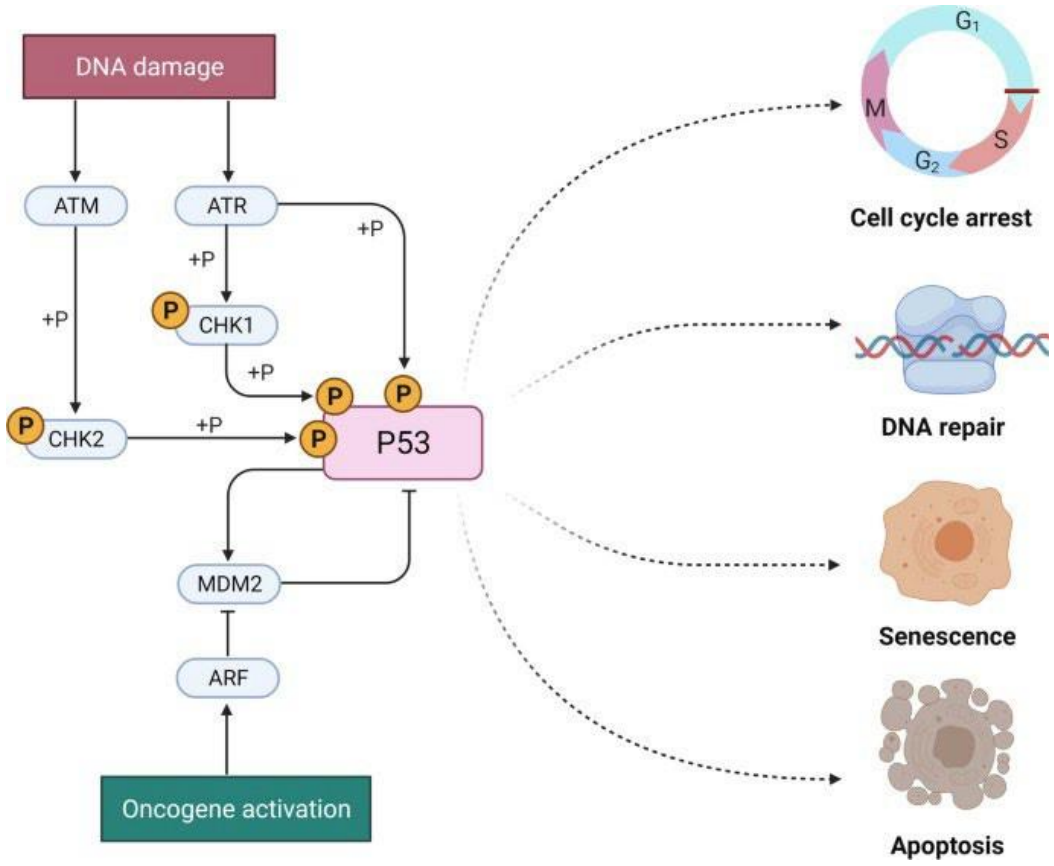

Figure 2.4: p53 and cancer progression (Marei et al., 2021)

2.5.2 Ki67 – cell proliferation marker

- The rate of cell proliferation in a tumor is usually considered to be important for prognosis; but, until recently, the pathologist's sole way to evaluate this was to count the number of mitotic figures, which is a challenging and potentially dangerous approach. Several antigens that are expressed exclusively by proliferating cells have now been identified; these can be shown immunocytochemically using monoclonal antibodies. Theoretically speaking, identifying these antigens allows for a far more precise

estimation of the number of proliferating cells than a mitotic count. Proliferating cell nuclear antigen (PCNA), which is expressed during the G1 and early S phases of the proliferative cycle, and Ki-67, which is expressed during the G2 and mitotic stages of the cycle, are the two proliferation antigens that have been investigated the most. Because PCNA has a lengthy half-life and may still be detectable in post-mitotic cells, Ki-67 is a more accurate measure of a tumor's growth fraction. However, the requirement to use fresh or snap frozen tissue initially restricted the research of Ki-67. However, the recently developed antibody MIB-1 can be used to detect the antigen in fixed paraffin-embedded tissue. One can measure or estimate the amount of cell nuclei staining positively for these proliferation markers using an image analysis system or by simply counting them (Yim & Park, 2007).

- Both PCNA and Ki-67 expressions are elevated in the upper levels of the cervical epithelium in cervical intraepithelial neoplasia compared to normal cervices. It is believed that this staining pattern, especially that for Ki-67, may be of considerable value in differentiating between CIN and non-neoplastic lesions that may mimic CIN. Contradictory findings have come from two investigations on PCNA expression in cervical carcinoma: one and another failing to demonstrate the predictive significance of this indicator (Yim & Park, 2007).

- Research on the correlation between the quantity of positively stained cells and prognosis in cervical carcinoma has mostly been unsuccessful. However, in one study, the Ki-67 index was found to be significantly correlated with tumor size, lymphatic spread, and disease-free interval in patients with stage I disease. According to research by Garzetti et al. (1996), the PCNA index in endometrial adenocarcinomas has been found to correlate with tumor grade, depth of myometrial invasion, and recurrence risk. PCNA staining has also been proposed as a means of pre-operatively identifying high- risk patients. In one study, Ki-67 expression in endometrial carcinomas was found to be connected with grade but not with stage or depth of myometrial invasion; however, in another, it became a highly significant indication of tumor recurrence. On the other hand, several researchers have discovered that endometrial neoplasm staining for PCNA or Ki-67 has little predictive significance (Yim & Park, 2007).

- Ki-67 localisation is cell cycle regulated. In interphase, it is localized to perinucleolar

and pericentromeric heterochromatin (A, red), and regulates chromatin compaction. In mitosis, Ki-67 relocates to the surface of chromosomes and is responsible for formation of the perichromosomal layer (B, red). Through extensive protein-protein interactions (C) and, possibly, an involvement in phase separation of subnuclear compartments (D), Ki-67 organizes heterochromatin and regulates transcriptional programmes (E). This allows cellular plasticity (F) and, in cancer cells, is required to maintain a hybrid EMT-state phenotype (G). As a result, Ki-67 is important for cell transformation, tumour development (H) and metastasis (I), as well as anti-tumour immune responses (J). P, phosphorylation (Andrés Sánchez et al., 2022).

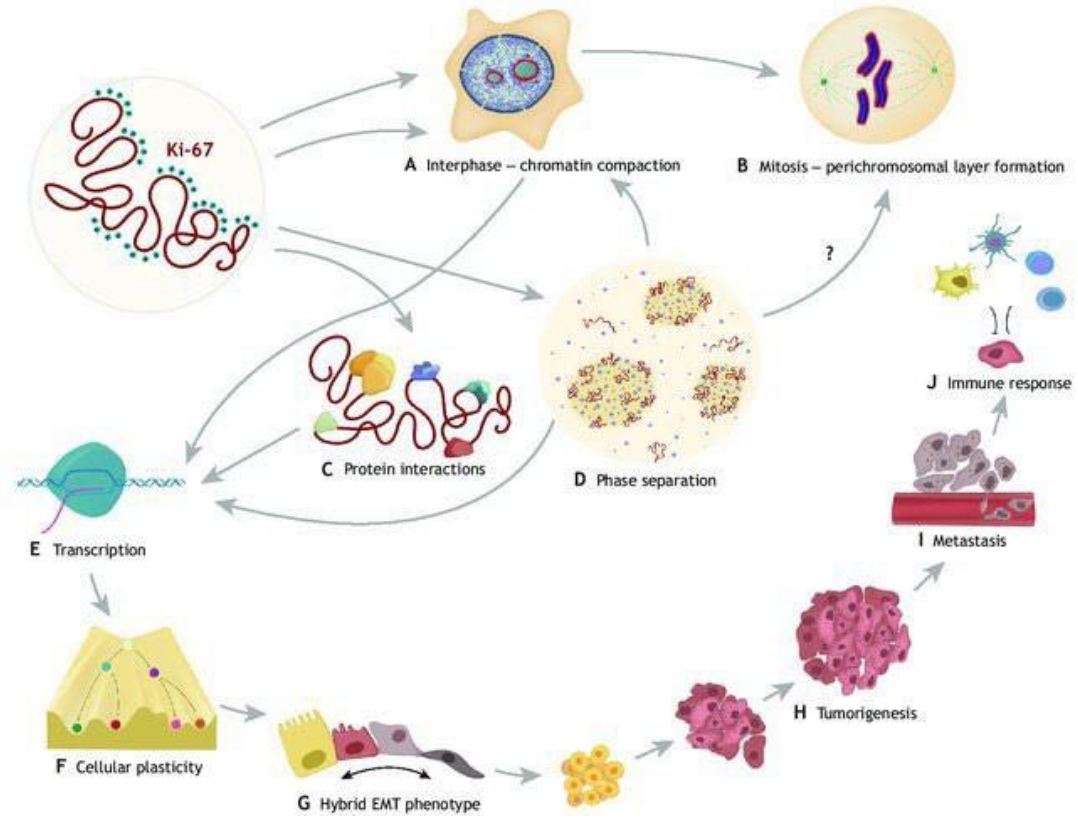

Figure 2.5: Overview of Ki-67 localisation and functions (Andrés Sánchez, Fisher, & Krasinska, 2022)

2.6 Human Papilloma Virus

- Based on statistics from the World Health Organization (WHO), common cancers remain one of the leading causes of mortality worldwide, resulting in 8.2 million deaths in 2012. Unfortunately, this trend has not changed in recent years. It has been found that viral infections contribute to 15-20% of all human cancers, with several viruses playing significant roles in the development of malignant cancers. For the past two decades, it

has become increasingly apparent that some viruses are crucial in the development of human cancers. Around 15% to 20% of cancer cases are associated with viral infections. Oncogenic viruses can facilitate various stages of carcinogenesis (McLaughlin-Drubin & Munger, 2008).

- The human papillomavirus (HPV), a virus that can be transmitted through sexual contact, has been found to contribute to cancer cases, including cervical cancer. In fact, high-risk HPV DNA has been detected in 99.7% of cervical cancer samples, indicating a strong connection between the virus and the development of this type of cancer (Walboomers et al., 1999).
- Within 12 to 24 months of exposure to the virus, 90% of HPV infections are cleared or become inactive. However, infections by the high-risk HPV types persist which then increase the risk of progression to cervical cancer (Chan, Aimagambetova, Ukybassova, Kongrtay, & Azizan, 2019).
- Human papillomavirus (HPV) is the most common viral infection of the reproductive tract. Cervical cancer is caused by sexually acquired infection with certain types of HPV, there are more than 100 types of HPV, of which forty types are known to infect the genital tract and spread through sexual contact. Among these, persistent infection/integration of any of the 15 genotypes, namely, HPV-16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, and 82 are implicated in causation of Carcinoma Cervix and are designated as high-risk (HR) HPVs. HPV-16 and 18 together are responsible for about 70% cases of Carcinoma Cervix occurring in every region of the world. (Global strategy to accelerate the elimination of cervical cancer as a public health problem; Prakash et al., 2016).

2.6.1 Taxonomy of Papillomaviridae

- The comparisons of virus sequences have established the basis for classifying papillomaviruses (PVs) into different groups, which are now officially recognized by the International Committee on Taxonomy of Viruses (ICTV). The classification of PVs is based on traditional criteria, specifically, the degree of variation in their L1 genes. PVs are categorized into different genera, represented by Greek letters, based on their position in the phylogenetic tree. Members of the same genus have at least 60% similarity in their nucleic acid sequence (Suntsova & Buzdin, 2020).

- Previously, PVs were classified as members of the Papovaviridae family along with polyomaviruses and simian vacuolating viruses. However, all PVs are now categorized in the Papillomaviridae family and are distinct from Polyomaviridae, eliminating the term Papovaviridae (Martini et al., 2007).

- PVs are highly host and tissue specific, and are rarely transmitted between species (Mistry, Qureshi, Talole, & Deshmukh, 2008). There are over 100 recognised species of PV (Kocjan et al., 2013). All PVs have similar genomic organizations, and phylogenetic studies strongly suggest that PVs do not recombine and have maintained their basic genomic organization (Willemsen & Bravo, 2019).

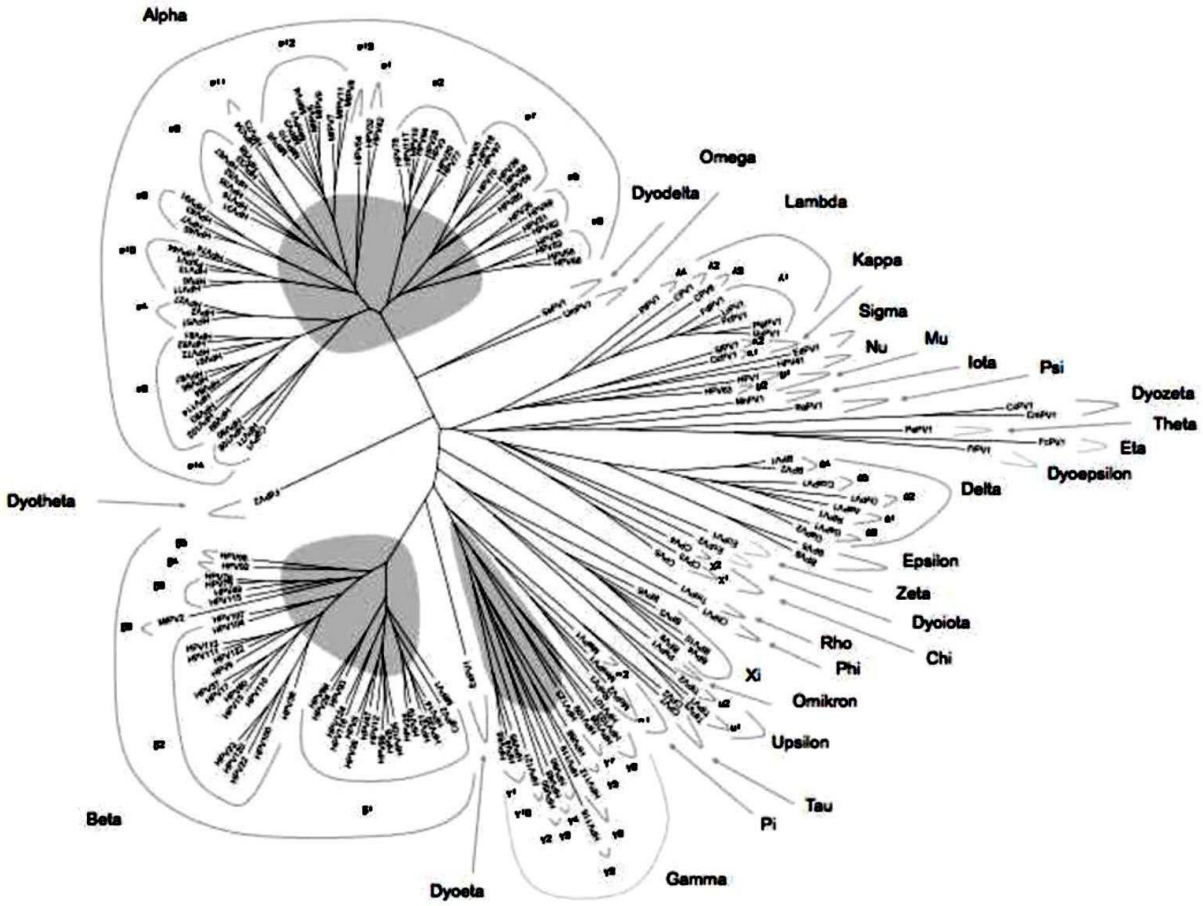

Figure 2.6: Phylogenetic tree of Papillomaviruses (Bernard et al., 2010)

Commonly used names	**ICTV term**
Taxonomic level	
Family: Papillomaviridae	**Family**: Papillomaviridae

Genus: alpha papillomavirus	**Genus**: *Alphapapillomavirus*
Species: alpha papillomavirus 9	**Species**: HPV-16
Types: HPV-16, -31, -33, etc	**Strains**: HPV16, 31, 33, etc.
Type species n/a	**Type species**: A term that identifies a papillomavirus typical of a genus

Table 2.2: Comparison of commonly used and ICTV Papillomavirus nomenclature (Bernard et al., 2010)

2.6.2 Current classification of Human Papillomaviruses (HPVs)

- More than 170 HPV types have been completely sequenced by the end of the year 2013, and these belong to five genera namely Alpha-papillomavirus, Betapapillomavirus, Gammapapillomavirus, Mupapillomavirus and Nupapillomavi- us. At least 202 different putative HPV types exist that are pending for full genome characterization and these are 5 alphapapillomaviruses, 37 betapapillomaviruses, 159 gammapapillomaviruses and 1 mupapillomavirus (Chouhy, Bolatti, Pérez, & Giri, 2013).

2.6.3 Structure of Genome

- Human papillomavirus (HPV) is a small virus that is 55 nm in diameter and lacks an envelope. Its capsid has 72 capsomers and is composed of at least two proteins, L1 and L2. Each capsomer consists of five L1 proteins, which are the major capsid protein. Additionally, each virion capsid has about 12 copies of the minor capsid protein, L2. When viewed by electron microscopy, the virus is said to resemble a golf ball (Figure 2.7) (Buck et al., 2008).

- The HPV genome is a circular, double-stranded DNA molecule of approximately 7,900 bp length that contains histones. All protein-coding open reading frames are located on a single strand (Favre, Orth, Croissant, & Yaniv, 1975).

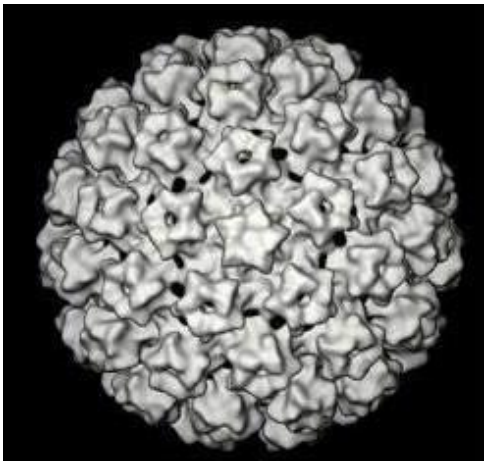

Figure 2.7: Electron micrograph of Human Papillomavirus

(Buck et al., 2008)

- The genome can be divided into three regions, each with a specific function (as shown in Figure 2.3). The first region is called the Upstream Regulatory Region (URR), also known as the Long Control Region (LCR), and is a noncoding region that spans 400 to 1,000 base pairs. The URR contains the p97 core promoter and both enhancer and silencer sequences that influence DNA replication by regulating the transcription of ORFs. This region also has the highest degree of variations in the viral genome, as reported by Apt et al. in 1996. The second region is called the Early Region and is made up of ORFs E1, E2, E4, E5, E6, and E7. These ORFs play a crucial role in viral replication and oncogenesis. The third region is called the Late Region and encodes the L1 and L2 structural proteins, which form the viral capsid (Jo et al., 2005).

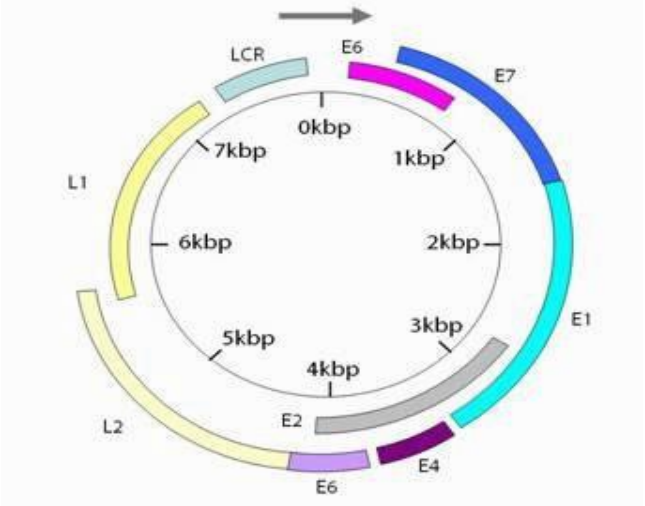

Figure 2.8: Genomic Structure of Human Papillomavirus (Jo et al., 2005)

2.6.4 Transmission of HPV

- HPV is primarily transmitted through skin-to-skin contact, and research shows that sexual activity can increase the risk of contracting genital HPV infection and cervical cancer. Although the virus is highly resistant to heat and desiccation, it can still be transmitted through nonsexual means, such as by prolonged exposure to contaminated clothing or other fomites (Okunade, 2020).

- The study found that certain types of human papillomavirus (HPV) tend to infect either the skin or the internal squamous mucosa. Researchers collected swab samples from five different skin sites of renal transplant recipients, dialysis patients, and healthy controls of the same age and sex. They analyzed the samples for HPV DNA and discovered 20 known types and 30 potential new types by cloning and sequencing 33 samples from 13 individuals. The study demonstrated that normal human skin contains a variety of PVs (Chen et al., 2022).

- There are different types of HPV infections that affect the lining of the mouth, throat, respiratory tract, or anogenital epithelium. These infections are mainly transmitted sexually. The outcome of mucosal HPV infection depends on the type of HPV involved. Anogenital warts (condyloma acuminatum) can appear on or around the genitals and anus of both men and women. Some people may have a latent or inactive infection, which means they may not experience any noticeable symptoms, and the infected area remains cytologically normal. However, others may develop penile, urethral, bladder, vaginal, vulvar, or cervical intraepithelial neoplasia (CIN) and invasive carcinoma due to certain types of HPV infections (Burd, 2003).

- Over the years, it has been observed that members of the Alphapapillomaviruses genus are responsible for a wide range of lesions, as shown in Table 2.3.

Species (Common use)	**Species (ICTV)**	**HPV genotype(s)**	**Cancer Risk**	**Common epithelial type infection and clinical manifestations**
1	HPV32	32, 42	Low	Mucosal

2	HPV10	3, 10, 28, 29, 77, 78, 94, 117	Low	Cutaneous > Mucosal
3	HPV61	61, 62, 72, 81, 83, 89, 84, 86, 87, 102, 114	Low	Mucosal
4	HPV2	2, 27, 57	Low	Cutaneous warts of Skin; Genital lesions of children
5	HPV26	26, 51, 69, 82	Low and High	Mucosal
6	HPV53	30, 53, 56, 66	Low and High	Mucosal
7	HPV18	18, 45, 39, 59, 68, 70, 85, 97	High	Mucosal; HPV18– invasive cervical carcinoma, particularly Adenocarcinoma
8	HPV7	7, 40, 43, 91	Low	Mucosal and cutaneous; HPV7 –Butcher's warts
9	HPV16	16, 31, 33, 35, 52, 58, 67	High	HPV16 –most common type in invasive cancers
10	HPV6	6, 11, 13, 44, 74	Low	Benign genital warts; Recurrent respiratory papillomatosis, HPV6–Verrucous carcinoma

Table 2.3: Members of the Alphapapillomaviruses genus ("Alphapapillomaviruses,")

- It is important to note that HPV infection with certain mucosal types can remain active for an extended period of over 10-15 years. During this time, the virus can cause changes in the infected cells, which may lead to the development of penile, urethral, bladder, vaginal, vulvar, or cervical intraepithelial neoplasia. Studies have shown that women in whom HPV DNA was detected had a higher chance of developing SIL within two years

(15-28%) as compared to those in whom HPV DNA was not detected (1- 3%). Additionally, there is a greater risk of progression for HPV-16 and -18 (approximately 40%) than for other HPV types (Cubie, 2013).

2.6.5 Replication of HPV (In vivo)

- The human papillomavirus (HPV) infects the basal cells of stratified squamous epithelium, while other cell types are less susceptible. Mild abrasion or microtrauma of the epidermis is likely required for the HPV infection to occur in the basal layer. Different HPV types may require different epithelial cell receptors for attachment. For example, α6-Integrin has been proposed as the epithelial cell receptor for HPV-6, while HPV-16 and HPV-33 attach to host cells via cell surface heparan sulfate. The L1 major capsid protein of HPVs interacts with heparin and glycosaminoglycans on human keratinocytes (Feller, Khammissa, Wood, & Lemmer, 2009).

- It is not yet known which cellular factors are required for the uptake of virions. After entering the host cell, HPV begins to replicate as basal cells differentiate and move towards the surface of the epithelium (as shown in Figure 2.9). Viral replication in the basal layers is believed to be unproductive, and the virus establishes itself as a low copy number episome by utilizing the host DNA replication machinery to synthesize its DNA, on average, once per cell cycle (Feller et al., 2009; Flores, Allen-Hoffmann, Lee, Sattler, & Lambert, 1999; Gilbert, Brewer, Reiter, Ng, & Smith, 2011).

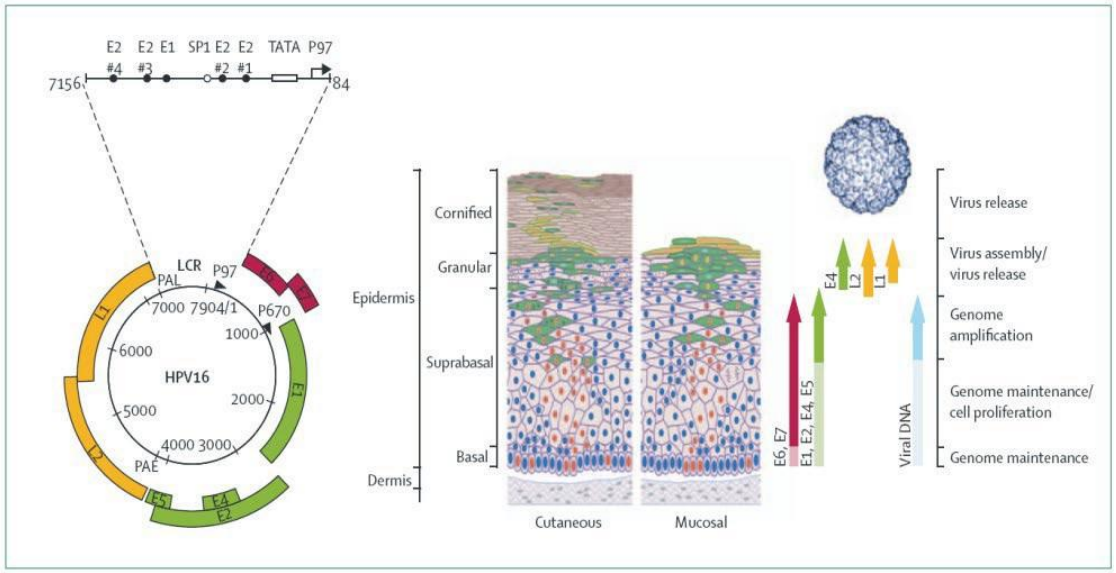

Figure 2.9: Organization of the HPV genome and the virus life cycle ("Organization of the HPV genome ")

- In the upper layers of the epithelium, the keratinocytes become specialized and the

human papillomavirus (HPV) changes its DNA replication to a rolling circle mode. This results in the amplification of viral DNA to high copy number, followed by the synthesis of capsid proteins and viral assembly (Flores et al., 1999). Since HPV encodes only 8 to 10 proteins, it relies on host cell factors to regulate viral transcription and replication. The replication of HPV starts with host cell factors that interact with the LCR region of the HPV genome and initiate transcription of the viral E6 and E7 genes (McKinney, Hussmann, & McBride, 2015).

2.6.6 Replication of HPV (In vitro)

- Efforts to replicate HPV in standard cell cultures have been unsuccessful, primarily due to the fact that the replication process is closely connected to the differentiation process of keratinocytes. Additionally, it has been difficult to recreate the stratified structure of the epithelium in vitro. However, some HPVs have been successfully propagated in xenograft biological models or organotypic raft culture systems. These systems have proven to be valuable tools for studying HPV replication and host cell interactions. HPV-6, -11, -16, and -40 have been successfully replicated in human skin and cervical tissues that have been transplanted into athymic (nude) or severe combined immunodeficiency (SCID) mice (Moody, 2017).

- Two epithelial cell lines derived from HPV-infected patients, the W12E cells containing HPV-16, and the CIN612-9E cells containing HPV-31b, have been successfully cultured on rafts. These HPV raft cultures are reproducible and recreate the morphological and physiological differentiation process of epithelial cells in vitro, allowing HPV replication. The process involves growing epithelial cell lines containing latent HPV with a collagen matrix on a solid support. The cells form stratified layers and display characteristics of normal terminal differentiation (Moody, 2017).

- To prepare raft culture systems, a base is created using type 1 collagen derived from bovine tendon. Human foreskin fibroblasts are mixed with collagen and placed on the base, allowing them to contract. Epithelial cells are pre-cultured on Swiss 3T3 fibroblast feeders and then added to the collagen plug. After four days of incubation, the raft is placed on a cotton pad and fed with cornification medium containing 12-O- tetradecanoyl phorbol-13-acetate (TPA) to induce differentiation. This differentiation leads to the replication of HPV in the suprabasal cells (Berthod, Hayek, Damour, & Collombel, 1993).

2.6.7 Risk factors

- Research has identified several risk factors for cervical cancer, including early age at first sexual intercourse, multiple sexual partners, high-risk male partner, family history, cigarette smoking, oral contraceptives, certain sexually transmitted infections like Chlamydia and Herpes simplex type 2 virus, and socioeconomic factors. Initially, these were considered the primary risk factors for cervical cancer. However, with the discovery of integrated HPV-16 and -18 genome in biopsy specimens and cell line derived from Carcinoma cervix, the role of HPV in cervical cancer has been recognized as a significant risk factor (Joffe et al., 1992).

- Multiple sexual partners, early age during sexual activity, a history of STDs, and genital warts in the individual or their partner increase the risk of HPV infection. Condoms may not provide adequate protection since HPV can be transmitted by contact with infected scrotal or anal tissues. Age is also a critical factor in determining the risk of HPV infection. The squamocolumnar junction undergoes continuous metaplastic changes, with the highest activity during puberty and first pregnancy, and lowest after menopause (Repp et al., 2012).

- HPV infection is common in sexually active young women between 18 and 30 years old. Cervical cancer tends to develop in women over 35 years old, suggesting slow progression from an earlier infection. High-risk HPV types increase the risk of cervical cancer, but other factors also play a role. Smoking and long-term use of oral contraceptives increase the risk of high-grade cervical disease. Impaired cell-mediated immunity, such as with renal transplantation or HIV disease, increases the risk of acquiring and progressing HPV infection. Multiple pregnancies are a significant independent risk factor among women with histopathologic (Scott, Nakagawa, & Moscicki, 2001).

- Smoking and parity are among the factors that independently influence the risk of cervical cancer, along with HPV infection. Smoking induces immune suppression and mutagenic activity in cervical cells, contributing to the persistence of HPV and malignant transformation. Smoking is the most significant risk factor for higher grades of cervical disease, but shows little or no relationship with low grades of cervical disease (Carrillo-Beltrán et al., 2023).

- It has been observed that multiple pregnancies could increase the risk for women who have histopathologic evidence of HPV infection in biopsy specimens and those who have moderate to high grade cervical disease. On the other hand, factors such as alcohol consumption and diet have not been proven to have a significant impact. Earlier, it was believed that an increase in viral load might be a risk factor for malignant transformation. However, High-risk HPVs of all types can induce malignant tumors even when present at low levels. Studies using quantitative type-specific PCR for high- risk HPV-16, -18, -31, -33, and -45 and low-risk HPV-6 and -11 have shown that HPV- 16 can reach much higher viral loads than other types, and only for HPV-16 does increase viral load correlate with increased severity of cervical disease (N. S. L. Yeo- Teh, Y. Ito, & S. Jha, 2018).
- The oncogenic potential of distinct HPV variants appears to vary by geographical region and ethnic background of the population under study. HPV-16, for example, has five naturally occurring phylogenetic clusters defined by sequence variation in its L1, L2, and LCR regions, namely European (E), Asian (As), Asian-American (AA), African-1 (Af1), and African-2 (Af2). Additionally, intratypic sequence variation has been discovered in HPV-16's E2, E4, E5, E6, and E7 genes. The LCR region contains multiple E2 binding sites and transcription factor binding sites. Therefore, nucleotide sequence variation in the LCR, E2, E6, and E7 genes may have functional significance (T. Li, Yang, Zhang, Wang, & Mei, 2023).
- Genetic predisposition plays a significant role in cervical cancer, accounting for 27% of underlying factors. Shared familial environment has a minimal effect (2%), observed only between sisters. (Magnusson et al. 2000) (Ramachandran & Dörk, 2021).

2.6.8 Pathogenesis of HPV

- HPV infection is linked to cervical neoplasia development and is necessary but not sufficient in the cause of cervical carcinoma. The most common high-risk HPV types found in CIN II-III and cervical squamous cell carcinomas are HPV-16, -18, -45, -31 and -33. Viral persistence is required for neoplastic progression and early high viral loads increase the risk. High-risk HPVs can induce malignant tumors even at low levels (Broccolo et al., 2013; Suprynowicz et al., 2008).
- The integration of HR-HPV genome into host cell genomes is critical for malignant transformation. Integration often causes breakage and deletions in the E2 reading frame,

which results in increasing levels of oncogenes E6 and E7. Inhibition of E6 and E7 expression through induction of BPV E2 protein has been shown to inhibit cell growth in specific cervical cancer cell lines (Norman, 2015).

- The E6 and E7 oncoproteins play significant roles in causing malignant transformation and are consistently expressed in malignant tissues. These proteins work by deactivating the p53 and pRb tumor suppressor proteins, respectively (Livingstone et al., 1992; Vousden, 1993). As the levels of E6 and E7 expression increase, they cause genetic instability and increase the risk of cellular changes, which ultimately leads to a selective growth advantage (Tomaić, 2016).

- In 1976, Zur Hausen hypothesized that HPV plays a role in cervical cancer pathogenesis. In 1981, he and his team cloned anogenital HPV, and in 1983-84, they discovered and named HPV-16 and HPV-18 in cervical cancers. In 2008, zur Hausen won the Nobel Prize for his discovery that human papillomaviruses cause cervical cancer (Louvanto, 2011).

- E6 and E7 genes hinder host cell growth by binding and deactivating tumor suppressors, cell cycle regulators, and kinases. E6 and E7 genes hinder host cell growth by binding and deactivating tumor suppressors, cell cycle regulators, and kinases. (Figure 2.10) (Yim & Park, 2005).

2.6.9 HPV genotypes

- The research conducted in India on HPV testing has primarily focused on identifying the virus for screening purposes. Previous studies have found that HPV 16/18 was present in 76.4% of 72 cases of cervical malignancy, in less than half of 24 cases of cervical intraepithelial neoplasia, and in none of the 4 normal cervical tissues tested, as reported by Menon et al. in 1995 (Travasso, Anand, Samarth, Deshpande, & Kumar- Sinha, 2008).

- Munirajan et al. (1998) found that out of 43 cases of Carcinoma cervix of stage IIB/IIIB, 70% were detected to have HPV. Out of these, 23 cases (53%) had HPV16, 4 cases (13.3%) had HPV18, and 1 case each had HPV33, 35, and 58. In a hospital-based case-control study from Chennai, HPV was detected through PCR in all 205 invasive cervical carcinoma cases except one and in 27.7% of the 213 age-matched controls. The study reported twenty-three different HPV types, with HPV16 being the most commonly found type, followed by types 18 and 33 (Kaliff et al., 2018).

- According to a study conducted in Andhra Pradesh, HPV16 and 18 were found to be the most common HR HPV among cancer tissues. However, in the cervical samples of women in the Medchal community, HPV 52 was observed to be the most common HR HPV. This suggests that there is a variation in the epidemiological distribution of the virus (Sontakke et al., 2019).
- A study conducted on cervical cancer tissues in the Western UP and Delhi region found that out of 90 samples tested with L1 consensus primers, 65 (72.2%) were positive for HPV. Further genotyping with type-specific primers revealed that 92.3% (n=60) of the positive cases were caused by HPV-16, while 7.7% (n=5) were caused by HPV-18 (Fan et al., 2018).
- A study conducted on cervical samples collected from TMH in Mumbai aimed to genotype HPV using a novel pyrosequencing method. The study found that among the cervical cancer samples, 96.9% had high-risk HPVs, including HPV16 (73.8%), HPV18 (10.77%), HPV33 (3.07%), HPV31 (1.53%), and HPV45 (1.53%) (Fan et al., 2018).
- A study was recently conducted in Guwahati, Assam to detect HPV in cervical cancer patients. The study used Nested Multiplex PCR assays for the detection of 13 high-risk and 5 low-risk HPV types. Out of 107 samples, HPV was confirmed in 105 samples. The study revealed the presence of six carcinogenic HPV types, namely HPV-16 (88%), HPV-18 (15%), HPV-31 (4%), HPV-45 (3%), HPV-59 (4%), and HPV-58 (1%). Additionally, one non-carcinogenic HPV type, HPV-6/11 (6%), was also detected (Mane et al., 2012).
- A study conducted in Pune examined the prevalence of HPV genotypes in cervical intraepithelial neoplasia in HIV-infected women. The most prevalent carcinogenic HPV genotypes were HPV-16, HPV-56, HPV-18, HPV-39, HPV-35, HPV-51, HPV-31, HPV-59, HPV-33, HPV-58, HPV-68, HPV-45, and HPV-52 in a declining order of occurrence (Mane et al. 2012). In a similar study, HPV genotype distribution was observed in HIV-positive females, where HPV-16 was the most common type, detected in 42% of HPV-positive women. This was followed by HPV-45 (15%), HPV- 18/52/31/58 (11.5% each), and HPV-33 (7.6%). In the control group, the corresponding figures were as follows: HPV-16 (66.6%), HPV-45/-18/-31 (16.6% each), and HPV- 33/-58/-68 (8.3% each). (Aggarwal et al. 2012 It was observed that most HR HPV genotypes are prevalent in the

Indian population (Mane et al., 2012).

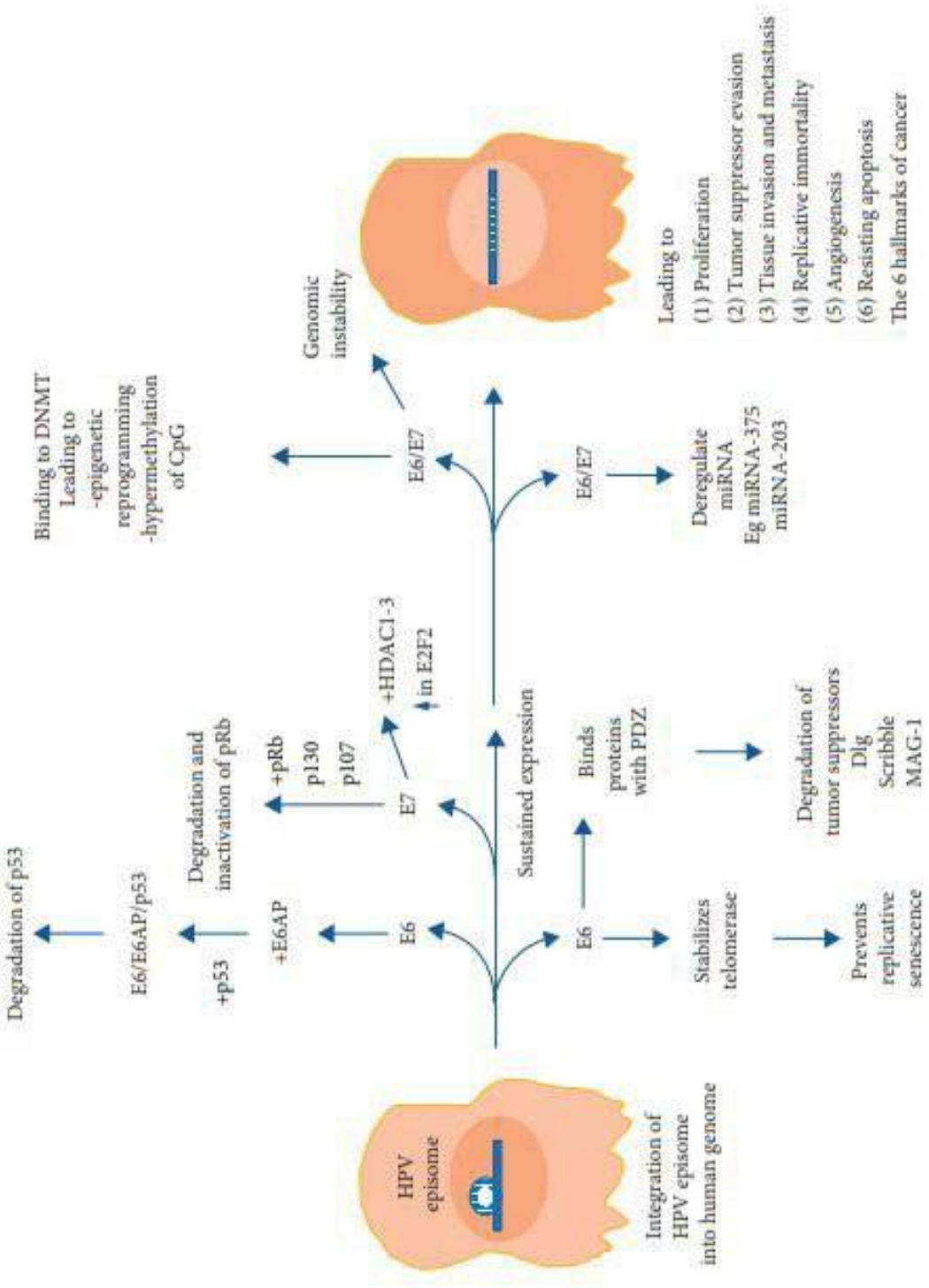

Figure 2.10: Cervical Cancer Genesis (Yim & Park, 2005).

- Cervical cancer genesis involves HPV gene integration that leads to uncontrolled cellular division and proliferation by impacting and dysregulating various pathways, including the inactivation and degradation of p53 and pRB.

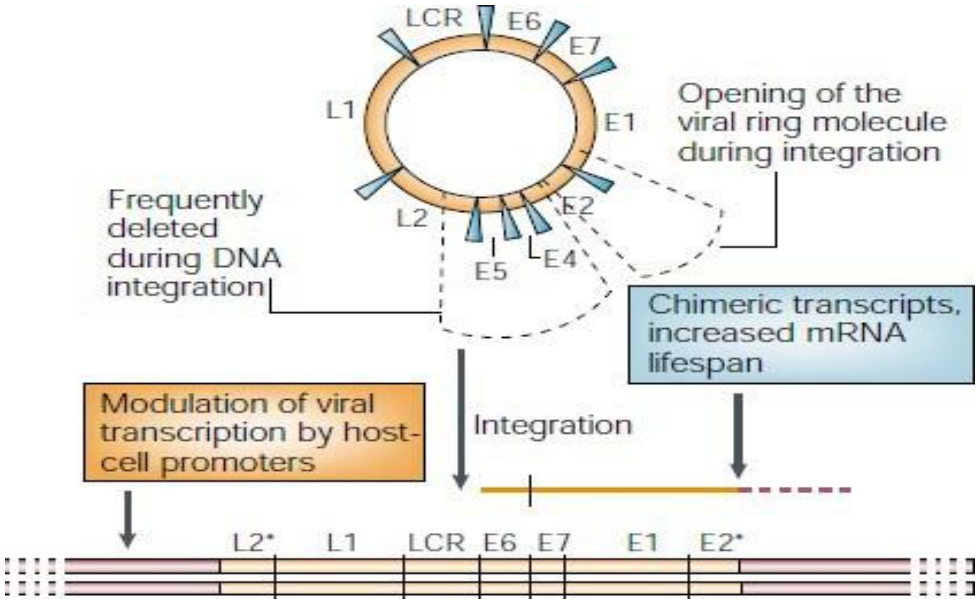

Figure 2.11: The organization of circular HPV DNA and its integration into host cell (Louvanto, 2011)

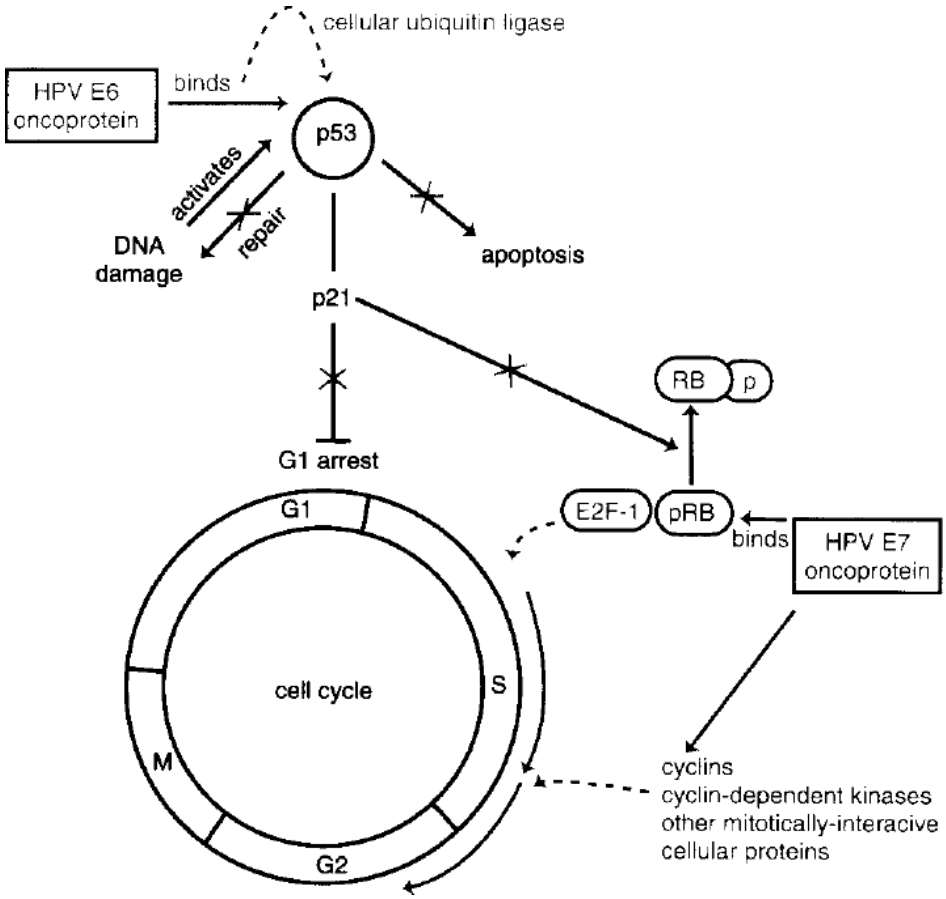

Figure 2.12: Effect of oncogenic HPV leading to cellular transformation (Burd, 2003, pp. 1-17)

2.6.10 HPV DNA testing

- In April 2014, the FDA approved the Cobas 4800 HPV test (Roche) for primary cervical cancer screening in women aged 25 years or older. This was the first HPV DNA test to be approved that can be used alone to detect high-risk HPV. The test is recommended as a first-line screening tool. It can specifically identify HPV -16 and -18, which are responsible for about 70% of all cervical cancers. Additionally, it detects 12 other high-risk genotypes (-31, -33, -35, -39, -45, -51, -52, -56, -58, -59, -66, and -68)

- simultaneously. The FDA's decision was based on the findings of the ATHENA study, which evaluated the Cobas 4800 HPV test among 47,208 U.S. women 21 years old and older who were undergoing routine cervical cancer screening (Arbyn et al., 2021; Zhao & Yang, 2012).

- The trial results have shown that even though women who tested positive for HPV 16 and/or 18 with a normal Pap test result, 1 in 10 women above the age of 30 had cervical pre-cancer. The Cobas HPV Test is recommended for women who test positive for HPV 16 or HPV 18. If a woman tests positive for 1 or more of the 12 other HR HPV types, then a Pap test is recommended to determine the need for a colposcopy. It is recommended that healthcare professionals use the Cobas HPV test results together with other relevant information, such as patient screening history, risk factors and current guidelines. These analyses validate the 2006 ASCCP guidelines for HPV-16/- 18 genotyping, recommending that HPV-16/HPV-18 positive women with negative cytology be referred to colposcopy (Perkins et al., 2021).

2.6.11 Policy decision

- In India, designing Cervical Cancer Screening Program (CCSP) guidelines that can screen most cases of High-grade Cervical Intraepithelial Neoplasia (CIN) and invasive cervical carcinoma is challenging. Prevention is essential in any disease control program, and the most cost-effective long-term method of cancer control. Although Pap smear screening is recommended for all sexually active women, it's not feasible in India for mass screening. One strategy is a hospital-based approach where cytological screening was conducted on women attending gynecologic OPDs of six hospitals in Delhi. A single lifetime cytology screening reduced the cumulative incidence of cervical cancer by 10.2% (Agarwal, Murthy, Sharma, Sharma, & Das, 1995).

- A community-based approach was conducted in Barsi to screen women above the age of 30 for cancer. However, only 8.3% of the women attended the screening camps. To make screening for cervical cancer successful, a proper referral system is important. Secondary and tertiary health institutions must be equipped to manage the cases detected by screening. There is an urgent need to initiate screening programs across the country, for which guidelines have been laid down by an Expert Committee group (Maxim, Niebo, & Utell, 2014; Nene, Jayant, Malvi, Dale, & Deshpande, 1994).
- India lacks an organized screening program for cervical cancer, with most cancer centers only providing opportunistic screening services. In contrast, many developed countries have seen a decline in cervical cancer incidence and mortality rates due to cytology screening. However, implementing such screening programs in India is challenging due to inadequate infrastructure, logistics issues, quality assurance concerns, and high screening costs (Srivastava, Misra, Srivastava, Das, & Gupta, 2018).
- Conventional Pap cytology and HPV DNA screening tests are not available in rural China and India. VIA and VILI showed variable sensitivity and specificity for detecting CIN2 or greater. A novel rapid HPV DNA test was found to be more accurate than VIA and approaching HC2 in a clinical endpoint study in Shanxi, China. Efforts are now being made to introduce an affordable HPV DNA test as a primary screening method in low-resource settings (Koliopoulos et al., 2017).
- Japan and South Korea's screening programs reduced cervical cancer. Japan saw an increase in young women despite Cytology. HPV DNA testing improves screening for CIN. Use HPV DNA testing as the primary screening test if resources are sufficient. In limited resource countries, use a simple HPV DNA test and immediate 'screen and treat' for HPV-positive patients (Konno et al., 2008).
- Loss of follow-up is a common issue in conventional cytology-based screening programs for cervical cancer. To address this, Denny et al. (2005) conducted a randomized controlled trial to assess the safety and efficacy of two alternative methods for cervical cancer screening. The first method involved cryotherapy treatment for women who tested positive for HPV DNA or VIA, while the second method was based on conventional cytology screening. The results showed that both screen-and-treat approaches are safe and effective, with a lower prevalence of HSIL compared to cytology-based screening at

6-12 months (Catarino, Petignat, Dongui, & Vassilakos, 2015).

- In India, Bangladesh, and China, there are no screening programs in place, but there are plans to establish demonstration centers for screening. VIA has been successful in pilot projects in India and is being introduced more widely in these countries, along with the possibility of HPV DNA testing (Bhatla et al., 2021).
- A study in India found that VIA (Visual Inspection with Acetic acid) is effective in preventing cervical cancer in developing countries, with proper training and quality assurance. Screening women once in a lifetime at the age of 35 with a one or two visit schedule involving VIA or HPV DNA testing significantly reduces lifetime risk of cervical cancer (Sankaranarayanan et al., 2007). Preventing cervical cancer requires both prophylactic vaccination and screening. Vaccination benefits younger women, but screening should be the focus for most regions due to cost. To be most effective, vaccination should be combined with sustainable screening programs (McGraw & Ferrante, 2014).