2

REVIEW OF LITERATURE

2.1. Cervical cancer

Globally, cervical cancer (CC) continues to be the leading cause of disease and death. Chronic high-risk human papillomavirus (HPV) infection is the cause of CC development (González-Rodríguez *et al*., 2024). In India, cervical cancer is among the top five cancers in terms of both incidence and mortality (Bray *et al*., 2024). Through microlesions caused by sexual transmission, HPV can eventually cause carcinogenesis in basal cells from the epithelium (Graham, 2024; Van Gerwen *et al*., 2022).

Patients with early-stage specifically on FIGO stage IA-IB1, cervical cancer are typically treated with radical hysterectomy, dissection of the lymph nodes, radiation, and/or chemotherapy (Liang *et al*., 2024). Patients with locally advanced cervical cancer are typically treated with external beam radiation and brachytherapy in addition to concurrent cisplatin-based chemotherapy (Ang and Chan, 2024; Makovec, 2019). When brachytherapy is substituted for external beam radiation for the treatment of cervical cancer with the goal of curing it, the outcome is not satisfactory (Han *et al*., 2024). In many developing countries, the 3- to 5-year survival rate from cervical cancer is less than 50% when considering all stages combined (Sathishkumar *et al*., 2024). Cervical cancer frequently results in localised disease progression that causes severe suffering, such as ureteral obstruction, pain, and fistulas (Li *et al*., 2024; Small *et al*., 2017).

Cervical cancer is highly preventable and treatable if detected early. However, in resource-limited countries, prevention efforts are often poor. Key barriers include lack of knowledge, limited awareness about prevention, difficulty accessing care due to cost or location, poor service quality, and insufficient support from family. Interestingly, studies in low- and middle-income countries (LMICs) show that awareness alone is not enough for women getting screened.

2.2. Types of cervical cancer

The cervix is bordered by stratified squamous epithelium that surrounds the exocervix and mucus-secreting columnar epithelium found in the endocervical canal. The region known as the squamocolumnar junction, which separates these two cell populations, is thought to be more prone to viral neoplastic transformation.

About 75% of instances of invasive cervical carcinoma are squamous cell carcinomas, which are the most common type of tumours that arise in the ectocervix. On the other hand, adenocarcinomas are more common in tumours that originate from the endocervix. Common histological subtypes of cervical carcinomas include squamous cell carcinoma (keratinizing, non-keratinizing, papillary, basaloid, warty, verrucous, squamotransitional, or lymphoepithelioma-like); adenocarcinoma (endocervical, mucinous, villoglandular, or endometrioid); clear cell adenocarcinoma; serous carcinoma; adenosquamous carcinoma; glassy cell carcinoma; adenoid basal carcinoma; small cell carcinoma, or undifferentiated carcinoma (Small *et al*., 2017; Carcangiu *et al*., 2014).

2.3. The biology of cervical cancer

The majority of cervical cancer cases are caused by human papillomavirus infection; 95% of malignant cervical lesions have HPV DNA detected in them (Rosendo-Chalma *et al*., 2024). Most HPV infections are temporary and will go away on their own. Persistent infection, however, may occasionally lead to the premalignant states of adenocarcinoma in situ or cervical intraepithelial neoplasia. For most women, the progression from dysplasia to aggressive cancer may take years or even decades if treatment is not received. However, this change might happen in less than a year in about 10% of patients (Vallejo-Ruiz *et al*., 2024). Furthermore, it appears that adenocarcinoma in situ is more challenging to identify with Papanicolaou testing, which may contribute to the rising frequency of this subtype of cervical cancer (Nishio *et al*., 2024).

There are several factors that have been proposed to raise the risk of developing a persistent infection and the subsequent malignant transformation. These factors include using oral contraceptives for an extended period of time,

smoking cigarettes, having high parity, and co-infection with either the human immunodeficiency virus or type 2 herpes simplex virus. In women with cervical cancer, the most prevalent HPV serotypes are 16, 18, 45, 31, 33, 52, 58, and 35, in decreasing order of frequency. It is estimated that HPV serotypes 16 and 18 account for almost 70% of cases (Alrefai *et al*., 2024).

2.4. Clinical presentation and diagnosis of cervical cancer

Cervical cancer can be asymptomatic in its early stages and may be discovered by a pelvic exam or routine screening. The symptoms of cervical carcinoma recurrence are often non-specific, making the clinical diagnosis difficult (Adiga *et al*., 2021; Gadducci *et al*., 2015). The most typical signs of cervical cancer include pain during sexual activity, bleeding other than during periods, postcoital bleeding, postmenopausal haemorrhage, and vaginal discharge that smells bad, is blood-stained, pelvic pain, abdominal pain, persistent back pain, urgency to urinate, white vaginal discharge, and malodorous vagina (Sudha *et al*., 2022).

The most reliable way to diagnose cervical cancer is to examine the histopathology of a cervical biopsy, using either a direct biopsy, a cone biopsy (conization), or an endocervical curettage for endocervical lesions. The three routes to it are cytology, colposcopy, and clinical assessment. The patient's presentation and the resources available will determine how the investigation is started, but a clinical evaluation is the best place to start. A direct biopsy must be carried out for the diagnosis in a clinical assessment if a lesion is apparent or if there is an irregular, persistent, or vegetated cervix in the speculum examination. In such instances, a biopsy suffices and the conization is not advised. Furthermore, a vaginal and rectal examination is required in this situation (Guimarães *et al*., 2022).

Conversely, patients who, like the majority of early-stage diagnosis, have abnormal cytology, positive DNA-HPV findings, and no visible cervix lesion should have colposcopy and suspicious region biopsies performed. In such instances, cytology plays a crucial role in directing the inquiry; specimens from the endocervix, ectocervix, and squamocolumnar junction must be present on the slide in order to be evaluated. To guide the biopsy, the colposcopy shows features (such

as aberrant vasculature, necrosis, or erosion) that signal tumoral invasion. It's crucial to use curettage to gain access to the endocervix during colposcopy. Because the endocervical curettage method can produce up to 75% false-negative findings, it is important to take into account the positive results and remember that a negative test does not rule out the possibility (Suzuki *et al*., 2017).

Ultimately, a cone biopsy is advised when stromal invasion cannot be excluded or quantified using a direct biopsy and colposcopy, when the colposcopy is insufficient, or when cytopathological analysis reveals an intra-epithelial high-grade lesion, or even when there is a dispute over the best course of action for a suspicious lesion. The examination of the histological type, maximum stromal invasion, tumour extent, and existence or absence of LVSI are all made possible by the cone biopsy (Nica *et al*., 2021). The CC type is classified as follows based on histopathologic diagnosis:

2.5. Risk factors of cervical cancer

Nearly all occurrences of cervical cancer are caused by chronic infection with high-risk oncogenic subtypes of the human papilloma virus (Torre *et al*., 2017). Consequently, risk factors include those linked to either getting HPV infection or having a severely compromised immune response to HPV infection (Huang *et al*., 2022). Early sexual age, having multiple partners or a high-risk partner, immunosuppression (such as after organ transplantation or immunodeficiency disorders like HIV), a history of STDs, a history of HPV-related vulvar or vaginal dysplasia, underscreening in nations with established cervical screening programmes, and non-attendance for screening are some of these risk factors (Cohen *et al*., 2019). In a cohort study of over 300,000 women, the European Prospective Investigation into Cancer and Nutrition, tobacco smoke was revealed to be a significant risk factor for both cervical precancer and cancer. After accounting for HPV status, smoking status, duration, and quantity were linked to twice the risk of high-grade dysplasia and cancer. Notably, quitting smoking was linked to a two-fold decrease in risk (Roura *et al*., 2014).

2.6. Prevention of cervical cancer

One way to think of prevention is through vaccination as primary and screening as secondary. Cervical exfoliative cytology was developed to detect cervical intraepithelial neoplasia, which can be treated to avoid the development of cervical cancer. This was made possible by the realisation that cervical neoplasia starts as an intraepithelial alteration and typically takes several years to develop into an invasive disease. Since high-risk HPV infection was found to be the cause of cervical cancer and prophylactic vaccination was developed in the 1990s, a more global approach to prevention through prophylactic vaccination is now possible (Small *et al*., 2017).

2.6.1. Primary prevention

Easing out of HPV infection is the first step in preventing cervical cancer. HPV infection can be avoided by refraining from sexual activity, practicing mutual monogamy among virgins, or using condoms—although these methods are not 100% effective. However, HPV vaccination is necessary for primary prevention of cervical cancer to be effective. Since their introduction in 2006, the first bivalent and quadrivalent HPV vaccines have demonstrated more than 90% efficiency in preventing HPV types 16 and 18, which are linked to high-grade cervical dysplasia (Lei *et al*., 2020; Crosbie *et al*., 2013).

2.6.2. Secondary prevention

Screening with HPV testing can result in a more accurate risk-based strategy because of the critical role that HPV plays in the development of cervical cancer. Randomised trials have shown that HPV testing is more sensitive than cytology and that screening intervals can be safely extended for women who test negative for HPV. Because HPV testing is not very specific, women must be sent to cytology for a colposcopy based on their circumstances. Limited data suggests that triaging high-risk HPV (hrHPV)-positive women utilising genotyping for HPV types 16 and 18 and reflex cytology for the 12 remaining hrHPV genotypes is a suitable way to manage these individuals (Huh *et al*., 2015). Women whose cytology results are negative provide a difficulty for primary HPV screening; however, different risk-based

approaches based on HPV type and persistence are being explored. Primary HPV testing is currently replacing primary cytology with a microscope and acetic acid in screening programmes across the globe (Small *et al*., 2017).

2.7. Treatment strategies for cervical cancer

Treatment strategies and results for cervical cancer patients are largely influenced by the disease stage at diagnosis (Ye *et al*., 2023). For individuals with cervical cancer, the 5-year survival rate can range from less than 20% in cases of distant or metastatic disease to over 90% in cases of early, localised illness (Marcus *et al*., 2021). Radiation therapy and concomitant chemotherapy are available for treating early-stage, locally invasive cervical cancer. Other treatment options include radical hysterectomy or radical trachelectomy along with pelvic lymphadenectomy. Systemic treatments are the mainstay of treatment for distantly metastatic cervical cancer (Serkies and Jassem, 2018).

2.7.1. Chemotherapy

Chemotherapy is an essential component of the standard treatment plan for cervical cancer. It is usually given as a stand-alone treatment for locally advanced disease, in combination with radiotherapy as previously discussed, or as an adjuvant therapy after surgery when poor prognostic tumour features increase the risk of recurrent disease. The platinum-based chemotherapy drug cisplatin has been the most successful single treatment for cervical cancer during the past three decades (Parveen *et al*., 2023). Notwithstanding the initial response of patients to cisplatin, there is often a report of growing resistance during therapy, which diminishes the effectiveness of further platinum-based second-line chemotherapeutics (Aldossary, 2019). As a result, research has shown that using cisplatin in combination with other medications may be more beneficial than using it alone (Burmeister *et al*., 2022).

2.7.2. Radiotherapy

Cervical cancer treatment is critical, and radiation therapy is predicted to cure it completely. Radiation therapy, which is usually given as external beam radiation and intracavitary brachytherapy, is an efficient treatment for cervical

cancer (Yamada *et al*., 2024). Two-dimensional planning based on bone structures dominated at first. The goal of radiation therapy is to minimise dosage to surrounding normal organs while administering a dose high enough to control local tumours. Due to their proximity to the uterus, the bladder and rectum may experience late unfavourable effects such cystitis and rectal haemorrhage. Since many patients with cervical cancer are young, attempts to decrease late adverse events are crucial since they lower the quality of life for patients (Soejima, 2023). Long *et al*. (2005) found that, whereas cisplatin alone had a 20% response rate, when coupled with topotecan, the response rate increased to 39%. For the treatment of cervical cancer, topotecan, paclitaxel, and other non-platinum-based chemotherapeutics such 5-fluorouracil and bleomycin are currently frequently utilised in addition to cisplatin. The median survival duration significantly and clinically meaningfully improves (Parveen *et al*., 2023).

Chemotherapy is mostly used for locally advanced cervical cancer and is frequently combined with radiation therapy (chemoradiotherapy). Although the goal of this regimen is to lessen illness recurrence, unfavourable side effects and persistent morbidity may occur. Chemoradiotherapy lowers the odds of both local and distant cervical cancer recurrences and improves overall and progression-free survival, according to a systematic review and meta-analysis (Kumar *et al*., 2018). Finally, although palliative chemotherapy may not be an effective means of reducing tumour size, it is used to enhance quality of life and treat symptoms of the disease. The success of chemotherapy is impacted by multidrug resistance in cancer cells, which makes the discovery and development of novel and improved medicines crucial (Porras *et al*., 2018).

2.7.2.1. External beam radiotherapy

The usual course of treatment for patients with cervical cancer is a mix of intracavitary brachytherapy (ICBT) and external beam radiation therapy (EBRT) for the pelvis (Soejima, 2023).

The advancement of computed tomography (CT) and advances in computer capacity have led to a significant advancement in EBRT planning during

the 1990s. An EBRT plan is usually developed using CT images as a basis. It is then given using intensity-modulated volumetric approaches, which involves administering six or more megavoltage X-rays to the entire pelvis, or multiple conformal fields, often known as three-dimensional conformal radiation, or 3D-CRT. The standard course of treatment for primary tumour and lymph node regions is EBRT at a dose of about 40–50 Gy; 1.8–2.0 Gy per fraction daily, followed by ICBT. While it is possible to conduct ICBT every day, it is typically conducted 1-2 times a week for four sessions (Suzumura *et al*., 2021).

Increased use of intensity-modulated radiotherapy (IMRT) or volumetric arc treatment (VMAT) has allowed for more accurate dose administration to a certain target during the past few decades due to advancements in imaging and calculation techniques. Using IMRT spread, it has been possible to reduce the dosages supplied to organs at risk without the requirement for centre shielding (Deng *et al*., 2017).

In patients receiving post-operative radiation therapy for cervical cancer, a phase III trial compared image-guided-IMRT (IG-IMRT) with 3D-CRT. The results indicated that IG-IMRT decreased late gastrointestinal toxicity while 3D-CRT did not vary in terms of efficacy outcomes (Chopra *et al*., 2021).

The available literature does not provide enough data to support the effectiveness of IMRT, and more research is required to decide whether or not this therapy option is appropriate for treating cervical cancer. Because of minute changes in the target's size, shape, or anatomical placements, the treatment plan in IMRT cannot be carried out to the required standard. Treatment uncertainties arise from the positional and volumetric alterations that the pelvic organs are prone to over time. Image-guided radiation therapy (IGRT) is being used more frequently and regularly in clinical settings. Optimising therapy delivery uncertainty requires image guidance (Yamada *et al*., 2024).

2.7.2.2. Brachytherapy

Brachytherapy is an extremely accurate form of radiotherapy in which the radiation source is positioned with an applicator in direct contact with the target

volume to provide a conformal dose distribution. For a long time, before external radiation techniques and surgery were developed, brachytherapy was a prominent treatment option for deep-seated cancers in soft tissue, the head and neck region, and the breast (Berger *et al*., 2023). Although its importance has been steadily declining, brachytherapy is still an essential part of the treatment for cervical cancers. Brachytherapy has rekindled interest due to the current cervical cancer situation in Romania, and it is an affordable and easily accessible treatment option.

Intracavitary brachytherapy is a vital and advantageous local therapy choice. It can increase the survival rate of patients with cervical cancer without raising side effects by generating a steep dose gradient, giving a high dosage to the tumour and a lower dose to the surrounding OARs (Bockel *et al*., 2021).

Brachytherapy is advantageous due to its dosimetric properties, which allow it to spare nearby organs including the bladder, rectum, sigmoid, and small bowel by delivering a rapidly falling dose at the disease site that is both high and conformal locally (Pötter *et al*., 2021). In many countries, brachytherapy is still not available. The usage of brachytherapy is decreasing, even in countries where it is widely available.

2.7.2.3. Charged particle radiotherapies

Charged-particle radiotherapy (RT), including as proton beam and carbon ion therapy, has become widely employed in clinical practice in recent decades. The energy of photons declines as the beam passes through the tissues, whereas protons and carbon ions lose their majority of energy near the end of their route, a phenomenon known as the Bragg Peak (Sakurai *et al*., 2016). The maximal dose deposition within the target, low entrance, and steep dose fall-off are thus beneficial physical properties of particle beam therapy. As a result, charged-particle radiotherapy can expose less normal tissue while raising the therapeutic ratio.

Proton therapy could be beneficial as a definitive/post-operative radiation therapy for cervical cancer. Carbon-ion radiation therapy maximises anticancer effects while minimising negative effects on healthy tissue. Moreover, their strong linear energy transfer confers biological advantages (Yamada *et al*., 2024).

Although there are little prospective clinical data and less information about the clinical benefit of charged-particle therapy, it may be safe and beneficial in treating cervical cancer. Therefore, larger, randomised trials are required.

2.8. Knowledge about HPV and cervical cancer

Among all sexually transmitted diseases, human papillomaviral infection is the most common (Cunha *et al*., 2020). Regarding HPV knowledge, many women are unaware of the connection between HPV infection and cervical cancer (Mingo *et al*., 2012). A recent study conducted among educated women (n=2100) in India revealed that the registered participants from both urban and rural areas possessed partial knowledge about cervical cancer (63.1% and 71.3%, respectively) (Sudha *et al*., 2022).

A study of female students at Wollega University, western Ethiopia, revealed that more than half (54.4%) of participants had heard about cervical cancer and its risk factors (Tilahun *et al*., 2019). In Bangladesh, a study of middle-aged women found that 81% were aware of cervical cancer (Islam *et al*., 2015). The same kind of results were observed in another study in Bangladesh. There 71.8 percent of the participants knew about cervical cancer (Banik *et al*., 2020). It is not shocking that literacy and place of residence are related to CC knowledge.

A study on KAP of cancer cervix among female students at Adama Science and Technology University, Ethiopia, revealed that three-fifths (60.6%) of the students had heard about cervical cancer (Tadesse *et al*., 2022). Similar results were found in other studies conducted in central Ethiopia, where nearly two-thirds (68.8%) of the 414 respondents had heard of cervical cancer (Gebisa *et al*., 2022). In Kuwait, 89.7% (n=224) of the studied population had heard of cervical cancer (Badawy *et al*., 2022).

Qayum *et al*., (2021) conducted a study in Bangladesh, and their results revealed that 87% of the respondents knew about cervical cancer. Williams *et al*., (2019) surveyed that the awareness and beliefs about cervical cancer among Ghanaian Women with diverse education levels (n=288). The percentage of people who were aware of cervical cancer was 56.9%.

In Cameroon, a study of female respondents on cervical cancer revealed that fifty-eight percent (58%) of the participants had good knowledge of cervical cancer (Nkfusai *et al*., 2019). In Ghana, a study of female respondents on cervical cancer revealed that 9.7% had a high level of knowledge, 20.6% had a moderate level of knowledge, and 69.7% had insufficient understanding (Gyamfua *et al*., 2019). The same kind of observation regarding enough understanding of cervical cancer was noted in Kolkata, India. Out of 202 study participants, cervical cancer was known to 15%, while HPV was known to 36% of women. In accordance with 28% of respondents, HPV causes cervical cancer (Montgomery *et al*., 2015).

Tiiti *et al*., 2022 studied women attending gynaecology clinics at a tertiary hospital in Pretoria, South Africa, to assess their knowledge and awareness of HPV and cervical cancer. Their study showed that most participants (73.8%) in their study sample were unaware of what HPV was. Only 45.6% of those surveyed knew that HPV is a cause of cervical cancer. A little over 60% of the participants were familiar with HPV.

Many previous reports statistics showed that less than half of the survey participants from various geographical populations were uninformed of cervical cancer, whereas the majority had heard of it. In comparison to their educational background, educated women should also focus on the initial preventive strategy. Women who are aware of cervical cancer are more likely to take preventive action by getting screened early and seeking medical assistance.

2.9. Knowledge about symptoms

In the study conducted in South Africa, the study participants were asked whether women with vaginal discharge and/or bleeding need to be screened for cervical cancer; the majority (58.8%) of participants responded "No," and 13.7% said "Don't know" in regards to the signs and symptoms of cervical cancer (Tiiti *et al*., 2022). Out of 667, 22% and 31.6% of students indicated vaginal blood and vaginal foul-smelling discharge during sexual intercourse as symptoms of cervical cancer (Tadesse *et al*., 2022).

A study conducted in Saudi Arabia exposed that most participants (n = 1196) correctly identified vaginal bleeding as a symptom of cervical cancer; similarly, 43.7% and 19.3% correctly identified dyspareunia and leg pain as signs of cervical cancer (Zahid *et al*., 2022). A recent study in Central Ethiopia revealed that 45.2% did not know the sign and symptoms of cervical cancer (Gebisa *et al*., 2022).

According to a study done in Bangladesh with 956 women, symptoms of cervical cancer include lower abdominal heaviness, foul-smelling vaginal discharge, weight loss, bleeding between periods, abdominal pain after sexual activity, bleeding after sexual activity, and vaginal bleeding after menopause (Qayum *et al*., 2021).

Lack of symptoms or 'no reason' was the primary reason for not undergoing screening for cervical cancer. Knowledge about cervical cancer's signs and causes can help lessen the disease's burden. Women unaware of the symptoms of cervical cancer are less likely to seek medical attention for such signs, which could result in cervical cancer not being discovered in its early stages (Qayum *et al*., 2021).

2.10. Recognizing risk factors

Human papillomavirus (HPV) is the most important risk factor for getting cervical cancer. Additional risk factors include long-term oral contraceptives, immune-suppressive illnesses, and numerous pregnancies (Sudha *et al*., 2022). Participants ' non-attendance at screening programs is a significant reason for rising cervical cancer occurrence (Andrae *et al*., 2008).

According to the Alam *et al*., 2022 study, women who lived in urban regions were more aware of the risk factors than women who lived in rural areas. The most prevalent risk factors identified by Bangladeshi women were early initiation of sexual activity, multiple sexual partners, and long-term use of oral contraceptives. The least recognized risk factors in rural and urban locations were not getting a routine Pap screening test and having HPV. The beneficiaries awareness of the disease impacts cancer prevention and screening methods. In one study, 49% of participants were unaware of the cervical cancer risk factors in Central Ethiopia (Gebisa *et al*., 2022).

According to a study by Qayum *et al*., 2021 conducted in Bangladesh, risk factors included getting married early (55%), sexually transmitted illnesses (42%), unhygienic cervix conditions (33%), having several sexual partners (15%), having children more than five times (14%), the Human Papilloma Virus (13%), persistent use of contraceptives > 5 years (11%), habit of smoking (9%), and habit of using alcohol or drugs (7%). Only 13% knew HPV as a risk factor for cervical cancer. In accordance with Badawy *et al*., 2022, most students do not see HPV infection as a substantial risk factor for cervical cancer, with just 25.72% (n=65) of students admitting that HPV infection is a risk factor. Research on nursing students in Saudi Arabia revealed similar results, showing that only 12.3% (n=16) had a good grasp of HPV infection and its connection to cervical cancer (Eittah *et al*., 2020). Tiiti *et al*., 2022 reported that the participants were questioned regarding the risk variables that can develop cervical cancer. Among the participants, only 18.8% correctly identified the risk factors. Only 16.0% of people correctly identified one risk factor, 2.7% correctly identified two, and 0.2% correctly identified three. STIs (8.6%), HPV (4.6%), and having multiple partners (4.0%) were the risk factors that were most often reported.

The assessment made clear that multiple sexual partners, long-term oral contraceptive usage, early marriage, unclean cervix conditions, and multiple pregnancies were all listed as significant risk factors for cervical cancer. HPV infection is also a risk factor, although study participants were unaware of the HPV infection and it was regarded as a substantial risk factor.

2.11. Prevention strategies – Overview

The broad implementation of cytological screening programs makes cervical cancer easily avoidable. Compared to women who had never been examined before the study, those who had previously used Pap screening services were more likely to be knowledgeable about HPV. The reason these women knew more may have been revealed during the screening process. There was a link between not getting screened and not being aware of the cervical cancer risk factors (Tiiti *et al*., 2022).

Titii *et al*. 2022 found that women who had previously received Pap smear screening were more likely to have a very good awareness of HPV (60.6%) than those who had not been checked (39.4%). Nevertheless, fewer than half of the participants (47.1%) reported having had a cervical cancer screening in the past, and of those who did, the majority (43.0%) had done so within the previous five years. According to Subba *et al*., 2022, just one-tenth of the doctors and less than 1% of the nurses have ever completed screening.

In Bangladesh, only 8.7% of women knew that a Pap smear is a cervical cancer screening test (Alam *et al*., 2022). Similar results were found in an earlier study conducted in India, where 7% of participants reported that CC could be identified through a Pap smear (Patra *et al*., 2017). Terefe and Gaym (2008) investigated KAP of cancer cervix in patients seeking treatment for reproductive health at three teaching institutions in Addis Abeba, Ethiopia. Most respondents said they were unaware of Pap smear screening. Those who knew about the exam got their information from medical institutes. The younger generation knew more than, the older ones did. These authors contend that the city's health education must be improved.

Around 35% of professionals should have advised eligible beneficiaries to undergo screening (Subba *et al*., 2022). Shekhar *et al*. reported that nearly 90% had never referred patients for screening. This shows a poor attitude toward cancer screening. Over half of the participants knew how to prevent cervical cancer, including stopping smoking, avoiding many sexual partners, and avoiding early sexual activity (Tadesse *et al*., 2022). At the same time, a Spanish study found that early detection and HPV vaccination can prevent 67% of cervical cancer (Navarro-Illana *et al*., 2014).

According to Tadesse *et al*. (2022), 370 people (55.5%) and 297 people (44.5%), respectively, exhibited unfavourable attitudes toward cervical cancer screening. The perception of the respondents, held by around two-thirds, was that cervical cancer might affect any woman. Most responders (73.2%) felt that screening can help prevent cervical cancer. From the observation of different study

population responses, this review stated that numerous variables, such as low levels of education and awareness, low levels of perceived risk, delayed signs and symptoms in the early stages, social stigma, cancer fear, cost, familial commitments, and humiliation, can be blamed for the low uptake of cervical cancer screening.

2.12. Factors responsible for HPV persistence and cervical carcinogenesis

Cervical cancer, like many other forms of cancer, is a chronic, complex disease brought on by a confluence of environmental factors as well as inherited genetic elements (Nelson and Mirabello, 2023; Hanahan, 2022). HPV infections have been shown to be inadequate on their own to cause cancer, despite being a significant environmental risk factor (Okunade, 2020). The fact that 60% of HPV infections spontaneously resolve within a year and 90% do so within two lends credence to this theory, with relatively few cases showing an inherent predisposition to develop into precancer or cancer (Bruno *et al*., 2024). In order to gain a better knowledge of host–virus interactions (Tempera and Lieberman, 2021; Lieberman, 2016) and the general aetiology of cervical carcinogenesis (Bowden *et al*., 2021; Hu *et al*., 2015), it is imperative that efforts be made to uncover inherited genetic risk factors (Dai *et al*., 2017).

2.13. Genetic and epigenetic factors involved in HPV-host interactions

The human papillomavirus family has five genera (α, β, γ, μ, and ν), 48 species, and 206 types (Kombe Kombe *et al*., 2021). HPV types contribute differently to cervical carcinogenesis. Thirteen high-risk (HR) HPV types, fourteen possibly high-risk HPV types, and other low-risk HPV types represent the responsive classification related to oncogenic degree (Näsman *et al*., 2020). Of the HR HPV kinds, HPV16 was responsible for over half of cervical cancer cases worldwide, with HPV18 accounting for the second-highest percentage of cancer cases (16.5%) (Yu *et al*., 2020). Apart from genotypes, HPV intra typic variations are also relevant in terms of epidemiology and carcinogenicity in relation to cervical cancer (Hu and Ma, 2018). A global investigation found that, in contrast

to HPV16, HPV18 subtypes did not significantly correlate with the risk of cervical cancer or histological types (Rader *et al*., 2019).

Furthermore, it is important to do more research to determine how ethnic genome variability, geographic location, and behavioural factors affected the oncogenic differences among HPV intra types in order to provide thorough guidelines for therapeutic application.

2.14. HPV integration

Cervical carcinogenesis involves a crucial molecular step called integration of the HPV genome into the host chromosome (Hu *et al*., 2015). Several investigations have demonstrated that HPV integration typically entails the disruption of viral E1 and E2 open reading frames, leading to the overexpression of oncogenes E6 and E7 (Oyervides-Muñoz *et al*., 2018; Zhang *et al*., 2016). Multiple cellular targets of E6 and E7 facilitate the promotion of malignant transformation. For example, E6 binds to and degrades pro-apoptotic protein BAK and tumour suppressor p53, enhancing host cell resistance to apoptosis and allowing viral DNA replication (Szymonowicz and Chen, 2020). Contrarily, E7 promotes the cyclin-dependent kinase 2 (CDK2)/cyclin A and CDK2/cyclin E complexes inhibiting tumour suppressor retinoblastoma 1 (RB1) to release E2F transcription factors and preventing cell cycle arrest while promoting proliferation (Mir and Sofi, 2023).

Genome-wide profiling of HPV integration sites is becoming practical and affordable with the advancement of next-generation sequencing (Zhang *et al*., 2016). Recent technological developments enable researchers to analyse HPV integrations in bigger samples with extreme sensitivity, providing fresh insights into the underlying mechanisms of HPV integrations. A unique multiplex technique called TEN16 was described by Xu *et al*. in 2013 for the sequence determination of HPV16 DNA integration sites based on next-generation sequencing. This strategy allowed for the simultaneous investigation of HPV16 integration sites in a single combination of around 50 tumour samples (McBride and Warburton, 2017).

2.15. Role of somatic mutations

A significant component of researching cervical carcinogenesis has been examining somatic mutations of the host genome during HPV-induced carcinogenesis, in addition to HPV integrations into the human genome of the host genes. Analysis of DNA mutations is crucial for distinguishing cancerous from non-cancerous cells and for directing treatment and diagnostic protocols. The most thorough genomic landscape paper to date was released in the Nature Journal and used NGS analysis to identify both novel and known high frequency mutations. The authors discovered that the most common mutations in squamous cell carcinoma were EP300 (16%), FBXW7 (15%), PIK3CA (14%), HLA-B (9%), and p53 (9%), whereas PIK3CA (16%), ELF3 (13%), KRAS (8%), and CBFB (8%) were found in adenocarcinoma (Ojesina *et al*., 2014).

Notably, driver mutations in cervical malignancies were recently found to be present in the oncogenes HLA-B, EP300, and FBXW7 (Ojesina *et al*., 2014). In addition to these new driver mutations, previous genetic studies using polymerase chain reaction (PCR) and Sanger sequencing (Xiang *et al*., 2015) or spectrometry-based mutation analysis (Hu and Ma, 2018) have already reported some significant common mutations, such as the oncogenes PIK3CA, EGFR, KRAS, and the gene suppressors PTEN, p53, STK11, and MAPK genes (Spaans *et al*., 2015; Frumovitz *et al*., 2016; Chakraborty *et al*., 2015). Numerous researchers have also validated the above results in various cervical cancer samples from various demographics with to advancements in the NGS test (Inaki and Liu, 2012).

Certain genetic alterations have a significant potential use as early cervical cancer screening biomarker. As a result, a wide range of research has examined the somatic mutation spectrum in CINs to cervical malignancies and discovered possible early diagnostic gene mutation markers, including the gene suppressors PTCH1 and EGFR, as well as the oncogene EGFR and PIK3CA (El Hamdani *et al*., 2010; Tornesello *et al*., 2014; Verlaat *et al*., 2015). In both the normal and ASCUS cytology states, no mutations were observed; however, in the cases of LSIL, HSIL, and SCC, the rate of mutation detection was rising (Pereira *et al*., 2024).

2.16. Challenges in NGS data integration

Along with accumulation NGS data of cervical cancer, molecular characterization of different levels from genome to metabolism becomes gradually clear. However, achieving management of NGS data by integrative analysis proposes huge challenge but inevitable task to classify virus-induced carcinogenetic subgroups by comprehensive molecular features, which is the guidance of ultimate clinical application of unbiased NGS strategy. Recently, the Cancer Genome Atlas Research Network conducted a multi-omic analysis of invasive cervical cancer on 228 extended samples (Iseas *et al*., 2024; Janiszewska *et al*., 2024).

It combines multiple platforms including whole-genome sequencing (WGS), whole-exome sequencing (WES), RNA-seq, microRNA-sequencing (miRNA-seq), DNA methylation profiling, and reverse phase protein array (RPPA) to classify molecular subtypes of cervical cancers (Siegel *et al*., 2021). Generally, this research defines three clusters keratin-low squamous, keratin-high squamous, and adenocarcinoma rich with different molecular features mainly based on mRNA expression analysis (Meijer and Steenbergen, 2017). And for the first time, this study predicts endometrial-like (UCEC-like) cancers with ARID1A, KRAS and PTEN mutated, low copy number, low CpG island hypermethylated (CIMP-low), and RPPA hormone-associated features (Siegel *et al*., 2021). As for high-risk HPV-related carcinogenesis, it first reports type-associated molecular signature except integration (Meijer and Steenbergen, 2017). Of note, the most important value of this article is the intention of serving clinical translation and reasoning. By structural variation analysis, the TCGA researchers identified BCAR4, CD274 (PD-L1), and PDCD1LG2 (PD-L2) linked rearrangement events. PD-L1 and PD-L2 are two important immune checkpoints as well as promising immunotherapy targets. Furthermore, the integrative analysis revealed >70% genomic alterations in either P13K-MAPK or TGFβ pathway, also indicating potential therapeutic agents. Besides, other contribution of this paper includes five new hypermutated genes and strong correlation of APOBEC somatic mutation (Siegel *et al*., 2021).

2.17. Breakthrough approaches and solutions for cervical cancer early treatment and prevention

Pap cytology has been used for secondary cervical cancer screening for almost 50 years. The Bethesda System was established in 1988, and newer techniques such as liquid-based cytology (LBC) and process automation emerged in the 2000s (Plagens-Rotman *et al*., 2023). Alternative cotesting of HPV and Pap smears, as well as HPV genotyping, were developed in tandem with the growing prevalence of HPV infection, particularly high-risk HPV strains, and the maturation of detection methods (Stoler *et al*., 2023). A notable invention that came after the HPV test was the prophylactic HPV vaccination, which was authorised in the 2000s. The FDA has approved the quadrivalent vaccine Gardasil, which prevents infection with HPV6/11 added, the bivalent vaccine Cervarix against HR-HPV16/18, which is responsible for 70% of cervical cancer cases, and the nine-valent vaccine Gardasil9 against an additional five varieties, 31, 33, 45, 52, and 58. The primary prevention of HPV-related cervical cancer with the HPV vaccine showed remarkable promise, and numerous nations have successively started national immunisation programmes for females between the ages of 9 and 25 before they begin engaging in sexual activity. The existing prevention and screening guidelines are not yet sufficiently prepared to distinguish between girls who have had HPV vaccinations and those who have not, even though the vaccinated girls have reached screening age (Aggarwal *et al*., 2024).

2.18. Cervical screening using current HPV testing methods

Since prolonged exposure to high-risk HPV strains is a prerequisite for the development of cervical cancer, HPV detection has progressively replaced cervical cytology as the main screening technique for the disease in recent years. HPV test development is still expanding quickly. If HPV is to be found in cervical tissues, at least 193 distinct HPV tests are currently available (Poljak *et al*., 2024). Comprehensive clinical data are desperately needed to assess the effectiveness of novel approaches.

A popular method for identifying HPV infections, HSIL, and cervical cancer is the Hybrid Capture 2 (HC2) HPV DNA test (Taro *et al*., 2024). The FDA authorised the Cervista HPV HR test in 2009. According to a number of clinical investigations, the Cervista HPV HR test may have the advantage of having less cross-reactivity with other HPV types and may be able to detect high-risk HPV with greater accuracy than the HC2 test (Bennett, 2024). In 2011, the FDA authorised the Cobas 4800 HPV test. The HPV E6/E7 oncogene mRNA test has recently been replaced by the HPV E6/E7 mRNA test. Research indicates that whereas E6/E7 mRNA expression may be minimal during short-term infections, it is overexpressed during long-term infections (Kannappan *et al*., 2021).

2.19. Prospects for NGS-based HPV testing in the future

Developing new NGS technologies have the potential to get beyond these restrictions in the near future. Research on coinfection between HPV types and HPV integration sites in the human genome has already been conducted utilising TEN16 or HIVID technique (McBride and Warburton, 2017; Hu *et al*., 2015).

NGS-based HPV screening may be used in the following future scenarios:

(i) Identifying HPV integration sites in the human genome for risk stratification;

(ii) Accurately identifying HPV types in cervical lesions;

(iii) Conducting epidemiological surveillance of the distribution of both low- and high-risk HPV types; and

(iv) Finding novel HPV genotypes.

Though it's evident that the technique has benefits and drawbacks, statistics on NGS-based HPV detection are still missing. More crucially, virus integration state and specific location based on chimeric readings and other methods can be detected with newly developed software. Even while softwares like BATVI (Tennakoon and Sung, 2017), ViralFusionSeq (Li *et al*., 2013), Virus-Clip(Ho *et al*., 2015), VirusFinder (Wang *et al*., 2013), VirusSeq (Chen *et al*., 2013), Vy-PER (Forster *et al*., 2015) has the ability to detect viruses related to cancer with high

sensitivity and specificity in a variety of NGS data, such as whole-genome sequencing, targeted sequencing, and RNA-seq; implementing this software in clinical practice is still very difficult.

Most software still depends on PCR-based Sanger sequencing to verify accuracy because of the complicated variance caused by HPV integration, which makes standardisation problematic in many situations. Importantly, for most users, particularly clinicians, who lack bioinformatic knowledge and skills, the application is severely limited, and the transformation period will be far longer.

Ultimately, it is feasible that NGS-based assays will exceed traditional HPV testing assays like Cobas 4800 in terms of cost-effectiveness. However, before this technology can be applied to regular diagnoses, complete automation, standardisation of protocols for library preparation and sequencing, and the development of a sophisticated and user-friendly interpretation tool are needed (Tsakogiannis *et al*., 2017).

2.20. Immunotherapy of cervical cancer

The treatment of early-stage cervical disease has advanced along with the prevalence of HPV vaccination and cervical cancer screening; these factors taken together will continue to lower the incidence and death of cervical cancer (Siegel *et al*., 2014). Nonetheless, for patients with recurring, advanced-stage, or invasive cervical malignancies. There are few options available with traditional treatment, which includes radiation, surgery, and systemic chemotherapy. These patients continue to experience excruciating agony and negligible chances of recovery from their treatments, and they do not profit from the findings of studies on the interactions between the host and HPV during cancer. Even while HPV is the primary cause of nearly all cervical malignancies, additional tumor-promoting factors—especially the blatant evasion of immune surveillance—are required for the malignancy to proceed over decades after infection (Smola, 2017). Consequently, novel approaches like immunotherapy offer a promising new direction in the management of HPV-driven carcinogenesis. In an effort to increase

the survival rate of patients with few options, clinical studies and research on modified immunotherapy are still ongoing.

2.21. Genome editing tools for treatment of HPV infections

Recently, the particular DNA sequence of HPV has been cut using artificially built genome editing techniques such zinc finger nucleases (ZFNs), Tal-effector nucleases (TALENS), and RNA-guided engineering nucleases (RGENs or CRISPR/Cas9). Double-stranded DNA breaks (DSBs) caused by these specially designed endonucleases should, in most circumstances, initiate DNA repair pathways (NHEJ repair pathway), which will disrupt target viral oncogenes and eradicate HPV infections.

In the early stages of developing HPV-targeted genome editing tools, ZFNs were intended to damage the E6 or E7 genes of high-risk HPV strains 16 or 18. According to these studies, ZFNs have the ability to damage HPV genomes in vitro culture models and HPV-positive cell lines. Notably, a study revealed that injected ZFNs may even prevent HPV-positive tumour xenografts from growing, suggesting that they have anticancer therapeutic potential (Periwal, 2017).

With the advent of CRISPR, this extensively used approach has been extended to target the E6 or E7 genes of HPV types 6, 11, 16, or 18. Numerous in vitro cell culture models and HPV-positive tumour xenograft models have demonstrated the antiviral efficacy of CRISPR/Cas9. When HPV E6 and E7 were disrupted, the corresponding viral proteins were downregulated, which in turn restored the expression of the tumour suppressors p53 and pRb (Mali *et al*., 2013).

Antiviral therapy based on genome editing may be important in the development of cervical cancer linked to HPV. Patients in precancerous stages, such as those with persistent HPV infections and the CINs associated with them, would be able to undergo HPV testing to identify the HPV subtype(s) and then choose the appropriate type-specific genome editing tools to treat the relevant HPV infection(s) and associated precancerous diseases if this therapy is appropriately combined with HPV testing. Instead of potentially overtreating these patients with cold knife conization, colposcopy-directed biopsies, and recurrent

screenings, this new "screen-and-immediately-treat" approach might be beneficial. As a result, extra expenses, patient worry, and unfavourable outcomes (such vaginal bleeding and cervical insufficiency) that may arise from recurrent screenings, colposcopy-directed biopsies, and cold knife conization should be prevented (Hu and Ma, 2018).

Cervical cancer should be precisely prevented, diagnosed, and treated in light of the novel concepts and technology for cancer therapies that are emerging. Understanding the molecular mechanism behind HPV persistence and cervical cancer can aid in predicting the prognosis of patients with HPV infections earlier on. The use of molecular classification based on genetic profiling and HPV integration may also be applied to precision medicine, which would enable doctors to concentrate more medical resources on high-risk patients whose conditions are worsening. This would significantly lessen the psychological and financial costs associated with HPV vaccination and cervical screening programmes going forward.