1. Introduction

1.1 Cancer

Cancer is a diseased state of cell due to abnormal cell growth with potential to invade other parts of the body. Cancer results from somatically acquired genetic and epigenetic changes that drive the cells to state of unregulated proliferation with altered morphology (Kim & Hahn, 2007). Carcinomas account for over 80% of human cancers with oral, skin, lung, colon, breast, prostate and uterus as the most frequent sites. Cancer is a multistep process which consists of three phases – initiation, promotion and progression (Chiang et al., 2006):

Initiation – This phase is rapid and irreversible event that occur when a normal cell is exposed to carcinogen, which causes unrepairable DNA damage (mutagenic).

Promotion – It denotes sustained clonal expansion of mutated cell to form actively proliferating state and is resistant to programmed cell death (apoptosis).

Progression – This stage involves cellular and molecular changes that occur from preneoplastic to the neoplastic state where more genetic instability and disruption of chromosome integrity is seen (Umar et al., 2001).

In 2005, 7.6 million people died of cancer out of 58 million deaths worldwide. More than 70% of all cancer deaths occur in low and middle-income countries, where resources available for prevention, diagnosis and treatment of cancer are limited or nonexistent. Based on projections, cancer deaths will continue to rise with an estimated 9 million people dying from cancer in 2015, and 11.4 million death in 2030 (Rebecca et al., 2015). Among the different types of prevailing cancer, the solid tumours are the predominant one and one of such localized tumour that occur in the glandular and epithelial cells is the cervical cancer (Figs. 1 & 2).

![Figure 1. Classification of solid tumour](image-url)
Cervical cancer

It is the cancer of the cervix – lower, narrow end of the uterus, extending into the upper end of the vagina. Cancer starts in the cells on the surface of the cervix- two types of cervix surface epithelium are squamous and columnar- squamous cell carcinoma begins in the thin, flat cells that line the cervix. Adenocarcinoma begins in cervical cells that make mucus and other fluids. Cervical cancer usually develops very slowly, which starts as a precancerous condition called dysplasia. Undetected precancerous changes can develop into cervical cancer which can spread to the bladder, intestines, lungs and liver. It can take years for precancerous condition to turn into cervical cancer. Histologic subtypes of invasive cervical cancer includes, Squamous cell carcinoma (~85%), Adenocarcinoma (~ 15% of cervical cancer in the UK), Adenosquamous carcinoma, Small cell carcinoma and neuroendocrine carcinoma. Though the incidence of squamous cell carcinoma has been reported in many cases, the adenocarcinoma of the cervix cases has been increasing in recent decades (Rebecca et al., 2015). Non – carcinoma malignancies which can rarely occur in the cervix include melanoma and lymphoma. Patients with cervical cancer do not usually have problems until the cancer develops to an advanced stage or metastatic.
The diagnosis of tumour ‘staging’ is based on semiquantitative assessment of the clinical gravity of the disease. A complete profile can be built up by assessing the size of the primary tumour, the extent of local lymph node involvement and the presence or absence of distant metastasis. In this tumour node metastasis (TNM) staging, the larger the primary tumour with more local nodes involvement then it is more advanced the stage with a concomitantly poorer prognosis. Significantly, the presence of metastatic disease immediately assigns the patient to the most advanced stage, irrespective of the size of the primary tumour, highlighting the importance of early detection and intervention so as to save the patient.

Cervical cancer is graded by the International Federation of Gynecology and Obstetrics (FIGO) staging system, which is based on clinical examination, rather than surgical findings. It allows only the following diagnostic tests to be used in determining the stage: palpation, inspection, colposcopy, endocervical curettage, hysteroscopy, cystoscopy, proctoscopy, intravenous urography and X-ray examination of the lungs and skeleton and cervical conization. The TNM staging system for cervical cancer is analogous to the FIGO stage.

**FIGO staging of cervical carcinomas**

**Stage 0** – full thickness involvement of the epithelium without invasion into the stroma (carcinoma in situ)

**Stage I**

Stage I is carcinoma strictly confined to the cervix; extension to the uterine corpus should be disregarded. The diagnosis of both stages IA1 and IA2 should be based on microscopic examination of removed tissue, preferably a cone, which must include the entire lesion.

**Stage IA:**

Invasive cancer identified only microscopically. Invasion is limited to measured stromal invasion with a maximum depth of 5 mm and not wider than 7 mm.

Stage IA1: Measured invasion of the stroma not greater than 3 mm in depth and not wider than 7 mm diameter.

Stage IA2: Measured invasion of stroma greater than 3 mm but no greater than 5 mm in depth and not wider than 7 mm in diameter.
Stage IB:  
Clinical lesions confined to the cervix or preclinical lesions greater than Stage IA. All gross lesions even with superficial invasion are stage IB cancers.  
Stage IB1: Clinical lesions not greater than 4 cm in size.  
Stage IB2: Clinical lesions greater than 4 cm in size.  

Stage II  
Stage II is carcinoma that extends beyond the cervix, but does not extend into the pelvic wall. The carcinoma involves the vagina, but not as far as the lower third.  
Stage IIA: No obvious parametrial involvement. Involvement upto upper 2/3rd of the vagina.  
Stage IIB: Obvious parametrial involvement, but not into the pelvic sidewall.  

Stage III  
Stage III is carcinoma that has extended into the pelvic sidewall. On rectal examination, there is no cancer-free space between the tumour and the pelvic sidewall. The tumour involves the lower third of the vagina. All cases with hydronephrosis or a non-functioning kidney are Stage III cancers.  
Stage IIIA: No extension into the pelvic sidewall but involvement of the lower third of the vagina.  
Stage IIIB: Extension into the pelvic sidewall or hydronephrosis or non-functioning kidney.  

Stage IV  
Stage IV is carcinoma that has extended beyond the true pelvis or has clinically involved the mucosa of the bladder and/or rectum.  
Stage IVA: Spread of the tumour into adjacent pelvic organs.  
Stage IVB: Spread to distant organs.  

Epidemiology  
Cervical cancer is the fourth most common cause of cancer and second in cause of female specific cancer related deaths among women. About 80% of cervical cancers occur in developing countries. In 2014, 12,360 new cases were diagnosed and about 4,020 have died of cervical cancer. An estimated 12,900 new cervical cancers and 4,100 cervical cancer deaths will occur in the United States in 2015.
Diethylstilbestrol (DES), which is a hormonal drug for women with high chances of miscarriage given between the years 1940 and 1971 were found to be of high risk not to the women taking drugs but to the women’s daughters. About 1 in 1000 of these women develop cervical cancer. Among those mothers took the drug during the first 16 weeks of pregnancy were found to be of higher risk. The drug however no longer in use. Family medical history can be a major factor for any female not just those mother’s used the diethylstilbestrol hormone medication but also women whose family has a history of cervical cancer (Herbert & Coffin, 2008).

**Etiology**

**There are few causes and risk factors for cervical cancer**

1. **Multiple sexual partners and early sexual activity**
   
   Cervical cancer-causing HPV types are nearly always transmitted as a result of sexual contact with an infected individual. The women who had many sexual partners generally prone to higher risk of HPV infection, which leads to cervical cancer. There is also a link between becoming sexually active at a young age and the occurrence of higher risk of cervical cancer. However, if a woman develops cervical cancer it does not always mean that she had several sexual partners or became sexually active earlier than most other females. It is just a risk factor. Women who only ever had one sexual partner can also develop cervical cancer.

2. **Other factors**
   
   Genetic mutations, multiple pregnancies, long-term use of the oral contraceptive pills, immune supression women infected with *Chlamydia, Gonorrhea or Syphilis* eventually led to higher risk of developing cervical cancer.

3. **Human Papilloma virus in cervical cancer**
   
   Human Papilloma Virus (HPV) infection appears to be involved in the development of more than 90% of cases of cervical cancers. Human papilloma virus (HPV) are a group of viruses which are associated with various proliferative diseases in the infected epithelium (Pfister, 1984). There are more than 70 HPV types have been reported. Among these HPV types *viz:* 6, 11, 16, and 18 are the most
common which are associated with lesions in the anogenital tract (Pfister, 1987). HPV belong to the papovaviridae family. They consist of 72-capsomere capsid containing the viral genome. Capsomers are composed of two structural proteins: the 57 kDa late proteins L1, which accounts for 80% of the viral particle, and the 43-53 kDa minor capsid protein L2.

Several clinical, molecular, and epidemiological investigations have identified that human papillomavirus (HPV) is the major cause of cervical cancer and cervical dysplasia (Walboomers et al., 1999). Estimates of the number of cervical cancer deaths are around 250,000 per year. The prevalence of genital HPV infection in the world is around 440 million. There are over 100 genotypes of HPV, 40 of which infect human mucosal areas of the upper digestive tract and the ano-genital tract. These are grouped into "high-risk" and "low-risk" types according to the degree of risk of development of cancer after infection with each genotype.

Genital HPV infection is extremely common and most often without any symptoms. A proportion of individuals infected with low-risk HPV types such as HPV-6 or HPV-11 will develop genital warts, whereas a subset of women with high-risk HPVs such as HPV-16 or HPV-18 will develop preneoplastic lesions of cervical intraepithelial neoplasia (CIN). Low-grade cervical dysplasias are common and most regress spontaneously. In contrast, the minority of lesions that progress to high-grade dysplasias tend to persist and/or progress to carcinomas in situ before becoming invasive cancers. The majority of adenocarcinomas of the cervix and of squamous cell cancers (SCC) of the vulva, vagina, penis and anus are caused by HPV-16 and HPV-18 (together accounting for about 70% of cases globally), the remaining 30% being due to other high-risk HPV types (such as HPV-31, 33, 35, 39, 45, 51, 66). The relative importance of different high-risk types varied between countries and regions, but HPV-16 has the greatest contribution to cervical cancer in all the regions. HPV is also associated with other types of cancers of the anus, head and neck, and rarely, recurrent respiratory papillomatosis in children (Dayyani et al., 2010).

The two proteins which play a significant role during the onset of this malignancy are; E6 and E7 proteins whose expression has been seen in the cell lines (Herdman et al., 2006). HPV 16 E7 proteins are small proteins consisting of 98 amino acids and zinc binding phosphoproteins are confined in the nucleus. This high risk
HPV E7 protein has a higher binding affinity for pRb protein (Darnell & Schroder, 2007). The HPV 16 is a small non-enveloped virus containing double stranded DNA as its genetic material. Its genome has seven functional coding regions in which E7 codes for viral protein that bind to the retinoblastoma tumour suppressor proteins. In the absence of Rb, E2F permits the cell to progress through the cell cycle without normal mitogenic signals (Conway & Christensen, 2009).

**Gene regulation in cervical cancer**

The combined action of both the viral proteins, E6 and E7, causes an expansion in the population of DNA replication-competent cells, in which the host cell DNA replication machinery is reactivated, allowing high-level amplification of the viral genome (Doorbar, 2006). Normally, the induction of such a replicative cycle in the host cell is highly controlled and efficient, but, for reasons that are still unclear, the controls sometimes break down and events can occur that lead to host cell immortalization and, ultimately, to cancer. During this process, the viral genome often becomes integrated into the host DNA, with the concomitant loss of expression of many viral gene products, effectively causing the ‘death’ of the virus as a replicating entity. However, in cases where the E6 and E7 proteins continue to be expressed the cells survive and, eventually, can undergo malignant transformation (Smotkin & Wettstein, 1986; Androphy et al., 1987; Banks et al., 1987).

Now it is known that these two proteins alone are responsible for the drive towards malignancy. They are invariably expressed in tumours and in cell lines derived from them, even many years after the initial transforming events, suggesting that they are required for the maintenance of the transformed phenotype. Indeed, this requirement has been confirmed in many studies using a variety of techniques to investigate the expression and/or activities of these proteins, including ribozymes (Alvarez-Salas et al., 1998), anti-sense RNA (Steele et al., 1992; von Knebel Doberitz et al., 1992), RNA interference (Butz et al., 2003; Yoshinouchi et al., 2003) and blocking peptides (Butz et al., 2000). In all these cases, the cells cease to grow and enter either apoptotic or senescent states.
Thus, both of these viral gene products represent ideal therapeutic targets for intervention in HPV-induced malignancy.

The two proteins have complementary activities, with E7 being largely responsible for driving cell proliferation and E6 for enhancing cell survival. This is exemplified by the cooperative activity of the two proteins in bringing about immortalization of keratinocytes, the natural target cells of the virus in vivo. Primary human keratinocytes have quite a short lifespan in tissue culture before they cease to proliferate and senesce. However, the combination of E6 and E7 is a very potent inducer of keratinocyte immortalization, whilst either protein alone exhibits only weak activity in such assays (Barbosa & Schlegel, 1989; Hawley-Nelson et al., 1989). Likewise, in the skin of transgenic mice, E7 is a potent inducer of benign cell proliferation, while the addition of E6 results in much more aggressively transformed lesions (Song et al., 2000). The precise mechanism by which E6 exerts its transforming activity is still under investigation; however, it seems very likely that the ability of E6 to target cell polarity regulators will be an important factor.

The E6 and E7 oncoproteins of high-risk HPVs, particularly HPV 16 and 18, bind respectively to the p53 and Retinoblastoma (Rb) tumour suppressor proteins, which are involved in the regulation of growth control. Moreover, the E6 proteins of high-risk HPVs bind more effectively to the p53 protein, leading to their degradation via a ubiquitin-mediated pathway, than those of lower-risk HPVs (Figs. 3 & 4).
Figure 3. Infection and replication of human papilloma virus in the epithelial cells

Figure 4. Schematic representation of the action of HPV oncoproteins E6/E7 and cell cycle regulatory proteins in cervical carcinogenesis (Young & Min, 2005).
Symptoms

During the early stages of cervical-carcinoma, the affected women may remain asymptomatic. Hence, women, especially perimenopausal, should undergo regular cervical smear tests. The most common symptoms of cervical cancer are:

- Bleeding between periods
- Bleeding during or after sexual intercourse (contact bleeding)
- Bleeding in post-menopausal women
- Discomfort during sexual intercourse (dyspareunia)
- Foul smelling vaginal discharge
- Sanguineous vaginal discharge (discharge tinged with blood)
- Pelvic pain

Prevention

Avoidance of infection by human papilloma virus (HPV)

HPV is one of the main causes of carcinoma cervix (invasive and precancerous stages) and avoidance of exposure to HPV can prevent this disease. HPV can be transmitted sexually (spread during sex- including vaginal intercourse, anal intercourse, and oral sex) and it is not that the mode of infection is only through sexual contact.

Cervical cancer control

There are four basic components of cervical cancer control under the National Cancer Control Program:

1. Primary prevention
2. Early detection, through increased awareness and organized screening programs
3. Diagnosis and treatment
4. Palliative care for advanced disease

Treatment for cervical Cancer

The treatment of cervical cancer varies worldwide, largely due to access to surgeons skilled in radical pelvic surgery, and the emergence of "fertility-sparing therapy" in developed nations. Because cervical cancers are radiosensitive, radiation may be used in all stages where surgical options do not exist. Treatment options depend on the following:
• The stage of the cancer.
• The type of cervical cancer.
• The patient's desire to have children.
• The patient’s age.

Microinvasive cancer (stage IA) may be treated by hysterectomy (removal of the whole uterus including part of the vagina). For stage IA2, the lymph nodes are removed, as well. Alternatives include local surgical procedures such as a loop electrical excision procedure or cone biopsy. For IA1 disease, a cone biopsy (cervical conization) is considered curative.

• If a cone biopsy does not produce clear margins (findings on biopsy showing that the tumour is surrounded by cancer free tissue, suggesting all of the tumour is removed), one more possible treatment option for women who want to preserve their fertility is a trachelectomy. This attempts to surgically remove the cancer while preserving the ovaries and uterus, providing for a more conservative operation than a hysterectomy. It is a viable option for those in stage I cervical cancer which has not spread; as there are only a few doctors are skillful in this procedure. Even the most experienced surgeon cannot promise that a trachelectomy can be performed until after surgical microscopic examination, as the extent of the spread of cancer is unknown. If the surgeon is not able to microscopically confirm clear margins of cervical tissue once the woman is under general anesthesia in the operating room, a hysterectomy may still be needed. This can only be done during the same operation if the woman has given prior consent. Due to the possible risk of cancer spread to the lymph nodes in stage 1B cancers and some stage 1A cancers, the surgeon may also need to remove some lymph nodes from around the uterus for pathologic evaluation.

• A radical trachelectomy can be performed abdominally or vaginally and opinions are conflicting as to which one is better. A radical abdominal trachelectomy with lymphadenectomy usually requires only a 2-3 days of hospital stay with that most women recovered quickly (about six weeks). Complications are uncommon, although women who are able to conceive after surgery are susceptible to preterm labor and possible late miscarriage. One year gap is generally recommended before attempting to become pregnant after surgery. Recurrence in the residual cervix is very rare if the
cancer has been cleared with the trachelectomy. Yet, women are recommended to practice vigilant prevention and follow-up care including Pap screenings/colposcopy, with biopsies of the remaining lower uterine segment as needed (every 3–4 months for at least 5 years) to monitor for any recurrence in addition to minimizing any new exposures to HPV through safe sex practices until one is actively trying to conceive.

- Early stages (IB1 and IIA less than 4 cm) can be treated with radical hysterectomy with removal of the lymph nodes or radiation therapy. Radiation therapy is given as external beam radiotherapy to the pelvis and brachytherapy (internal radiation). Women treated with surgery who have high-risk features found on pathologic examination are given radiation therapy with or without chemotherapy to reduce the risk of relapse.

- Brachytherapy for cervical cancer

- Larger early-stage tumours (IB2 and IIA more than 4 cm) may be treated with radiation therapy and cisplatin-based chemotherapy, hysterectomy (which then usually requires adjuvant radiation therapy), or cisplatin chemotherapy followed by hysterectomy. When cisplatin is present, it is thought to be the most active single agent in periodic diseases.

### 1.3 Natural compounds in cancer therapy

Viral DNA integration with the host genome and expression of E6 & E7 porteurins result in malignancy of cervical cells. Antiviral therapy targeting the E6 & E7 oncoproteins results in remarkable reduction in cancer progression (Bharathi et al., 2009). The siRNA, therapeutic nucleic acids - antisense oligo DNA and ribozymes against E6/E7 mRNA are considered to be effective in controlling cancer pathogenesis (Dipalo et al., 2004). Intra bodies, therapeutic antibodies and indole derivative compounds (IDCs) are capable of blocking protein – protein interaction that are the possible ways of reducing the HPV replication and capsid protein production and hence the less occurrence of HPV in cells. However, these methods have limitations like narrow specificity and high cost (Keriel et al., 2009).

Recent studies have shown the potential cancer inhibitory property by secondary metabolites like polyphenolic - Anthocyanidin. The anthocyanidins are
aglycones of anthocyanins that impart color in fruits and vegetables. These dietary polyphenols show the free radical scavenging activity, antioxidant, anti-inflammatory and antitumour properties (Shih et al., 2005). Idaein chloride, also known as idein chloride, is an analogue of cyanidin 3-o-galactoside chloride, which exhibits powerful antioxidant property in HT29 clone 19A (Human colon cancer cell line) besides inhibitory role in cellular metabolism (Pool-Zobel et al., 1999). Likewise, Delphinidin and cyanidin exhibited significant radical scavenging activity, inhibition of cell proliferation and induced apoptosis on MCF7 cancer cell line (Tang et al., 2015) and the presence of active compounds like catechin, galangin, homogentisic acid, gallic acid kaempferol & cyanidin 3-glucoside in guava peel possess strong antioxidant and anticancer activity on different cancer cell lines (Chen et al., 2015). Many researchers have reported that the anthocyanin target the signaling networks in cancer cells thereby it induces cell death in a programmed manner (Sehitoglu et al., 2014).

In view of the above, the present study was aimed to target viral oncoproteins using an anthocyanidin-‘idaein chloride’, through in silico and in vitro analyses.