CHAPTER-III

REVIEW OF LITERATURE
Review of Literature:

3.1: Overview:
The cervix is the portion of the uterus which connects the body of the uterus to the birth canal. Cervical cancer is malignant neoplasm of cervix uteri or cervical area. Nearly all cervical cancers arise from the cells of the inner lining of the cervix. Most cervical cancers arise within the transformation zone of the cervix (Burghardt and Ostor, 1983). Cancer of this region comes on slowly over time and usually manifests itself as cells which go from normal to pre-cancer and then eventually turn into cancerous.

3.2: Incidence and Prevalence:
3.2.1: Worldwide:
Cervical cancer is the 3rd most common cancer in women worldwide, with 78% of cases occurring in developing countries where cervical cancer is the second most frequent cause of cancer death in women. Worldwide 527,624 new cervical cancer cases are diagnosed annually and an estimated 265,653 women die of cervical cancer every year (Ferlay J, 2015). It is the 10th most common cause of cancer death in women in Europe as a result of organized screening programs (Davies P et al., 2006). In many developing countries, however, cancer of the cervix has changed little in incidence, except for those countries that have achieved the demographic (epidemiological) transition with increasing affluence from industrialization. In such countries, there has been a fall in incidence of cancer of the cervix, and a rise in incidence in cancer of the breast, similar to changes that occurred in North America and Western Europe in the early part of the last century. When the geographical areas were considered Asia, Latin America, and Sub-Saharan Africa have recorded the highest incidence for cervical cancer (Yang et al., 2004). Whereas areas like N. America and N. Europe have decreased incidence considerably (Vizcaino, et al., 2000).

3.2.2: Indian Perspective:
It has been reported that in India among all types of genital cancer, carcinoma of cervix accounts for 80%, ovary 10% and uterine body 4-5%. (Paul Satya B et al., 2011). As per data of world health meeting for control of cervical cancer (Ferlay et al.,2015), cervical cancer ranks as the 2nd cause of female cancer as well as the 2nd cause of female cancer deaths in the reproductive age group in India. Age-standardized incidence rate is 22 per 100,000
women per year in different regions of India whereas in world it is 14 (Sankarnarayanan R et al., 2008; Ferlay et al., 2015). In India according to last update 20 September 2013 (Ferlay et al., 2015) it is estimated that 122,844 new cases of cervical cancer and 67,477 deaths occur each year. In India correspond to 26.4 per cent of all women dying of cervical cancer globally. Thus, cervical cancer is an important public health problem that deserves urgent attention. As of the 2001 census, trends in the demographic profile have transformed deeply. Both the birth and death rates have been turning down nationwide and the increase in health services has drawn out life expectancy to 62 years (S Malik, 2005). Whereas communicable diseases have in the past been major killers of the Indian population, but recently non-communicable diseases such as malignancy are coming into the forefront(S Malik, 2005).

Many new associations have crop up in the wake and there are several registry programs that help to catalogue data specific to cancer rates in India. The major resource of information comes from the National Cancer registry Program me (NCRP) which was set up in 1991. The NCRP collects numbers from key cities in Barshi, Bhopal, Delhi, Chennai, Mumbai and Bangalore who do the local data collection from the gynecology departments of many hospitals and community clinics (S Malik, 2005, Juneja et al., 2003). In older population based cancer registries (PBCR) (NCRP 2008) Barshi and Chennai PBCRs have always recorded the highest incidence of cervix cancer. Cancer of the cervix has been the most important cancer in women in India over the past two decades (A Nandakumar et al., 2009). All the urban population based cancer registries (PBCR) in India at Delhi, Chennai, Bhopal, Bangalore, Mumbai have shown a statistically significant decrease in the AARs of this site of cancer (A Nandakumar et al., 2009;NCRP 2009). This decline is despite the absence of any organized screening programme. The decline in the AAR varies from 42.3 (in 1982-83) to 22.3 (in 2004-05) per 100,000 in Chennai to a marginal decline in Barshi from 23.5 (in 1988-89) to 22.8. It accounted for 16 per cent of all cancers in women in the urban registries in 2005. However, it constitutes 37 per cent of the cancers in females in Barshi. The highest age specific incidence rate of 98.2 per 100,000 for cancer cervix was observed in the age group of 60-64 yr. Mostly resides in the rural areas, cancer cervix still comprises the number one cancer in either sex.

3.2.3: Northeast India Perspective:

Cervical cancer is the second leading cancer in the female in the Southern part of Assam (ICMR 2009, Silchar Medical College) but interesting fact is that stomach cancer is the leading female cancer in our neighboring state Mizoram. The report of the North Eastern
PBCRs (NCRP 2008) indicates an AAR of 25.4 per 100,000 in Aizawl district of Mizoram state followed by AAR in Imphal West district (20.5) and Kamrup Urban district (17.3) based in 2005-06 report. In the hospital based cancer registries, first is Bangalore and Chennai, the second most important site is Mumbai and Thiruvananthapuram and the third leading site is in Dibrugarh.

3.3: Anatomy of cervix:

![Fig.3.1: Anatomy of female reproductive system and cervix](image)

The cervix is the lower most part of the uterus. It extends from the histological internal os and ends at external os which opens into the vagina after perforating the anterior vaginal wall. It is almost cylindrical in shape and measures about 2.5 cm in length and diameter. Cervix has 2 parts, ectocervix i.e., the part which connects to vagina and endocervix or cervical canal. Ectocervix is lined by squamous epithelium and endocervix is lined by columnar epithelium. The area where endocervix meets ectocervix is called transitional zone where most of the cancer arises.
3.4: Natural history of cervical cancer:

![Diagram of the natural history of cervical cancer]

3.5: Patho physiology:

**Cytologically normal women**: No abnormal cells are observed on the surface of their cervix upon cytology.

**Cervical Intraepithelial Neoplasia (CIN) / Squamous Intraepithelial Lesions (SIL)**: SIL and CIN are two commonly used terms to describe precancerous lesions or the abnormal growth of squamous cells observed in the cervix. SIL is an unusual result derived from cervical cytological screening or Pap smear testing. CIN is a histological analysis made upon analysis of cervical tissue obtained by biopsy or surgical excision. The situation is graded as CIN 1, 2 or 3.

**Low-grade cervical intraepithelial lesions (LSIL/CIN-I)**: Low-grade cervical lesions are defined by early transformation in size, shape, and number of unusual cells formed on the surface of the cervix and may be referred to as mild dysplasia, LSIL, or CIN-I. Mild cervical dysplasia results when irregular cells are limited to the deepest one-third of the surface cell layer (known as the epithelium) that lines the cervix.

**High-grade cervical intraepithelial lesions (HSIL)**: High-grade cervical lesions are defined by a large number of precancerous cells on the surface of the cervix that are distinctly different from normal cells. They have the potential to become cancerous cells and invade deeper tissues of the cervix (WHO/ICO Information Centre, 2010). Moderate cervical dysplasia occurs when uncontrolled cell growth goes on with, and equal to two-thirds of the surface cell layer is abnormal.
**Carcinoma in situ (CIS):** Pre invasive malignancy limited to the epithelium without invasion of the basement membrane.

**Invasive cervical cancer (ICC) / cervical cancer:** If the high-grade precancerous cells invade the basement membrane is called ICC. ICC stages range from stage I to stage IV.

**Table 3.1: Correlation of dysplasia, CIN (WHO) and Bethesda system (D C Dutta, 2013)**

<table>
<thead>
<tr>
<th>Dysplasia</th>
<th>CIN</th>
<th>Limits of histological changes</th>
<th>Bethesda system</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild</td>
<td>CIN I</td>
<td>Basal one third</td>
<td>LSIL</td>
</tr>
<tr>
<td>Moderate</td>
<td>CIN II</td>
<td>Basal half to two-third</td>
<td>HSIL</td>
</tr>
<tr>
<td>Severe</td>
<td>CIN III</td>
<td>Whole thickness except one or two superficial layers</td>
<td>HSIL</td>
</tr>
<tr>
<td>CIS</td>
<td></td>
<td>Whole thickness</td>
<td></td>
</tr>
</tbody>
</table>

**3.6: Types of cervical cancer:**
1. **Squamous cell carcinoma (SCC)** - It is the most common type of cervical cancer, accounting for 85% to 90% of all patients. It arises from the squamous epithelium.
2. **Adenocarcinomas** develop from the columnar cells that line the mucous-producing glands of the cervix. Adenocarcinoma accounts for about 10% of all cervical cancers.
3. **Mixed carcinomas** (for example, adenosquamous carcinomas) combine features of both squamous cell carcinoma and adenocarcinoma.

**3.7: Clinical staging carcinoma cervix:**
The clinical staging of cervical cancer as recommended by Federation of Gynaecological oncology (FIGO) is divided into four stages.
Stage I-The carcinoma is firmly confined to the cervix
Stage II-cervical carcinoma invades further than the uterus, however not to the pelvic wall or to the lower third of the vagina.
Stage III-The tumor expands to the pelvic wall and / or involves lower third of vagina and / or causes hydronephrosis or nonfunctioning kidney.
Stage IV- The carcinoma has extended beyond the true pelvis or has involved (biopsy proven) the mucosa of bladder or rectum. A bulous oedema, as such, does not permit a case to be allowed to stage IV.

3.8: Etiology and associated Risk Factors:
HPV infection is a necessary cause of cervical malignancy, but it is not an enough cause. Other cofactors are essential for succession from cervical HPV infection to cancer. Besides HPV type, there are several cofactors that may contribute to the development of cervical cancer. The risk factor may include other sexually transmitted infections, like Chlamydia, herpes simplex virus-2, HIV infection, prolonged period use of oral contraceptive pill, hormonal factors which can compromise the immune system and makes the host more susceptible to cancer. While these factors definitively has a vital role but in Indian scenario, there are other issues that disproportionately affect poor rural people than urban members of the Indian population (S Malik, 2005). Genetic and immunological host factors and viral factors such as genotype of HPV, viral load and viral integration, are likely to be important factor but still more research is required. (Munoz N et al., 2006; Madeleine, M.M et al.,2007; Berrington de González A et al.,2004; Paul Satya B et al., 2011)

3.8.1: Smoking and Diet:
Cigarette smoking and diets that lack important vitamins have been linked to increased rates of cervical cancer. Tumors associated with cigarette smoking are most often manifested as squamous cell carcinomas and the same are found in the cervix also. One study shows that women who discontinue smoking experienced a reduction in the size of the lesion by approximately 20%. Diets rich in vitamin C and Beta-carotene have been considered protective agents against cervical malignancy. A lot of studies have approved a link between certain levels of micronutrients in the blood and a decreased risk of developing lesions or tumors (Juneja et al., 2003).

3.8.2: Socioeconomic Status:
Studies have shown that women who belong to the lower social classes are more likely to contact and sustain cervical cancer. Patients with poor socioeconomic tracts had significantly higher rates of late stage cancer diagnosis and lower rates of cancer survival (S Malik, 2005; Misra JS,et al., 2009; Vallikad E, 2006). Incidence of death rates also increased with populations who are illiterate or received little to no educational training. Women in the poor
economic classes were less likely to be screened and often times could not afford to go or see a doctor. Ultimately poor economic status is observed to be the confounding factor in higher cervical cancer rates (Juneja et al., 2003). Observations related to lower socioeconomic status and cervical cancer have already been made as poverty is the real context of India (Duggal R, Gangolli LV, 2005). In 2010, black women had the highest rate of getting cervical cancer, followed by Hispanic, white, American Indian/Alaska Native, and Asian/Pacific Islander women. (CDC 2010; Clegg LX, 2009) and that incidence of cervical cancer is highest in women with the lowest socioeconomic status (Clegg LX et al., 2009; Franceschi S et al., 2009). Reasons for ethnic and socio-economic differences in the incidence of cervical cancer can be difficult to determine because definitions of ethnic groups and socioeconomic status are not always consistent and because ethnicity may be confounded with socioeconomic status and other variables, which can or cannot be controlled for analyses (Pruitt SL et al., 2009).

3.8.3: Sexual Behavior:
Cancer cervix is higher with early age of marriage and increasing parity and prolonged sexual period. Cervical cancer and its association with early sexual activity and sexual promiscuity in particular, have been well established in a number of epidemiological studies in the West (Harris R W C et al., 1980; La Vecchia C et al., 1986; Herrero R et al., 1990) and in Asian populations (Donnan S P B 1989). Importance of early age at first coitus lies in the fact that intercourse introduces a carcinogenic agent to the cervical epithelium, which is most susceptible during adolescences (Rotkin I D et al., 1967). Role of HPV infection as the carcinogenic agent has prompted a reconsideration of the role of age at first coitus. Study done by Dasgupta A et al., has mentioned that 59.3% were married before completion of 18 years of age (Dasgupta A et al., 2002). Biswas et al., highlighted that cervical epithelium is more susceptible to carcinogenic agents, exposure to sexual activities and early pregnancy which are well known etiological factors for cancer cervix (Biswas et al., 1997). Mean age of marriage was reported as 20 years in study done by Varghese C et al. 1999. Another study shows that cervical cancer increases with the onset of early sexual activity and the incidence declines as the age of marriage increases (S Malik 2005). However, gather data on sexual behavior within the population of Indian women has proven to be practically difficult. Sex is not a suitable topic of conversation for women and many of them never see a gynecologist at all due to the fear and shame associated with female sexuality. As a result, substantive data on sexual action has not yet been established (Juneja et al., 2003).
3.8.4: Parity:
Multi parity is a well-known risk factor for cancer cervix, focusing not just on the frequency of coitus but also on the assault on cervix during childbirth. R Sultana from Bangladesh also found that the disease was associated with grand multi parity (R Sultana, 2012). Cervical cancer incidence was more pronounced and statistically highly significant when parity were in between 2 and 3 ($p<0.10$) (Misra JS et al., 2009). Sreejata et al. in their study, reported 23.5% of the population had three children and 13.7% had more than three children (Sreejata et al., 2002). Misra J S noticed that frequency of both SIL and the age at which women engage in their first sexual intercourse has also been cited as a risk factor for cervical cancer as damage might be caused to the cervix at a time when it is still developing. The risk of getting HPV and cervical cancer in women who initiated sexual intercourse around age 15 has been shown to be quite higher risk of those who did so after age 20 (Biswas LN et al., 1997). Male circumcision is associated with a reduced risk of penile HPV and less risk of cervical cancer is noticed in their current female partners (Castellsagué X et al., 2003; Castellsagué X et al., 2002).

3.8.5: Gender Dynamics:
Gender dynamics within Indian society also play a very important role in the onset of cervical cancer. Study shows that even with the high incidence cervical cancer in India, the role of sexual promiscuity in Indian study has not been well addressed as the rate of promiscuity among women is known to be very less (S Malik, 2005). The study by S Malik examined the sexual risk factors that recurred among the couples by interviewing each partner of the couple separately and in this case, male promiscuity was discovered to be responsible for the increased risk of cancer amongst women. They also concluded that many women grossly underestimated the number of sexual partners that their husbands had been involved with (Biswas LN et al., 1997). The risk of infection with HPV with ten or more partners has been reported to three to four times higher than the risk associated with just one partner (Juneja et al., 2003). Collaboration between public health professionals and sociologists in informing women about cervical cancer (in a culturally appropriate way) could potentially change the mortality rates for women.

3.8.6: Ethnicity:
Study from various part of the world was conducted to find out the ethnic and racial composition shows that there was no predominant racial or ethnic category (Boyer CB et al.,
2005; Bull SS et al., 2008). In a study of North Karnataka, population was mainly divided into different community and when these communities were considered it was seen that Hindus were of 97.66%, Muslims were 2.03%, and Christians were 0.2% respectively (Shanthala S et al., 2014). The study done by Michelle Kaku it was seen that Hindus were 71%, Muslims were 11% and Christians were 18% (Kaku, Mathew et al., 2008). The reason was explained that the increased number among Hindus may be due to decreased socio demographic status, improper hygiene, multiple sexual partners and increased use OC pills. It was seen that the practice of male circumcision may be the reason for the decreased number in certain community (S B Paul et al., 2011).

3.9: HPV infection:

![HPV virus](image)

**Fig.3. 2: HPV virus**

3.9.1: HPV virus:

Human papillomavirus (HPV) is a member of the papillomaviridae family of viruses that is capable of infecting humans. Like all papillomaviruses, HPV has icosahedral symmetry and are non-enveloped. Seventy-two capsomeres surround the genome, 12 pentamic and 60 hexameric capsomere arranged on a T=72 lattice. Capsomers are composed of two structural proteins, L1 and L 2. The HPV genome consists of eight kilobasepairs (Kbp) and is a double-stranded DNA molecule. HPV is a double-stranded DNA virus that is non-enveloped and has an icosahedral capsid; the virus replicates as an extrachromosomal DNA inside the nucleus of the host cell (Longworth and Laimins, 2004).
3.9.2: HPV genotypes:
HPVs are clinically classified as “low Risk” and “High Risk” type depending on the propensity of the HPV associated lesions to undergo malignant progression (Margaret et al., 2009). Variety of cancers including cervical cancer is being caused by high risk HPV genotypes. High risk types are HPV 16, 18, 31, 35, 39, 45, 51, 52, 56, 59, 66, 68, 69, 73 and 8. Depending upon the infection types HPVs can be divided into cutaneous types mucosal types. (EM Burd, 2003). Cutaneous types of HPV infect basal epithelial cells of the skin such as skins of the hands and feet. Mucosal types infect the inner lining of tissues such as lining of the oral cavity, respiratory tract, genital tract and anal epithelium (E M Burd, 2003). Apart from cervical cancer, HPV infection has been found to be associated with many other cancers like 40-60% of vaginal malignancy, VIN (vaginal intraepithelial neoplasias), and penile carcinomas, up to 50% of vulvar carcinomas and approximately 90% of anal carcinomas (Parkin et al., 2006; ME McLaughlin-Drubin, 2009).

Table 3.2: List of cancers related to HPV infections and HPV types (Shirish sukla et al., 2009):

<table>
<thead>
<tr>
<th>Organ</th>
<th>HPV types</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anogenital cancers</td>
<td></td>
</tr>
<tr>
<td>a) Uterine cervix</td>
<td>HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 59, 66, 71, 73, 6</td>
</tr>
<tr>
<td>b) Anus</td>
<td>HPV 16, 18, 29, 30</td>
</tr>
<tr>
<td>c) Penis</td>
<td>HPV 16, 18, 30</td>
</tr>
<tr>
<td>d) Ovary</td>
<td>HPV 16, 18, 30</td>
</tr>
</tbody>
</table>

3.9.3: HPV infection and cervical cancer:
The most important risk factor for development of cervical cancer is the infection with high risk human papillomavirus (Franceschi S et al., 2003; Gewin L et al., 2004). Human Papilloma Virus (HPV) which is mainly transmitted sexually, prevalence in cervical carcinomas has been estimated to be 99.7%, higher than previously thought (Walboomers JM et al., 1999). Cervical cancer is a multistep process that develops slowly over several years after persistent infection of epithelial cells with oncogenic types of HPV mainly type 16 and 18 (Koushik Chattopadhyay, 2011). It usually takes ten to fifteen years for HPV infection to
develop into cancer. HPV has multiple types both oncogenic and non oncogenic. There are more than 200 types of HPV have been recognized till now on the basis of DNA sequence data analysis, of which 85 HPV genotypes are well characterized and rest 120 are partially characterized potentially new (Zur Hausen, 2000). Another study reported that of about one hundred strains of the virus, approximately 30 (thirty) are transmitted sexually, out of these only a few have the probable to develop into malignancy (Eileen M. Burd, 2003). McIntosh et al has mentioned that 99.7% of cervical cancers are directly linked to infection by a “high risk” strain of HPV and HPV-16 is the most common “high risk” strain of the virus (McIntosh, 2000). Clinical and sub-clinical HPV infections are the most common sexually-transmitted diseases nowadays.

In India 85-90% cervical cancer cases are squamous cell variety (Shukla S et al., 2009). When compared to other parts of the world, the proportion of HPV16 is much lower and ranges only up to 70% when both HPV16 and 18 were considered (Bosch FX et al., 1996; Bosch FX et al., 2002). According to some studies in India, HPV type 16 alone in cervical cancer is 70 to 90% while occurrence of HPV type 18 varies from 3 to 20% and other high risk HPV types such as HPV 45, 33, 35, 52, 58, 59, 73 have also been reported and constitute only a minor group (Franceschi S, et al., 2003; Sowjanya AP et al., 2005; Bhatla N et al., 2006). HPV infection has been reported to be associated with greater than 99% of all cervical cancer cases, and high risk HPV types 16,18,31,33,45 has been identified in up to 97% of cervical cancer cases worldwide (Margaret E et al., 2009).

Some data are now emerging to suggest that HPV-16 behave differently from all other oncogenic and non-oncogenic HPV types. HPV 16 and 18 are estimated to account for about 70% of all cervical cancers and altogether HPV 16, 18, 45, 31, 33, 35, 52, 58 are responsible for about 90% of all cervical cancers worldwide (Bosch et al., 2002).

In addition to the most prevalent low-risk HPV types HPV 6 and 11, other types are 40, 42, 43, 44, 54, 61, 70, 72 and 81 (Villers et al., 2004). In addition to squamous cell carcinomas, HPV DNA have also been detected in most cervical adenocarcinomas or mixed variety like adenosquamous carcinomas, and carcinomas with neuroendocrine differentiations (Margaret E et al., 2009; ME McLaughlin-Drubin, 2009). Studies have also revealed that HPV 16 is most commonly associated with squamous cell carcinomas, while HPV18 is the predominant type found in adenocarcinomas and neuroendocrine carcinomas (Margaret E et al., 2009).
Studies have shown that the pick of HPV infection, particularly HPV16, appears to reach a later stage in the third decade of sexual life at 26-35 years in Indian women in contrast to 18-25 years in western country (Schiffman M et al., 2005; Das BC et al., 2008).

Generally HPV strains are divided into two categories; “high risk” and “low risk.” (Eileen M. Burd, 2003). But typically genital types are divided into three groups based on the frequency of association with malignant tumours. The low risk group includes types 6, 11, 42, 43, 44, which are common low-grade SIL (LG-SIL), less so in high-grade SIL (HG-SIL), and practically nonexistent in cancer specimens (EL Franco, 1995). Low risk strains of the virus typically cause benign changes or abnormalities in the external genitalia. The intermediate risk group is comprised of types 31, 33, 35, 51 and 52, whose combined frequencies of association increase within the SIL spectrum. “High risk” strains of the virus are those that have the ability to cause cervical cancer. HPV types 16, 18, 45 and 56, are considered high risk group which are powerfully associated with carcinomas and exhibit diverse behaviour with respect to HSIL (EL Franco, 1995). The high risk group poised of two different subgroups: high-risk/HPV 16, which seems to be evenly analytical of HSIL and cancer, and high risk/HPV 18/45/56, which are strongly associated with cancer.

Most of the women infected with HPV, clear the infection through natural means within two years and only a minority of women will develop persistent HPV infection that could eventually cause cervical intraepithelial neoplasia (CIN). It has been reviewed that HPV-16 and 18 infections are cleared more slowly than infection caused by other high risk types (Trottier H et al., 2008). HPV-16 DNA viral load is independent cause of cervical cancer. This indicates that additional risk factors play an important role in the development of cancer of the cervix.

When exposed to HPV, a woman’s immune system typically prevents the virus from doing injury. In a tiny group of women, though, the virus survives for years before it finally converts some cells on the surface of the cervix into cancer cells. 50% of cervical cancer cases occur in between ages 35 and 55. The HPV types that produce genital warts (lesions that are raised and bumpy, of flat and almost impossible to see) are different from those that cause cervical cancer. Women with a past history of genital warts have almost twice the risk of having an abnormal PAP smear than other women. Normally, cervical cells grow in an
arranged method but when control of cell growth is lost, cells divide too often and too quick in particular during HPV infection.

The fact that only a small proportion of HPV-infected individuals will eventually develop cancer of the cervix and the long latency period between primary infections and cancer emergence suggest that additional factors are involved in the development of the SIL. Majority of SIL and cancers develop within the transformation zone, indicating that other exogenous or endogenous factors specific to the anatomical milieu may be conducive to SIL and cancer development (E Guzmán-Olea et al., 2012). Similarly, the amplified frequency of SIL and cervical cancers in AIDS patients suggests the importance of the response of immunity and exclusively T lymphocytes, in the avoidance or restriction of HPV-infected lesions (Benton C et al.,1992;Giannini SL, 1998). Moreover, several studies have shown that antibody-mediated immune responses to HPV reflect more the tumor progression than a protective host mechanism against development of disease (Jochmus Kudielka I et al., 1989; Viscidi RP et al, 1993). In contrast to a cell-mediated immune response, if response of the antibody-mediated is inefficient may lead to tumor progression (Frazer I, Tindle R.1992, Giannini S L, 1998).

### 3.10: Role of immunity in cervical cancer:

Normally, when a foreign agent infects a cell, it cites a multiparty system to activate immune responses in order to protect the organism. When cell is infected the immune system starts with the construction of danger signals which produces antigens. The antigens are processed and create peptides which causes MHC class I or II to react with CD4+ or CD8+ T-cells, respectively. CD4+ and CD8+ T-cells generate interleukins (ILs), which activate wider immune responses (Stern et al., 2001).

Evidence has suggested that progress of HPV life cycle provoke immune system responses. There are several different factors which provoke immune system are: First, the target cell of HPV i.e. keratinocytes, are less likely to provoke a novel T-cell response than other types of host cells (Tristram, A., & Fiander, A. 2007). Second, HPV producing genes suppresses part of the human immune system. Down regulation of MHC class I molecules occurs due to the protein encoded by E5 (Tristram, A., & Fiander, A.2007). Presence of MHC class I molecules with fragments of invading elements detect T-cell and an immune response is being elicited. Third, the immune system usually detects capsid proteins in the lower layers of the epithelium (Tristram, A. & Fiander A. 2007). Capsid proteins are produced in the upper
layers of the epithelium, which is distant from the elements of the immune system response. Fourth, as HPV infection does not involve the blood stream, so the mechanisms for the immune system to detect an infection are limited (Tristram A, & Fiander A, 2007). Lastly, as HPV infection does not cause lyses of the host cells so induction of danger signals does not occur (Tristram A, & Fiander A, 2007).

3.10.1: Th1/Th2:
The abbreviations Th1 (T helper cell type 1) and Th2 (T helper cell type 2) can be considered as Th1 or Th2 primary effector cells. If they are "resting" but polarized (i.e., committed to a Th type), they could be considered Th1 or Th2 memory cells as they form Th1 or Th2 memory effector cells when reactivated (Ahmed, R and D Gray 1996; Dutton, R.W. et al., 1998).

Immunity is the result of interplay between two "immune" systems: the innate immune system that initially encounters antigen and the adaptive immune system of T and B cells. These T and B cells will respond accordingly to information provided by the innate system (Medzhitov, R. & C.A. Janeway 1997; Fearon, D.T& R.M. Locksley 1996; Borghans, J.A.M. et al., 1999). Once antigen has been detected by an innate immune cell, this in turn is communicated to T and B cells of the adaptive immune system (Medzhitov, R. and C.A. Janeway 1997; Borghans, J.A.M. et al., 1999). The signals are sent in relation to the context and molecular nature of antigenic epitopes. The signals indicate whether antigen should be attacked or not. The induction of T cells of adaptive immune leads to change of T cells from a naive phenotype to either a memory phenotype or an effector functional type. The Th1/Th2 phenotype reveals the result of naive T cell activation (Medzhitov, R. and C.A. Janeway 1997; Borghans, J.A.M. et al., 1999). T and B cells will respond by secreting specific cytokines.

It is well established that immune responses can be principally divided into a type I or a type II. The factors mediating immune responses are type of antigen-presenting cell (APC), co-stimulatory molecules, the type and concentration of antigen and the cytokine milieu (SL Giannini, 1998, Seder RA, Paul WE.1994). These two responses are associated with either CD4 T helper cells that produce type I (IL-2, IL-12, IFN-γ and TNF-β) (M Clerici et al., 1997) or type II (IL-4, IL-5, IL-6, IL-10 and IL-13) (Mosmann TR, Sad S, 1996; SL Giannini, 1998). In addition, several cytokines have been shown to contribute to the initiation
or suppression of these immune responses, for example IL-10, IL-4, IL-12 and/or transforming growth factor-beta 1 (TGF-β1). These cytokines have been shown to be produced by numerous types of cells, including dendritic cells (DC), keratinocytes and macrophages (Aragane Y et al., 1994)

Cytokines are functional small peptides which under physiological conditions control the “cell-to-cell communication” within the various body tissues i.e. paracrine function. Sometimes, when the cell which secretes a cytokine also has the receptors for the same cytokine on its cell membrane, the cytokine may control the function of the very cell from which it originated i.e. autocrine function, whilst less commonly cytokines spill over into general circulation and can affect distant tissues and organs, i.e. endocrine function (Platanias, 2005; Chedrese, 2009).

Cytokines are also called as interleukins, monokines, lymphokines, chemokines and growth factors. It has been observed that the local tissue or circulating cytokine levels is altered in a number of cancers, including gynecological cancers (Murooka et al., 2005; Heikkela et al., 2008). Recent literature reveals a significant association between deregulation of some cytokines and the incidence of cervical precancerous lesion (LSIL, HSIL), progression from precancerous to carcinoma “in situ”, further invasion and also ends stage metastasis. (EY Chen et al., 2010).

A shift from Th1 type to a Th2 type cytokine response was observed when healthy control or LG-SIL was compared to HG-SIL or cervical cancer. Qualitative analysis of the immune response in human tumors has been well clarified by the Th1/Th2 model of immune regulation that was developed in the mouse (MosmannTR& CoffmanRI,1987) and the model were later extended to include humans also. ClericiM, et al. (1997) introduced a functional definition of type 1 and type 2 cytokines and defined type 1 cytokines as those that mainly induce cell-mediated immunity and type 2 cytokines as those that predominantly stimulate humeral immunity. They have observed that a decline in type 1 cytokine production and an increase in type 2 cytokine productions (M Clerici et al., 1997). In cervical cancer patients, Tartour et al., 1998 analyzed intra tumor expression of IFN mRNA in tumor biopsy specimens as a prognostic factor. They showed that poor prognosis and tumor recurrence were associated with the detection of a low number of IFN-γ mRNA copies. T-helper responses are linked to clearance of HPV infection and regression of cervical intraepithelial
neoplasia. CTL lines raised against HPV epitopes can eradicate established HPV induced tumor in mice. An augmented immune response is correlated with squamous intraepithelial lesions (SILs) and they found a change in IL-12/IL-10 ratios from blood samples of HPV positive and HPV negative patients (Giannini S. L et al., 1998). IL-10 inhibits immune responses by inhibiting IL-12 production which is associated with the progression of immune responses against tumor growth. This shows that an infection by HPV is associated with a decline in immune responses against cancerous cells. Dissemination and progressive growth of HPV induced lesions, can be, at least, partially related to escape from local cytokine mediated surveillance (AF Nicol et al., 2005). Patients with HPV-associated neoplasms with a type 1 cytokine profile have shown a better clinical outcome compared with others exhibiting a type 2 cytokine profiles. Nevertheless, type 2 cytokines are major one whether locally or in the peripheral circulation, which may promote the growth of cervical SIL and neoplastic change (AF Nicol et al., 2005). Recent studies found that HIV/HPV co-infection predicts the highest IL-10 concentrations whereas co-infection with HIV, HPV and other STD predicts the highest IL-12 concentrations. Concomitant infection of the genital tract with HIV and other STD might influence the local concentrations of some immunoregulatory cytokines (AF Nicol et al., 2005).

There are almost 50 cytokines identified now and some of these or their receptors are significantly altered in carcinogenesis and metastasis of cervical cancer. Cytokines like IL-6, IL-8, IL12, ILR4, IL-4, IL-10 etc have been shown to serve as potential biomarkers to assess the risk of invasive cancer and metastasis (R S Jayshree et al., 2009). Persistent HPV infection with high risk HPV types and the cervical carcinogenesis have been reviewed earlier in details (Dutcher et al., 1988; Clerici et al., 1998; Hausen, 2000; Markowska, 2007; Boccardo et al., 2010). Many cytokines are markedly altered in cervical precancerous lesion and cancer, more so in advanced cancer with metastasis. Excess of some of these, e.g. IL-6, IL-17, and IL-8 is associated with tumor growth whilst some are associated with inhibition of HPV.

Tumor immunology is one of the most promising fields in cancer research, aiming to direct the immune system to attack tumor antigens. However, this global has not yet been reached. The importance of tumor-specific immune responses for treatment of malignant diseases is a matter of continued research. Immune response-based therapy in cervical cancer has not yet shown significant therapeutic effects. Several studies have examined the use of IFN with or
without combination chemotherapy in patients with recurrent cervical cancer (Hallum AV et al., 1995; Gonzales-de-Leon C, et al., 1995).

Many studies has discovered qualitative and quantitative differences in host inflammatory response both in epithelium and in stromal tissue in HPV infected women (Tay et al., 1987; Viac et al., 1990; Coleman et al., 1994; Davidson et al., 1997). Type 1 cytokines exert potent antitumor activity, as summarized by the following observations (M Clerici et al., 1998):

First, IL-12 is a potent activator of cellular immunity, has antitumor and anti-metastatic activities against many murine tumors. Up regulation of IFN-γ production is also done by IL-12.
Second, IL-2, IL-12, and IFN-γ activate defense mechanisms.
Third, IL-2 induces the transformation of NK cells into lymphokine activated killer cells.
Fourth, IL-12 prevents angiogenesis induced by human tumor cell lines in vivo.
Fifth, IFN-γ augments the presentation of antigenic peptides to TH lymphocytes.
Sixth, IFN-γ directly inhibits the growth of cervical carcinoma cell lines.

Studies suggest that the concentration of antigen presented to the T cell during primary activation influences its choice. The presence of some cytokines (such as the ones mentioned above) will also influence the response that will eventually be generated, but more understanding is required in these fields.

Classification of cytokines:
- Interleukins – produced exclusively by leukocytes.
- Lymphokines – produced by lymphocytes.
- Monokines – produced exclusively by monocytes.
- Interferons – involved in antiviral responses.

Viral infectious agents exploit the cytokine repertoire to evade immune responses of the host. Activities of cytokines affected by virus-encoded factors works in at least four different ways:
- By preventing synthesis and release of cytokines from infected cells.
- By meddling with the interaction between cytokines and their receptors.
- By inhibiting signal transmission pathways of cytokines.
- By synthesizing virus-encoded cytokines that antagonize the effects of host cytokines mediating antiviral processes (viroceptor and virokines).
The many specific activities of individual cytokines have been the basis for current concepts of therapeutic intervention—predominantly, hematopoietic malfunctions, and tumor therapy.

3.10.2: TNF-α:

TNF is a protein consisting of 157 amino acids and is synthesized as a membrane-bound protein (pro-TNF) (Wang X et al., 2008). TNF act against viral infection like a defense mechanism in the host. Tumor necrosis factor alpha (TNF-α) is a multifunctional cytokine that plays important roles in diverse cellular events such as cell survival, proliferation, differentiation, and deaths. (Wang X et al., 2008). TNF-α is a Th1 pro-inflammatory cytokine, predominantly secreted by activated macrophages in response to acute inflammation (Paradkar et al., 2014). Tumor necrosis factor-alpha (TNF-α) gene is located within the major histocompatibility complex (MHC) on chromosome 6, between HLA class I and II regions and it is a potent pro-inflammatory cytokine playing an important role in the development of the immune response (Balkwill F, Lobito AA et al., 2011, VJ Sindhava et al., 2013). Although the regulated release of TNF-α may exert normal physiologic effects, the uncontrolled production of TNF-α may lead to abnormal function of organ (Strieter RM et al., 1993). The blood level of TNF-α is also increased in some solid tumors (Ardizzoia A et al., 1992). Previous study has confirmed the evidence supporting a pivotal role of TNF-α in tumor promotion by stimulating the proliferation of cervical cells immortalized and transformed by HPV (Woodworth CD, 1995, Moore et al., 1999, Gaiotti D et al., 2000). Therefore, TNF-α expression levels may influence HPV infection and subsequent HPV associated cancer development. The potential roles of TNF-α as an important and pleiotropic cytokine that plays a critical role in immune regulation through prominent anti-inflammatory and immune regulatory activities, its genetic variants may affect the host immune system and HPV infection and consequently the HPV-associated cancer development (Trimble CL et al., 2010). In addition, high serum TNF-α level in cancer patients have been detected (Abrahamsson et al., 1993) and were associated with a poor disease outcome (Nakashima et al., 1998). Hence, TNF-α expression levels may contribute to the pathogenesis and promoting malignant progression of cervical cancer (Feng Pan et al., 2012). Tumor necrosis factor (TNF-α) has been implicated in both direct and indirect control of HPV infection. HPV harboring cervical keratinocytes constitutively produce active TNF-α (Malejczzyk J et al., 1992). Direct control of HPV infection by TNF-α occurs by induction of apoptosis in HPV-infected cells and cervical cancer cells (Basile JR et al., 2001; Suk K et al., 2001). Stimulation of the inflammatory response through up-regulation of vascular adhesion
molecules and chemokines (Malejczyk J et al., 1997) arresting growth of HPV infected keratinocytes, and down-regulation of HPV gene transcription (Vieira KB, 1996; Kyo S et al., 1994). Up regulation of HLA class 1 mediated by TNF-α in nonprofessional antigen presenting cells represents indirect control (Hallermalm K et al., 2001). It has been seen that regulation of levels of TNF-α locally and systemically occurs at the genetic level (Hajeer AH, Hutchinson, 2000) by posttranscriptional effects and feedback inhibition occurs by TNF-α receptors (Peschon JJ et al., 1998). In regard to cancer, TNF acts in two ways. Action of TNF-α could be either pro or anti-tumorigenic. On one way, TNF act as a promoter of endogenous tumor, because TNF encourage the growth, proliferation, and invasion. Other way, TNF can kill a cancerous cell. The killing capacity of TNF renders it a potential cancer therapeutic drug, although more experimentation is needed to reduce its toxicity for systematic TNF administration (WangX et al., 2008). On the cellular level, TNF exerts its effects through its receptors to activate distinct signaling pathways that regulate survival of a cell, proliferation, or death. As a result, complex roles for TNF in cancer have emerged. On one hand, its anticancer property is mainly through inducing death of a cancer cell; other way TNF stimulates proliferation of cell, survival of cell, migration and angiogenesis in most cancer cells that are resistant to TNF-induced cytotoxicity, resulting in tumor promotion (WangX et al., 2008). The aforementioned crosstalk among the TNF-α induced pathways plays a key role in the biological effects of TNF-α on cancer.

**TNF-α in carcinogenesis:** A growing body of epidemiological and clinical data supports the concept that chronic inflammation promotes tumor development. Pro inflammatory cytokine TNF-α is able to act as an endogenous tumor promoter to bridge between inflammation and carcinogenesis. In fact, current information has shown that TNF is involved in all aspects of carcinogenesis as summarized below: survival, proliferation, cellular transformation, invasion, angiogenesis, and metastasis (MS Wu et al., 2004; Shishodia et al., 2003; C Lin, 2014; Moore et al., 1999).

**Elevated TNF-α expression levels in tumor patients:** Numerous reports have shown that the serum TNF-α concentration is increased in different cancer patients (Ferrajoli A et al., 2002; Ahmed MI et al., 2001). TNF-α expression was also expressed at higher levels in various pre-neoplastic and tumor tissues (Ferrajoli A et al., 2002; Ahmed MI et al., 2001; Szlosarek PW et al., 2006). Further, the increased TNF-α expression level in pre-cancerous and tumor cells was associated with the progression of malignant diseases such as breast...
cancer, and cervical carcinoma (Ferrajoli A, et al., 2002; Ahmed MI et al., 2001; Michalaki V et al., 2004; Wang Xia and Yong Lin, 2008). The serum TNF-α concentration was markedly decreased during chemotherapy in breast and prostate cancer patients, the extent of which was well-correlated with the extent of therapy responses, suggesting that serum TNF-α level could be an indicator for chemotherapy response and prognosis (Ferrajoli A et al., 2002; Michalaki V et al., 2004; Berberoglu U et al., 2004).

The role of TNF-α in tumor promotion and growth: The tumor promoting role of TNF-α has also been demonstrated in various mouse tumor models. There is rising proof indicating that patho-physiological concentrations of endogenous TNF-α promote tumor genesis and expansion (Popivanova BK et al., 2008). NF-κB activation is critical for TNF-induced tumor back-up, as JB6 mouse epidermal cells (P-cells) are transformation resistant and TNF were unable to induce NF-κB activation (Hu J et al., 2004).

A potential anti-oncogenic effect of TNF: TNF promoted tumorigenesis which is evidence proved. In some experimental systems TNF exhibited an anti-oncogenic effect. Despite the reported role of TNF in chronic inflammation, TNF in mice were significantly more susceptible to 3’-methylcholanthrene (MCA) induced skin sarcoma (Swann JB et al., 2008). The antitumor role of TNF may involve immune responses that prevent tumor formation, for example, promoting tumor stroma destruction by cytotoxic T lymphocyte (CTL) or tumor infiltrating macrophages (Nakagawa J et al., 2007; Dace DS et al., 2007; Zhang B et al., 2008) and activating tumor infiltrating dendritic cells (DC), thereby triggering a potent adaptive immune response leading to tumor rejection (Larmonier N et al., 2007). Previous reports indicated that TNF-α promotes the progression of cell cycle by increasing cyclin-dependent kinase activity and HPV16E6/E7 RNA expression in HPV-immortalized keratinocytes (Gaiotti D et al., 2000; E Boccardo et al., 2010). In addition, it has been shown that TNF-α enhances the transcription and stability of the epidermal growth factor (EGF) receptor, and cell proliferation or tumorigenesis follows (Gaiotti D et al., 2000) Recently, it was found that the level of serum TNF-α in women with cervical intraepithelial neoplasia (CIN) was much higher than in healthy controls (Szlosarek P et al., 2006; Mocellin S et al., 2005). TNF-α level have been found to be increased in local tissue specimens from cervical cancer and hence blocking antibodies have found therapeutic application (P H Paradkar et al., 2014). TNF polymorphism is associated with increased risk of cervical cancer (Liu et al., 2012). TNF-α has been reported to be involved in orchestrating an antitumor immune
response against HPV expressing cervical cancer cells, but results involving different study population belonging to different ethnicity and different pathological types are equivocal and inconclusive. Hence, loss of TNF-α could be advantageous for tumor cells to escape immune clearance.

3.10.3: Interleukin 10:
Interleukin-10 (IL-10), also known as human cytokine synthesis inhibitory factor (CSIF), is an anti-inflammatory cytokine. IL-10 is encoded by the IL10 gene (Eskdale J et al., 1997). IL-10 is released by cytotoxic T-cells to inhibit the action of NK cells during the immune response to viral infection (Eskdale J et al., 1997). IL-10 is a cytokine with pleiotropic effects in immune regulation and inflammation. It also enhances B cell proliferation, survival as well as antibody production. Moreover IL-10 can obstruct NF-κB activity, and is implicated in the regulation of the JAK-STAT signaling pathway.

IL-10 interacts with Interleukin 10 receptor, alpha subunit (Ho AS, et al., 1993, Tan JC, Braun S et al., 1995; Josephson K, et al., 2001; Josephson K, McPherson DT, 2001). The occurrence and progression of tumor is due to the loss of tumor antigenicity and suppression of immune system. Cytokines contribute to this phenomenon. Tumor associated suppression of immune system may be mediated by IL-10 (Germain et al., 1998). The role of IL-10 in cervical cancer is not clear. Thus, it is necessary to analyze the biological role of IL-10.

Cytokine profile in cervical cancer patients: The initiation and maintenance of cell mediated immunity (CMI) is associated with CD4+ T helper cells producing either Th1 (IL-12, IFN-γ, TNF-α) or Th2 cytokine (IL-4, IL-6, IL-10). An interesting study (Clerici M et al., 1997), found an increase levels of IL-10 in HPV expressed CIN indicating that the enhanced IL-10 production was supported by immune competent cells such as tumor-infiltrating lymphocytes in some cases. Expression of major histocompatibility complex (MHC) class I expression is down-modulated by IL-10, thereby inhibiting tumor antigen presentation to CD8+ CTLs (Matsuda et al., 1994; Beissert et al., 1995; AF Nicol et al., 2005). IL-10 was found maximum in CIN III and invasive carcinoma. This suggests a viral activation of the systemic cytokine network in HPV positive women. Development of SIL or cancer is preferentially associated with type II or immuno suppressive cytokine in particular IL-10 (Giannini et al., 1998; BS Chagas et al., 2013). IL-10 is an immune-modulatory cytokine with both suppressing and enhancing properties on different types of the immune cells (Moore et
IL-10 functionally inhibits antigen presenting cells. IL-10 blocks the cytokine synthesis of Th1 type T cells, activated monocytes and natural killer cells. IL-10 stimulates and/or enhances the proliferation of B cells, monocytes and mast cells and decreases the cytotoxic T lymphocytes generations (Moore et al., 1993 & Yang et al., 1995). IL-10 down regulates tumor specific immune response by one of the following mechanisms:

i. Directly suppressing IFN-γ and IL-12 production thereby preventing the activation of CTLs and natural killer cells (Mosmann and Coffman, 1987).

ii. Reducing MHC expression on the surface of tumor cells (Moore et al., 1993; M Clerici et al., 1998)

iii. Inhibiting tumor antigen presentation by professional APCs (Beissert et al., 1995)

**Anti-Inflammatory effect of IL-10:** Another possible role of IL-10 in promoting cancer is in the inhibition of Th1 cytokine TNF. TNF-α is necessary for the activation of Langerhans cells and is capable of inducing apoptosis in virus infected cells (Wong et al., 1992). However, it does not induce apoptosis in all cells (Mota et al., 1999). Most cells are protected from apoptosis caused by TNF-α by a separate proliferative pathway involving the activation of NF-kB. HPV infected cells contain E6 protein which also activates the NF-kB production thus, preventing the cells from the apoptosis caused by TNF-α (Carter et al., 1999; Marconi et al., 1999; Carter et al., 2000; Gentry et al., 2000 & Manna et al., 2000). IL-10 in combination with IL-2, a Th1 cytokine was able to consistently increase the cytotoxicity. Administration of IL-10 in combination with IL-2 after antigen stimulation consistently increases the intracellular expression of Th-1 cytokine (Alessandro et al., 2000, 2011). But unfortunately there is no in vivo proof for this as there is always a decrease in Th1 cytokine level when IL-10 level is high (Alessandro et al., 2000). IL-10, E6 and E7 oncoproteins have the same effect on the TNF-α, IFN-γ and CD8+ T cells. But the possible link between these three proteins is not known. If the link between these proteins is established then the exact role of IL-10 can be formulated. However, the role of IL-10 in promoting cancer of the uterine cervix may depend upon the microenvironment around the cervix (Yan Wang et al., 2013). Multifunctional cytokine IL-10 shows immunosuppression as well as anti-angiogenic properties (Yan Wang et al., 2013). Previous studies suggested that increased IL-10 levels may control inflammatory responses and cancer development (Caruso C et al., 2004) and constitute a risk factor for carcinogenesis. Moreover, in other types of cancer, lower IL-10 expression may be a risk factor for disease or disease progression (Howell WM and Rose-Zerilli MJ, 2006; Yan Wang et al., 2013). Furthermore, an increase in the levels of
IL-10 was observed in cervical cancer and CIN grade III patients, in comparison to early CIN grades and healthy controls (Sharma A et al., 2007; Yan Wang et al., 2013). The genotype predisposing to the production of high levels of IL-10 is more commonly observed in cervical cancer patients, compared to healthy women (Stanczuk GA, et al., 2001; Yan Wang et al., 2013). However, findings of a previous study suggested that there is increase of chemokine receptor IL-2 and IL-4, rather than IL-10 or Fas ligand, increases the risk of cervical cancer (Ivansson EL et al., 2007; Brower V, 2005) supporting the clinical use of IL-10 in combination with IL-2 in the treatment of cervical cancer (Santin AD, et al., 2000). These studies suggested that IL-10 expression may play an important role in the development of cervical cancer. The twin biological function of IL-10 as antiinflammatory (potentially cancerpromoting) and antiangiogenic (potentially cancerinhibiting) agent reflects the conflicting data in cervical cancer (Yan Wang et al., 2013).

**IL-10 exerts a tumor promoting effect in cervical cancer:** IL-10 mRNA and/or protein have been found to be enhanced in several types of malignancy in human such as ovarian cancer, hepatocellular carcinoma in addition to squamous cell carcinoma and basal cell carcinoma of the skin, (Nakagomi H, et al., 1995; Pisa P, et al., 1992; Chan SL, et al., 2011) human gliomas (Huettner C et al., 1995) and melanoma (Kruger-Krasagakes S, et al., 1994). Furthermore, IL-10 is elevated in squamous intraepithelial lesions (SILs), which are considered as pre neoplastic stages of cervical cancer (The 1988 Bethesda System) as well as in cervical cancer. For example, it has been shown that mononuclear cells collected from peripheral blood samples of patients with cervical SIL and true cervical cancer patients, produced higher levels of IL-10 (Giannini SL, et al., 1998; Clerici M, et al., 1997; Jacobs N, et al.,1998; Mota F et al.,1999). Serum levels of IL-10 were observed to be significantly higher in women with invasive cervical cancer and high grade CIN as compared to controls (Feng et al., 2012). They did not find any difference in serum levels of IL-10 in healthy women who were HR HPV positive or HR HPV negative indicating that defective immune responses are more contributory to the carcinogenetic process rather than the infection per say. The cytokine of immunosuppressive quality may play an important role in creating a microenvironment that favors progressive cervical disease and immune evasion by high-risk HPV (Syrjanen S, et al., 2009) and may also explain the immunosuppressive state of cervical cancer patients (Bhairavabhotla RK et al., 2007). The ability to reduce the expression of IL-10 may be effective in the treatment of cervical cancer though the mechanism by which IL-10 induced tumor promoting effect in cervical cancer is still complicated (Yan Wang et
al., 2013). Certain investigators have hypothesized that higher IL-10 levels encourage HPV intensification, viral replication and malignant conversion of infected cells in women, which offers a probable explanation for some women with HPV developing cervical cancer, whereas others do not (Brower V; 2005; Yan Wang et al., 2013). In particular, IL-10 is highly expressed in tumor cells and its expression is directly proportional to the development of HPV positive cervical cancer, signifying an vital role of HPV proteins in the expression of IL-10 (Yan Wang et al., 2013). Furthermore, IL-10 is highly expressed in the tumor cells of all patients and its expression is directly proportional to the development of HPV positive cervical malignancy, signifying a diverse association between IL-10, HPV and progression of cervical cancer (Yan Wang et al., 2013; Kirvis Torres Poveda et al., 2014; Bermudez-Morales VH et al., 2008). The elevated expression of IL-10 may allow for persistent level of virus and transformation of cervical epithelial cells and, consequently, cancer development (Yan Wang et al., 2013; Kirvis Torres-Poveda et al., 2014; Bermudez-Morales VH et al., 2011). The detection of IL-10 and TNF-α in cervical secretions may be an important indicator of local immune response of cervical lesions induced by HPV infection (Azar KK et al., 2004; Yan Wang et al., 2013). The maintenance of IL-10 expression may contribute to the initiation of SIL, by allowing HPV to weaken the innate immunological surveillance and the competent tumor escape mechanisms (Giannini SL, et al., 1998; Yan Wang et al., 2013). Two viral oncoproteins (E6 and E7) of HPV-16 play an energetic role in the malignant growth properties of cervical cancer cells and may be ideal targets for antigene therapy (Yan Wang et al., 2013; Madrigal M, et al., 1997). When E6 oncoprotein activity is high, IL-10 is found to promote tumor growth (Vinueselvi P et al., 2008). Additionally, the HPV E2 protein binds to the regulatory region of the human IL-10 gene (-2054 nt) and induces the expression of elevated levels of IL-10 mRNA in HPV infected cells.

**IL-10 exerts a tumor inhibiting effect in cervical cancer:** The dual biological function of IL-10 as an anti-inflammatory (potentially cancer promoting) and anti-angiogenic (potentially cancer inhibiting) agent reflects the conflicting data in cervical cancer. In almost all cervical cancer cases IL-10 levels were found to be high (Yan Wang et al., 2013; Clerici M, et al., 1997; Jacobs N, et al., 1998; Mota F et al., 1999). However, several gene transfection studies on IL-10 have demonstrated that IL-10 has the ability to inhibit tumor growth and metastasis in several types of cancer, but the mechanisms have yet to be elucidated. IL-10 might act by inhibiting angiogenetic factors, such as IL-1β, TNF-α, IL-6,
vascular endothelial growth factor, and metalloproteinases, or by augmenting NK cell dependent tumor cell lysis (Yan Wang et al., 2013; Kundu N and Fulton AM, 1997).

A small synthetic peptide derived from IL-10 may increase tumor sensitivity to NK cells in human melanomas (Yan Wang et al., 2013) which may prove relevant in the designing of future strategies for cancer immune therapy (Kurte M, et al., 2004). Furthermore, decreased IL-10 levels are also associated with a higher risk of cervical cancer (Brower V, 2005). Previous studies suggested that higher levels of IL-10 may prevent cervical neoplasia by assisting in the elimination of HPV (Farzaneh F, et al., 2006). Under pathological circumstances it can lead to immunosuppression and improper clearance of viruses like HPV and other pathogens and persistence of these infections and tumors. It decreases TH1 types of cytokines (IL-12, IFN-γ) and increases TH2 types of cytokines (IL-4, IL-5, and IL-13) (PH Paradkar et al., 2014). IL-10 also decreases the pro inflammatory cytokines IL-12, IL-2, IL-6, IL-1β, GM-CSF, TNF-α and IFN-γ (Bijjiga et al., 2013; Asadullah et al., 2003; PH Paradkar et al., 2014). The sources of IL-10 have been identified as TH2 cells, monocytes, macrophages, B lymphocytes, eosinophil, mast cells and possibly keratinocytes. Multiple factors including cytokines, NF-kB, and stress can lead to IL-10 release from different sites. IL-10 activity is mediated through IL-10 receptors which are situated mostly on immune cells. IL-10 may affect its actions by inhibiting NF-kB activity through dual mechanism; i) inhibition of IKK activity; ii) Blocking the binding of NF-kB to DNA. IL-10 also inhibits VEGF and angiogenesis (Lin A et al., 2002). Local levels of IL-10 in cervico-vaginal secretions were increased in women with HPV associated CIN (PH Paradkar et al., 2014). IL-10 polymorphism has been reported and correlated with some immune disorders and cervical cancer (Shekari et al., 2011). Interleukin-10 deficiencies are observed in some diseases and a recombinant analogue is undergoing clinical trial in various chronic diseases (K Asadullah et al., 2003). The effect of IL-10 on tumors is variable and both inhibition and promotion of tumor growth have been reported (Asadullah et al., 2003).

3.10.4: Interleukin 12:

IL-12 is an interleukin that is produced in response to antigenic stimulation by dendritic cells, monocyte or macrophages and human B- lymphoblastoid cells (Kalinski P et al., 1997; Dorman SE, Holland SM, 2000). IL-12 is involved in the differentiation of naive T cells into Th1 cells (Hsieh CS et al., 1993). It induces the production of IFN-γ, favors the differentiation of Th1cells and forms a link between innate resistance and adaptive
immunity (Trinchieri G, 2003). Studies showed that IL-12 had a central role in Th1 responses (Kobayashi M, et al. 1989; Hsieh CS, et al. 1993) and it is required for optimal Th1 cell development during the immune response to pathogens (Kubin M et al, 1994).

**Basic biology and immune stimulatory effects of IL-12:** Initially IL-12 was described as “Natural killer-stimulating factor” and “cytotoxic lymphocyte maturation factor” and has since been reported to have important effects on the generation of an adaptive immune response (Kobayashi M, et al., 1989; Wolf SF, et al., 1991). It is a potent activator of NK cells. The effects of IL-12 on T cells include enhanced cytotoxicity and CD4+ T cell differentiation into type-1 helper T cells (Th1) (Hsieh CS, et al., 1993).

**IL-12 in the tumor microenvironment:** The wide-ranging effects of IL-12 have profound impacts upon the tumor microenvironment; acting directly on tumor cells, influencing the surrounding stroma or structure of tumor and modulating the infiltrating immune cells. For tumor eradication or inhibition combine effect of recruitment of lymphocytes, direct effects on tumor cells to decrease angiogenesis as well as activation of tumor infiltrating lymphocytes are required.

An important tumor microenvironment modulatory effect by IL-12 is the inhibition of angiogenesis. Besides antiviral activity IL-12 is also vital for the host resistance against the tumors. The activity of IL-12 as antitumor activity has been widely reported in mouse models, where it inhibit tumor genesis and induce regression of established tumors (Brunda MJ, et al., 1993; Nanni P, et al., 2001). The major antitumor activities of IL-12 rely on its ability to promote Th1 adaptive immunity and CTL responses (Trinchieri G, 2003). IL-12 plays an important role in the activities of natural killer cells and T lymphocytes. IL-12 exerts anti-angiogenic activity by blocking the formation of new blood vessels. The anti-angiogenic activity occurs by increasing the production of interferon gamma, which in turn increases the production of a chemokine which mediates this anti-angiogenic effect. As IL-12 has got its ability to induce immune responses and anti-angiogenic activity, research is going on as a possible anti-cancer drug. IL-12 appears to be immune protective for cervical cancer. The reduced expression of the cytokine IL-12 in cervical biopsy specimens from invasive cancer cases was associated with a reduced immune response and high IL-1 and IL-6 levels as seen by immunocytochemistry (P HParadkar et al., 2014).
3.10.5: INF-γ:
IFN-γ is a pleiotropic cytokine secreted by type-1 helper (Th1) T cells, cytotoxic T cells, and stimulated NK (natural killer) cells in response to antigenic stimulation and involved in activation of macrophages and endothelial cells (D Ma, 2014; Woodman, J. P., Dimier, et al., 1991). Production of IFN-γ is related to the induction of reaction in T lymphocytes, and enhances an immune response against malignant cells (D Ma, 2014). Interferon is glycoprotein that is synthesized by a variety of cells in response to viral infection, immune stimulation, and certain chemical inducers. They appear to act in a paracrine capacity within the immune system, with a wide range of effects that mimic the diversity of the endocrine system. 20 (Twenty) interferon have been identified in human till now; they can be classified into three groups (Revel M, 1984; D Goldstein, 1988). Most of the subtypes belong to the alpha class, two subtypes of beta (1 and 2) have been described, and there is only one gamma species. Alpha and beta interferon were both originally named according to their early method of production (namely, leukocyte derived alpha and fibroblast-derived beta), but in fact they appear to be synthesized by virtually every cell in the body. Gamma interferon, however, is a lymphokine secreted solely by T cells. The gamma gene, by contrast, is on chromosome 12 and shows no homology with other interferon. The receptor is found on chromosome 66 and appears to be different from that of alpha and beta interferon.

Modes of Action: The exact physiological roles of each of the interferon remain unclear. In terms of their use in cancer therapy, however, they have been studied for both their direct (cytotoxic) and immune (stimulatory) effects. The predominant mechanism responsible for the documented anticancer activity has not been identified. Interferon-gamma (IFN-γ) is a cytokine that plays an important role physiologically in promoting response of innate and adaptive immunity. The absent or insufficient production of IFN-γ lead to response of the cell considerably predisposes the host to microbial infection, implicating physiological importance of this cytokine in preventing infectious disease (R Gangwar et al, 2009). At the cellular level, interferon appears to act by gene activation. This is associated with decreased synthesis of a number of proteins as well as the synthesis of new proteins. A protein kinase phosphorylates proteins P1 and elongation initiation factor EIF2a in turn inhibits the binding of transfer RNA to the ribosome. This inhibition is characterized by discrimination between cellular and viral mRNA (Senn CC, 1984; Samuel C: 1986). These systems, which appear to inhibit viral replication, may also be involved in the inhibition of tumor protein synthesis and probably contribute to the prolongation of the cell cycle and the increased percentage of cells
in the GO phase observed with interferon (Revel M, 1984). Interferon also initiates the production of a series of new proteins, the functions of which are still unknown. Other cellular changes occur in the cytoskeleton due to alterations in tubulin production. The cell membrane is altered, as shown by increased expression of tumor-associated antigens, human lymphocyte antigens (HLAs) class I and II, and beta-2 micro globulin in both tumor and normal cells. (Goldstein D, Laszlo J, 1988) in some situations, interferon acts as a differentiating agent, causing malignant clonogenic cells to differentiate and lose the capacity to divide.

NK cell direct cytotoxicity and antibody dependent cellular cytotoxicity are modulated by the interferon (Paulnock DM et al., 1985). Other immune-augmenting activities common to all the interferon include stimulation of HLA class I expression, activation of B cells, and augmentation of other lymphokines such as interleukin-2 (IL-2) and tumor necrosis factor (TNF). Gamma interferon appears to have a wider variety of immune-stimulatory effects than do alpha or beta interferon, (Vilcek J, et al, 1985) including enhanced expression of class II antigens and Fc-fragment receptors and activation of macrophages in addition to the NK cell activation common to all interferon. The optimum dose for immune stimulation is unclear, but in the case of alpha interferon, it does seem that lower doses achieve higher NK activity (Laszlo J, et al: 1983). In cervical cancer patients, decreased intra-tumour expression of IFN-γ has been reported to be associated with the poor prognosis (Tartour E et al., 1998; R Gangwar et al., 2009).

Interferons (IFNs) have been used to treat diseases caused by human papillomaviruses (HPVs), such as condyloma acuminata and cervical intraepithelial neoplasia (CIN), with mixed results (Gross 1997 and Bornstein et al. 1993). The molecular basis for this discrepancy in the efficacy of IFN treatment has not been determined, but it has been noticed that expression of viral oncogenes, especially E7, is considerably higher in the non-responders than in the responders (Arany et al., 1995).

Some investigators have speculated that down regulation of HPV oncogene expression could be the reason for the positive clinical outcomes observed with IFN treatment (Nawa et al., 1990; De Marco & Marcante, 1993; Agarwal C et al., 1994). If this is the case, an explanation for the observed discrepancy in the efficacy of IFN therapy could be that IFN is unable to suppress HPV expression universally in all cases.
Interferon-gamma (INF-γ) enhances susceptibility of cervical cancer cells to lysis by tumor-specific cytotoxic T cells. Immunological imbalance created by infiltrating inflammatory cells may contribute to cancer growth and spread of cervical carcinogenesis (Jacobs, N., et al, 1998). The cytokine network of several common tumors is rich in inflammatory cytokines, growth factors, and chemokines (Smyth, M. J. et al. 2004). Evidences says that inflammatory cytokines and chemokines, occurs due to presence of the tumor cells may contribute directly to malignant progression, these are TNF-α, and IFN-γ (D Ma, 2014). The local production of cytokines within the tumor microenvironment can prevent the effectors’ response (Venetsanakos, E., J. et al., 1997) and cytokines also can mediate the activities of immune cells in the fight against malignant cells. (Mocellin, S et al., 2001). IFN-γ/TNF-a synergism also has been reported in biological responses other than tumor cell killing (Kyoungho Suki, et al., 1994). For instance, the two cytokines synergistically up-regulated the expression of numerous genes, like IL-10, and major histocompatibility complex class I heavy chain (Ohmori Yand Hamilton T A, 1995). However, the molecular mechanism of the synergism between the two cytokines is not clearly understood. It has been reported that IFN-γ increases the expression of TNF-α receptors (Ruggiero, V., et al., 1986). However, because the sensitivity of the cells to TNF-α is not simply correlated with the level of TNF-α receptor expression (Tsujimoto M et al., 1986) up-regulation of TNF-α receptor alone does not adequately explain the cytokine synergism in the anti-tumor action.

3.10.6: NF-κB:
NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) is a protein complex that controls transcription of DNA. It is found in roughly every animal cell types and is concerned in cellular responses to stimuli such as bacterial or viral antigens, free radicals, stress, ultraviolet irradiation, cytokines, oxidized LDL, and (Gilmore TD 2006; Brasier AR 2006; Perkins ND January 2007; Gilmore TD 1999; Tian B, Brasier AR 2003). Inaccurate parameter of NF-κB has been associated to viral infection, inflammatory, and autoimmune diseases, cancer, septic shock, and improper immune development. NF-κB has also been concerned in processes of synaptic plasticity and remembrance (Tian B, Brasier AR 2003, Levenson JM, et al, 2004). To sum up, NF-κB can be understand to be a protein responsible for cytokine production and survival of cell. NF-κB is important in regulating cellular responses because it belongs to the category of "rapid-acting" primary transcription factors.
These factors are present in cells in an inactive state and do not require new protein synthesis in order to become activated.

NF-KB is a central pro-inflammatory molecule regulating the immune response to infection. Constitutive activation of NF-κB signaling is a key event in virus- and non-virus-induced carcinogenesis. NF-κB regulates the expression of a large number of genes associated with inflammation [Jo MJ et.al. 2013; Park SW et.al. 2012], tissue damage and repair [Ling H et al. 2013; Zhang J et.al. 2013], cell differentiation [Hyldahl RD et.al. 2013; Limpert AS et.al. 2013], apoptosis [Ivanov VN et.al. 2000; Zhang W et.al 2013], tumor growth [Sung B et.al. 2010; Jin HR et.al 2012] and improper development of immunity (Emily L. et al, 2013). The HPV-16 E6 and E7 proteins regulate NF-kB, but conflicting evidence exists as to whether they stimulate (Hussain et al., 2011; James, Lee, and Klingelhutz, 2006; Nees et al., 2001; Xu et al., 2010) or suppress activation (Havard et al., 2002; Havard et al., 2005; Huang and McCance, 2002; Perea, Massimi, and Banks, 2000; Spitkovsky et al., 2002). In this regard, the NF-kB activation pathway is dependent of the type of cell and context of the signal.

**NF-kB cancer promoter:** Rising evidences support a major role of NF-kB in cancer development. The expression of genes which is regulated by NF-kB plays a key role in the proliferation of cell, or migration of cell and apoptosis (X Dolcet et al, 2005). Activation of NF-kB promotes malignant development and progression in several animal models (Erez et al., 2010; Greten et al., 2004; Pikarsky et al., 2004), and in many human malignancy including cancer of the cervix (Karin, 2006; Prusty BK et al, 2005; Xavier Dolcet et al 2005; Pallavi S et al, 2015; Prusty BK et al, 2005). NF-kB has been identified to be a vital link between chronic inflammation and cancer (Karin, 2009). Further, recently it has been established by works using in vitro systems that TNF-α induction is dependent of NF-kB activation and expression (Wang X et al, 2008).

**NF-kB cancer inhibitor:** Study shows that down regulation of NF-kB might represent a unique mechanism by which HPV interferes with innate immunity and promotes persistent infection and progress to cancer (Erik R. Vandermark et al, 2012). In early stage of carcinogenesis NF-κB may play an inhibitory rather than promotional role (Arnott CH et al, 2002). Inhibition of NF-κB in human keratinocytes was reported to promote development of malignant human epidermal lesions resembling squamous cell carcinoma in mice (M Brown, 2008). These contradictory results suggest that the cancer promoting role of NF-κB in
certain contexts may be tissue dependent, or may involve additional steps that are not well understood (M Brown et al, 2008). Hence NF-κB has been rightly stated as a two edge sword [Pikarsky E et al, 2006].

Moreover, NF-κB activity is inhibited by HPV-16 E6 and E7 proteins in cells cultured from the transformation zone of cervix, the site of most of the cancer cervix. Thus, NF-κB has the potential to act as a tumor suppressor in cervical cells (Georgopoulos, Proffitt, and Blair, 2000). Decreased NF-κB activation might represent a unique mechanism for the virus to interfere with the host immune response and promote persistent infection (KS Ahn, 2005). It is hence also been publicized that NF-κB act as a tumour suppressor under some conditions, indicating paradoxical function (Perkins 2006). Data have also shown that NF-κB activity enhances tumor cell sensitivity to apoptosis and senescence. Inhibition of NF-κB may suppress Fas-mediated apoptosis to impair host immune cell-mediated tumour suppression.

3.11: HPV detection technique:
Techniques to detect the presence of HPV in cervical cells have involved considerably, from simple scoring of cytological signs of HPV to immunocytochemical staining, nucleic acid hybridization methods and in recent times, PCR (polymerase chain reaction). Modern PCR protocols based on the so-called consensus primers (Because they amplify defined regions in genes that are highly conserved across HPV types) have sensitivity and specificity for epidemiologic studies (Vanderbrule et al, 1990). Use of PCR has almost solved the problem of misclassification of viral status in epidemiologic studies. The American cancer society now recommends that HPV screening be initiated within 3 years of the onset of vaginal intercourse or less than 21 years. Biological studies of HPV indicating that there is little risk of a significant precancerous lesion going undetected within the first 3 to 5 years after the onset of sexual activity (Moscicki 2012). Cervical HPV diagnosis is mostly performed by Papanicolaou stained smears (Pap-test) which is a cytological screening that detects changes in cellular morphology in cervical cancer screening programme. There are many limitations of PAP, like low sensitivity, error in diagnosis and inter-screener variations in PAP test, therefore, the confirmatory diagnosis of HPV is done only by molecular hybridization methods, of which the polymerase chain reaction (PCR) is the most sensitive (Cope J. U et al,1997; Das BC et al, 2000). A recent study by Nonogaki et al. 2004 has compared the performance of HPV DNA detection by
PCR and Hybrid Capture II (HCII) found that both tests yielded concordant results in 76.5%.

Another study by Nieminen et al, 2004 studied the strength of the high risk HPV DNA detection by HCII and conventional Pap smear screening, and the finding was that Pap smear, as a screening test is less sensitive but clearly more specific than HCII. Other reports, using lone finding of high risk genotypes of HPV was more sensitive and less specific than cytological screening for the identification of subsequent diagnosis of CIN III.

Though, the mixture of both techniques did not significantly improve the performance of HPV test (revised by Villa et al 2002). The overall prevalence of HPV 16 in invasive cervical cancer, based on primer MY09/11 PCR assay, targeting a 450 base pair (bp) fragment within the HPV L1, is around 50%. In 7% of these tests in cervical carcinomas there been a failure of HPV DNA detection due to either the absence of HPV DNA or false-negative HPV results (revised by Bosch et al. 1995). However, Walboomers and Meijer (1997) suggested that this methodology would lead to the identification of HPV DNA in virtually 100% of cervical cancer.

In reality additional analysis of the tumors, initially thought to be negative for HPV, recognized HPV DNA with a prevalence rate of 99.6% (Walboomers et al. 1999). This could be the major concern about the HPV identification. Around 10% of these HPV infections lead to other condition like vulval warts, papillomas or dysplasia; but the commonly is not connected to clinical consequences. About 11% of women with evidence of HPV infection as the only abnormality of cervical cytology will already have CIN by colposcopic biopsy; furthermore, usually after 10 months of initial screening 33% will develop cervical intraepithelial neoplasia (CIN). In the general population, only 2-3% of women will develop dysplasia, despite a high prevalence rate of HPV infection (Ho GY et al. 1998).

3.12: Role of Immunohistochemistry in cervical cancer:
The role of HPV in the development of cervical cancer is unshakable. HPV infection lead to a number of alterations in gene or protein expression within the HPV infected host cells. Proteins produced by infection with high risk HPV is the E6 and E7 oncoprotein. E7
oncoprotein binds to the retinoblastoma gene product (RB), thereby leading to its functional inactivation. Since expression of the cyclin-dependent kinase inhibitor gene p16 (INK4a) is under negative feedback control of functional RB, overexpression of p16 (INK4a) ultimately occurs in cells infected by high-risk HPV. As the p16 (INK4a) protein can be detected immunohistochemically, it offers a reasonable substitute indicator for high-risk genotype HPV; principally since p16 (INK4a) protein is not usually expressed in normal squamous epithelium of cervix. On immunohistochemical staining, expression of p16 (INK4a) protein is observed both within the nucleus and the cytoplasm of the affected cells (Sano T et al, 1998). In 1998, Sano and colleagues studied a series of 139 formalin fixed paraffin embedded cervical and genital biopsies for p16 (INK4a) using immunohistochemistry, and interpreted the findings with the results of HPV typing performed on the same samples. Marked overexpression of p16 (INK4a) protein (reflected by diffuse and strong immunostaining) was found in all pre neoplastic lesions that showed infection by high and intermediate risk HPV subtypes (16, 18, 31, 33, 52, and 58), and also in all frank cervical cancer. Lesions that were associated with low-risk HPV types 6 and 11, such as low-grade SIL, showed focal and frail staining of the immunohistochemistry slide for p16 (INK4a). In a similar study reported in 2001 by Klaes and associates, marked overexpression of p16 (INK4a) was detected in all CIN 1 lesions (n = 47) except 7 cases that were associated with low-risk HPV types. In addition, p16 (INK4a) protein was overexpressed in all cases of CIN 2 (n = 32) and CIN 3 (n = 60), as well as 58 of 60 (97%) invasive cervical carcinomas. As such, these results suggest that overexpression of p16 (INK4a) assists in the identification of high-risk HPV-related cervical squamous lesions. Keating et al. reported the immunohistochemical study results with p16 (INK4a) were correlated with histologic diagnoses and with results of HPV status by PCR analysis. Immunohistochemical overexpression of p16 protein associated with intact retinoblastoma protein expression in cervical cancer and cervical intraepithelial neoplasia (Pathology International, 1998). Altered expressions of receptor for advanced glycation end-products (RAGE) and its ligand (S100A9) are observed in many cancers and play a key role in inflammation associated cancer which plays an important role in the development of SCC. Moreover, the expressions of S100A9 and RAGE in SCC tumor cells were closely associated with histological differentiation (Xuejie Zhu et al, 2013). Abnormal immune expression pattern of ubiquitin and telomerase is common in HPV-positive cervical cancer, indicating the existence of an intense degradation of proteins, subsequent cellular immortalization and maintenance of the malignant phenotype (Virchows Arch. 2009).