CHAPTER-I

INTRODUCTION
**Introduction:**

Cervical cancer remains a major cause of illness and all cancer-related deaths among women in developing countries (Jemal et al., 2010). It is one of the major significant cancers of all female neoplasms in developing countries, while in western countries it ranks as the tenth most common malignant disease (Parkin et al., 1993). Cervical cancer is the second most common cancer in women worldwide (Ferlay J, 2015). Every year 529,000 new cases detected and 275,000 deaths occur yearly. India accounts for more than 25% of the global cervical cancer burden. In India, the age standardized cervical cancer incidence and mortality rates were 27 and 15 per 100,000 women (Ferlay et al., 2010). Cervical cancer is considered as a complex multi-step, multi-factor process, and carcinogenesis occur as a result of multiple gene-environmental interactions (Feng Pan et al., 2012). A number of epidemiologic and molecular biologic data revealed that high-risk human papillomavirus (HPV) infection is the most well established environmental risk factor which is confirmed in 95%-100% cases for cervical cancer (Zur Hausen, 2002). The other risk factors known to increase the incidence of cervical cancer are early sexual practice, early marriage, teenage delivery of the first baby, multiparty, multiple sexual partners, poor practice of personal hygiene, low socio economic status, herpes simplex virus type II, HIV positivity, use of oral contraceptive pill, tobacco smoking etc (Raychaudhuri and Mandal, 2010; D Das et al., 2013).

A consensus panel convened by the World Health Organization’s International Agency for Research on Cancer (IARC) has considered that HPV infection is the cause of cervical cancer both from the biologic and epidemiologic point of view (Franco et al, 1995). Many studies have consistently shown that HPV infection is the major risk factor for cervical cancer, with relative risks (RR) in the 20-70 range, which shows that the magnitude is higher than that for the association between smoking and lung cancer and is second only to that for the association between the chronic carrier state of hepatitis B infection and liver cancer (Franco et al., 1995).

HPV is a double stranded DNA virus with eight early genes (E1, E2, E3, E4, E5, E6, E7, E8) and two late genes (L1, L2). More than 200 types of HPV have been recognized till now on
the basis of DNA sequence data showing genomic differences. Out of these 85 HPV genotypes are well characterized and 110 are partially characterized new genotypes (Zur Hausen H, 2000). High risk HPV 16 and HPV18 are closely related with development of cervical malignancy and histopathologically maximum cases are squamous cell carcinoma variety (Shukla S et al., 2009). Nonetheless, the virus could be naturally cleared in 70-90% of individuals with HPV-infection, while a small proportion of patients with persistent HPV infection ultimately develop cervical cancer, indicating that HPV infection is necessary but not sufficient risk factor for the development and progression of cervical cancer (Walboomers et al., 1999). Along with HPV infection, alteration(s) in host genetic factors and its associated pathways plays a key role of any etiology leading to carcinogenesis. Therefore a thorough study of the key factors and molecular mechanism is important for planning future therapeutics. A substantial majority of squamous intraepithelial lesion (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, 2012).

At the early stages of cancer, there is stimulation of an active antitumor immune response; however, tumor development correlates with changes in the immunogenic properties of tumor cells. Hence defects or decreased efficiency in immune surveillance could contribute to an increased incidence of cervical malignancy (Smyth MJ et al., 2006). Furthermore, dynamic immune equilibrium in precancerous lesions, the cytokine profiles observed could vary based on the type of the precancerous lesion. Several cytokines that mediate the immune response have been implicated in the development of cancer (Dranoff, 2004). Host factors including cytokine modulation are also critical in regulating tumor growth. Analysis of T helper type 1 and type 2 cytokine profiles has been used to characterize the immune response in several human diseases, including HPV-associated diseases. A shift from T helper cell 1 (Th1) to T helper cell 2 (Th2) cytokines has been demonstrated by various authors during carcinogenesis in the cervical epithelia (Delgado, FG et al., 2009); whereas, other studies have suggested that cervical cancer progression is associated with specific immune failure in response to HPV-16 and that it is not related to a shift to Th2 cytokine profile (de Jong A et al., 2004). Cytokines which have already been studied includes type-1 cytokine like IFN-γ which shows immune-stimulatory function and capable of limiting tumour growth and type-2 cytokines like IL-4, IL-10 and IL-6 which shows immunosuppressive function and capable of stimulating
tumour growth. Already established report says that the immune responses against tumour were mainly mediated by Th1 cells. The anti-tumour immune response of the body would be affected, leading to the cancerous growth of the tumour if there is imbalance of Th1/Th2 ratio because of the increase Th2 cells production (Z Chen, 2013).

Tumor necrosis factor-alpha is a pro-inflammatory cytokine which plays a critical role in a wide range of inflammatory, autoimmune, and malignant diseases (Beutler and Bazzoni, 1998). TNF-α has been shown to be involved in the proliferation of tumor cells followed by invasion and metastasis occurs because it may have both tumor-necrotic and tumor-promoting functions (Mocellin S, 2008; Shishodia et al., 2003). The key role of TNF is in the regulation of immune cells. Tumor necrosis factor alpha (TNF-α) may be involved in orchestrating an antitumor immune response against HPV expressing cervical cancer cells as TNF-α is a multifunctional cytokine playing a key role in apoptosis and cell survival as well as in inflammation and immunity and is a member of a group of cytokines that stimulate the acute phase reaction (X Zhu, 2013). TNF-α may play a significant role in the immune response of cervical lesions (Balkwill F 2009; Walczak H 2011). It is produced chiefly by activate macrophages, NK cells and neurons. Hence, loss of TNF-α could be advantageous for tumor cells to escape immune clearance. 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, TNF-α 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.

Interleukin-10 (IL-10) is central immune-regulatory cytokine with important effects on B cells. IL-10 has exerted various biological roles based on the fact that their receptors are expressed by diverse cell population (Vicki, 2005). But in all types of advanced cancer IL-10 level was found to be apparently high (Mota et al., 1999). This dual biological function of IL-10 as anti-inflammatory (potentially cancer promoting) and anti angiogenic (cancer inhibiting agent) reflects the apparently conflicting data on impact of IL-10 directly.

It has been reported that IL-12 is important in the fate of CD8+ T cells where IL-12 promotes differentiation into functional effector cells and inhibits memory T cell formation (Pearce EL& Shen H, 2007). Induction of Interferon (IFN)-gamma from B, T and NK cells occurs following IL-12 production. (MJ Micallef et al., 1996 ; Lauwerys BR et al.,1999;
Yoshimoto T et al., 1997). IL-12 has positive feedback loops whereby IL-12 stimulates dendritic cells to produce more IL-12, thereby stimulating IFN-gamma production resulting in additional IL-12 produced by monocytes (Ma X et al., 1996; Grohmann U et al., 1998). IL-12 can obstruct the development of new blood vessels by increasing production of interferon gamma.

INF also exerts antiviral activity by enhancing the expression of major histocompatibility class I and II proteins, which is considered as mediator of immune recognition of viral antigens (Nees M, et al., 2001).

NF-kB (nuclear factor kappa-light-chain-enhancer of activated B cells) is a protein complex that controls transcription of DNA. NF-kB is found in almost all animal cell types and is involved in cellular responses to stimuli of bacterial or viral antigens. (Gilmore TD, 2006). NF-kB plays a key role in regulating the immune response to infection (k light chains are critical components of immunoglobulins). It has been proved that incorrect regulation of NF-kB leading to cancer, inflammation (Albensi BC & Mattson MP, 2000). The activation of NF-kB can suppress apoptosis, thus promoting chemo resistance and tumorogenesis. Aberrant activation of NF-kB is prevalent in cell lines (M Brown, 2008). Nuclear factor kappas B (NF-kB) etc have been shown to play a crucial role during development of cervical cancer (Fontaine V, et al., 2000). The inducible transcription factor NF-kB regulates expression of wide variety of cellular and viral genes during immune and inflammatory responses and initiation and progression of cancer (Greten FR, 2004; Georgopoulos NT, 2000). Evidence also indicate involvement of NF-kB transcription factor in controlling expression of numerous oncoproteins, cancer suppressor genes, various growth factors and cell adhesion molecules that play a key role in oncogenesis (Halbert CL, et al., 1991). Interestingly, however, most chemo preventive agents appear to suppress the activation of the NF-kB through inhibition of NF-kB signaling pathway. These chemo preventive agents also sensitize the tumors to chemotherapeutic agents through abrogation of NF-kB activation. Overall, these observations suggest that NF-kB is an ideal target for chemoprevention and chemo sensitization. Thus NF-kB is a critical sensor and integration of exogenous / endogenous signals leading to activation of cellular genes serves as a suitable target that can be used as a basis for anticancer drugs.
Thus, thorough study of the natural history of the sequence of HPV infection and associated gene environmental risk factors can lead to a more thorough understanding of how to prevent the disease in long term.