2.1 Global scenario cancer

Cancer remains as the major cause of death in both less and more developed countries of the world. One in 4 deaths in the United States is due to cancer (Siegel et al., 2013). Nearly, tens of millions of people are diagnosed with cancer each year and eventually half of them die from it. This is the second most common disease after cardiovascular disorders for maximum deaths in the world (Jemal et al., 2007). According to GLOBOCAN 2012 database, an estimated 14.1 million new cancer cases and 8.2 million cancer-related deaths occurred in 2012, compared with 12.7 million and 7.6 million, respectively, in 2008. The most commonly diagnosed cancers worldwide was those of lung (1.8 million, 13.0% of the total), which is the leading cause of death among males in both more developed and less developed countries. Among females breast (1.7 million, 11.9%) was the leading cause of cancer worldwide and in less developed countries. Prevalence estimates for 2012 show that there were 32.6 million people (over the age of 15 years) alive who had had a cancer diagnosed in the previous five years. Projections based on the GLOBOCAN 2012 estimates predict a substantive increase to 19.3 million new cancer cases per year by 2025 globally, due to growth and ageing of the global population (Ferlay et al., 2015). The estimated future burden could be much larger than the given above due to ageing and population growth, and increasing adoption of lifestyle risk factors such as smoking, poor diet, physical inactivity, and reproductive changes associated with urbanization and economic development (Ma and Yu, 2006).

Less developed countries account for only 57% (8 million) of new cases and 65% (5.3 million) of cancer deaths worldwide, in spite of their relatively larger share of the population. In less developed countries, liver, stomach and cervical cancers are most frequently diagnosed cancers, respectively, and leading causes of cancer death.

These cancers are predominantly attributable to infection, which accounts for 77%, 75%, and 100% of cases worldwide, respectively (de Martel et al., 2012). In more developed countries, bladder cancer among males and uterine cancer among females are frequently diagnosed. Prostate, colorectal, female breast and lung cancer incidence rates were also several times higher in more developed countries compared with less developed countries. Although incidence rates for all cancers combined are nearly twice as high in more developed than in less developed countries in both males and females, mortality rates are only 8% to 15% higher in more developed countries. This disparity reflects regional differences in the mix of cancers, which is affected by risk factors and detection practices, and/or the availability of treatment (Torre et al., 2015).

Frequencies of total cancer incidence in different continents revealed that Asia (4,878,952) had the highest incidence of cancer, followed by Europe (2,820,771), North America (1,570,520), Central/South America (766,575), Africa (649,760) and Oceanic (103,725) (Kamangar et al., 2006). Further, the pattern of cancer incidence is varying among different geographical regions of the world (Ferlay et al., 2010; Sharma et al., 2014). As per IARC, Cancer Incidence in Five Continents Vol. IX report (Ferlay et al., 2007) esophagus had the highest incidence in China, Zhongshan for both male and female (AAR-26.9, 10.1) followed by China Guangzhou City (AAR-22.2, 9.8), Gallbladder in Chile, Valdivia (AAR-12.3, 27.3), Lung in USA, USA Louis., New Orleans: Black, Louisiana: Black in Male (AAR 96.6, 91.7) and in Female USA, Kentucky (AAR 50.3), USA Pennsylvania: Black (AAR 46.8) while Stomach was highest in Japan Hiroshima (AAR-80.3, 30.2), followed by Japan Yamagata Prefecture (AAR-79.4, 31.3). These heterogeneity of cancer incidence is contributed by different demographic, ecological, environmental, cultural, and genetic variability (Wang et al., 2012).

2.1.1 India cancer scenario

No national registry exists that provides comprehensive cancer incidence or mortality data for India. However, the National Cancer Registry Programme (NCRP) was started by the India council of Medical Research (ICMR) with a network of cancer registries across India in December 1981. Till 2011 there were 25 Population Based Cancer Registries (PBCRs) that provides information from 30 geographical areas.

Of the 14.1 million new cancer cases and 8.2 million cancer deaths reported by worldwide nearly 1 million new cases and 7, 00,000 deaths occurred in India, which is home to 17% of global population (NCRP, 2013). Among males, cancer of the lung, mouth, oesophagus, colorectal, pharyngeal and stomach were the highest. Lung cancer was a rare cancer in the beginning of the 20th century but later on it become epidemic resulting in greater number of deaths than those caused by colorectal, breast and prostate cancers (Khuri et al., 2001). Lung cancer was the leading cancer in Bangalore (10.8), Bhopal (12.4), Chennai (12.6), Delhi (13.9), Kolkata (16.8), Tripura (16.1), Kollam (19.5) and Thiruvananthapuram (14.4). Oesophageal cancer is considered as the 6th most common cancer among males and 9th most common cancer among females globally (Kumar et al., 2007). Cancer of the oesophagus is the leading site in Assam and Meghalaya, which was much higher than any other parts of the world, AAR of oesophagus cancer for male in East Khasi Hills was observed as (71.4), followed by entire state of Meghalaya (46.2), Aizwal District (42.0), Kamrup urban (27.0) District of Assam and entire Mizoram state (26.0). Stomach cancer is the 4th most common malignancy in the world and the 2nd leading cause of cancer death (Sharma et al., 2014). Globally a total of 989,600 new stomach cancer cases and 738,000 deaths are estimated to have occurred in 2008, accounting for 8% of the total cases and 10% of

total deaths (Jemal et al., 2011). Stomach cancer was leading site in state of Mizoram (47.6).

Among females, cancer of the breast and cervix were the leading site of cancer in almost all the PBCRs. Breast cancer is the most common type of cancer diagnosed in women in developed countries and the 2nd most common type diagnosed in developing countries. Breast cancer has been described as an alarmingly health problem in India (Yeole and Kurkure, 2003) while cervical cancer is the 2nd most commonly diagnosed cancer and 3rd leading cause of cancer death among females in less developed countries (Torre et al., 2015). Cancer of the breast is followed by gallbladder as the leading cancer in Dibrugarh (7.7) among females. Lung cancer was the leading site of cancer in Manipur (11.9) and Mizoram (28.7). Cancer of the oesophagus is highest in East Khasi Hills (30.2), Meghalaya (19.8), and Kamrup Urban District of Assam (18.3). Cancer of the thyroid followed breast cancer in Kollam (7.3) and Thiruvananthapuram (10.0) in Kerela State.

2.1.2 Nasopharyngeal carcinoma: A global perspective

According to the estimates of the International Agency for Research on Cancer (IARC) there were 86,691 new cases of NPC and 50,831 deaths in 2012. NPC is about 2-3 times higher in males than in females globally (Table 2.1). There were 60896 (0.6%) and 25795 (0.4%) new NPC cases in male and females respectively, while 35756 (0.8%) and 15075 (0.4%) deaths were reported in males and females worldwide. Although NPC is considered rare worldwide, its incidence is high in southern China and Southeast Asia, particularly among the Cantonese population, making this type of cancer a leading cause of death in high-risk areas (Xue et al., 2013). The geographical disparities in the burden of NPC in relation to resource are

noteworthy, with an estimated 92% of new cases occurring within economically developing countries (Torre et al., 2015).

Table 2.1 Incidence, mortality and prevalence of NPC in the World

Case/Sex	Incidence (%)	Mortality (%)	5-years (%)
NPC in Male	60896 (0.8)	35756 (0.8)	161899 (1.1)
Total cancer in males	7427148	4653385	15362289
NPC in Female	25795 (0.4)	15075 (0.4)	66799 (0.4)
Total cancer in females	6663001	3548190	17182344
Both sexes	86691 (0.6)	50831 (0.6)	228698 (0.7)
Total (NPC)	14090149	8201575	32544633

Table 2.2 NPC (Age-adjusted incidence rate) by sex and world areas

S. no	**Region**	**Male**	**Female**
1	South-Easter Asia	6.4	2.4
2	Micronesia/Polynesia	2.6	1.3
3	Eastern Asia	2.5	1.0
4	Northern Africa	2.3	1.0
5	Eastern Africa	1.9	1.1
6	Middle Africa	1.3	0.6
7	Western Asia	1.3	0.5
8	Western Africa	0.7	0.4
9	Southern Europe	0.7	0.3
10	Northern America	0.7	0.3
11	Australia/ New Zealand	0.7	0.3
12	South-Central Asia	0.6	0.2
13	Central and Eastern Europe	0.6	0.2
14	South America	0.5	0.2
15	Western Europe	0.5	0.2
16	Melanesia	0.4	0.1
17	Southern Africa	0.4	0.2
18	Caribbean	0.4	0.2
19	Northern Europe	0.4	0.2

AdaPted from Global cancer database (GLOBOCAN 2012) httP://globocan.iarc.fr/

AAR- Age standardised rates per 100,000 populations

According to GLOBOCAN 2012 (Ferlay, et al. 2013), the estimated AAR world for NPC incidence among males and females in 2012 were 1.7 and 0.7, respectively (Table 2.2). The areas with the highest incidence were Southeast Asia (6.4

for males and 2.4 for females), the disease is the sixth most common cancer among males in the region. Rates were also elevated in Micronesia (3.3 and 2.0), East Asia (2.5 and 1.0), North Africa (2.3 and 1.0), and East Africa (1.9 and 1.1). Globally, the 3 highest incidence rates were estimated to be in Malaysia (10.6 for males and 3.9 for females), Singapore (9.7 and 3.2), and Indonesia (8.3 and 3.0) followed by Vietnam (7.7 and 3.4), and Brunei (7.6 and 1.5). Other populations with relatively high incidence of NPC were the Inuit of Alaska, Greenland, and North Canada, as well as Chinese and Filipinos living in the United States. Rates of NPC are considerably lower in remaining countries like Americas and Europe (Zanetti et al., 2010).

In 2012, AAR world for NPC mortality among males and females were 1.0 and 0.4, respectively. The areas with the highest mortality were Southeast Asia (3.8 for males and 1.4 for females), East Asia (1.5 and 0.6), East Africa (1.4 and 0.9), North Africa (1.4 and 0.6), and Micronesia (1.3 and 1.0). The countries with the highest mortality were Indonesia (5.0 and 1.6), Vietnam (4.8 and 2.0), Singapore (4.4 and 1.3), Malaysia (3.9 and 1.2), and Brunei (3.4 and 0.5) (Wei et al., 2014).

In India, NPC is also rare, except for the Hill States of Northeast India (Kataki et al., 2011). The Mongoloid race in this region has shown an increase in NPC incidence. According to the National Cancer Registry programme (NCRP), 2006–2008 the highest age-adjusted incidence rates (AARs) of NPC was reported in Mizoram (6/100,000) followed by Manipur State (5/100,000) and Nagaland State belonging to the ethnic groups of Mizo, Manipuri and Naga. As per earlier report of NCRP 2002, Nagaland state had the highest AARs followed by Manipur and Mizoram. The district-wise distribution of the age-adjusted incidence rates (AARs) of NPC in Kohima district in Nagaland states was 19.4/100000, among the highest AARs reported in the world (Sharma et al., 2011).

2.2 Environmental risk factors for NPC

Epidemiological studies in endemic areas have identified various risk factors associated with NPC risk. EBV infection is considered as the major viral risk factor contributing to NPC carcinogenesis. Among others the dietary and lifestyle habits of the individual also are major contributing factors to NPC development as reviewed by ChangAdami (2006) and JiaQin (2012) in different regions of the world.

2.2.1 Dietary factors: Preserved foods, herbal medicine, fresh fruits and vegetables

Among the non-viral risk factors consumption of salted preserved fish is most consistently and strongly associated with NPC (Tsao et al., 2014; Putera et al., 2015). Salted preserved fish is a traditional staple food in several NPC-endemic areas and Ho (1972) in the early 1970s, first identified it as a potential risk factor for NPC development. Salted preserved fish is a traditional weaning food among the Cantonese population, resulting in early and more frequent feeding of infants. Recent, studies by Yang et al. (2005); Guo et al. (2009); Jia et al. (2010), in Chinese populations reported higher risk of NPC with childhood intake of salted fish more than the adulthood consumption. Moreover, the duration and frequency of consumption were independently associated with elevated risk of NPC (Armstrong et al., 1983; Yu et al., 1988; Yu et al., 1989a; Guo et al., 2009; Ekburanawat et al., 2010). Previously, Yu et al. (1986) estimated that over 90% of young nasopharyngeal carcinoma cases in Hong Kong Chinese can be attributed to consumption of this food during childhood. The relative risk for having Cantonese-style salted fish as one of the first solid foods during weaning and early childhood was 7.5 (95% CI: 3.9-14.8) and 20.2 (95% CI: 6.8-60.2) respectively, and the relative risk for consuming the food at least once a week compared to less than once a month at age 10 years was 37.7 (95% CI: 14.1-100.4). However, the relative risk for consumption of salted fish during adulthood was 7.5

(95% CI: 0.9-65.3). Two independent studies in Guangxi, China reported an elevated risk of NPC among salted fish consumers. Yu et al. (1988) found an increased risk of NPC for weekly intake (RR=2.2; 95% CI: 0.7-7.6) during early childhood and during weaning (RR=2.6; 95% CI: 1.2-5.6). Similarly, 3.8 fold risk (95% CI: 0.7-7.6) of NPC was reported for monthly and weekly consumption during early childhood (Zheng et al., 1994c). Salt-preserved fish is a traditional favourite item in the Cantonese diet. Development of nasal and nasopharyngeal tumors in experimental rats had supported its carcinogenic potential (Huang et al., 1978; Yu et al., 1989b; Zheng et al., 1994a). Salt-preserved fish contain N-nitrosamine (Poirier et al., 1987; Zou et al., 1994), which might be a source of carcinogenic chemicals that can act on the nasopharynx of susceptible individuals.

Other preserved protein-containing food and animal fats, fruits and vegetables were also found have significant association with NPC risk in southern Chinese (Chen and Huang, 1997; Jia et al., 2010; Liu et al., 2013), Southeast Asians (Lee et al., 1994; Armstrong et al., 1998), and North Africans (Feng et al., 2007). In low-incidence northern Chinese (Ning et al., 1990) significant association were observed for consumption in childhood of salted shrimp paste (increased risk) and carrots (reduced risk), and the three dietary effects (i.e., those from consumption of salted fish, salted shrimp paste, and carrots) were independent of each other. While in low-incidence population of U.S. (Farrow et al., 1998) risk of non-keratinizing and undifferentiated tumors of the nasopharynx ranges from 1.99 to 4.59 fold in frequent consumers of preserved meats, which contain high levels of added nitrites. Similarly, Hung et al. (2004) demonstrated that men who consumed fermented bean products, salted food and preserved/pickled vegetables more than once a week after age 40 years had a 3.4-fold risk (95% CI: 1.9-6.2), 2.3-fold risk (95%CI: 1.2-4.2), and 2.5-fold risk (95% CI: 1.3-

4.5) of esophageal cancer, respectively, compared to men eating these items less than once a week. High incidence of NPC is reported in northeast states of India. Very scanty data are available on detailed dietary risk factors associated with NPC. However, a study conducted by Chelleng et al. (2000) from Nagaland found that smoked meat (OR=10.8; 95% CI: 3.4–39.0) was among the major contributing dietary factor for NPC development.

In NPC endemic areas herbal medicine is used either for clinical treatment, herbal tea, and/or soups. Lin et al. (1979) first revealed a significant association of herbal medicine and NPC risk in Taiwanese population. Furthermore, several case-control studies in Asia showed high risk of NPC in association with use of traditional herbal medicines. Hildesheim et al. (1992), reported a 16 fold risk of NPC association with used of herbal medicine in Philippines. Further, strong interaction between herbal medicine and EBV infection was also suggested. It was reported that exposure to herbal medicines among subjects testing strongly positive for anti-EBNA antibodies was associated with a significant 49-fold excess risk of NPC when cases were compared to controls. Similarly, West et al. (1993) showed ever-users of herbal medicines being at a 2.5-fold excess risk of NPC in Philippines. Chelleng et al. (2000) reported herbal medicine used as a nasal drop enhanced the risk of NPC upto 22 fold (95% CI: 6.8-71.4) in the Naga population of northeast India. A role of herbal plants in NPC development is biologically plausible because such plants might contain compounds e.g., phorbol esters, which is thought to be linked to nasopharyngeal carcinoma, either through its ability to reactivate the EBV infection or through a direct promoting effect on cells transformed by the EBV (Furukawa et al., 1986; Tomei et al., 1987; Zeng et al., 1994). In addition to herbal medicine use, the consumption of herbal tea and soups with complex herbal medicine ingredients is common in the Chinese populations, but

there is limited evidence to suggest an associated with NPC risk (Zheng et al., 1994c; Jia et al., 2010).

In contrast to preserved foods regular consumption of fresh fruits and vegetables showed protective effect towards NPC development (Chen and Huang, 1997; Armstrong et al., 1998; Feng et al., 2007; Jia et al., 2010; Polesel et al., 2013). These findings were consistent with other cancers (Key et al., 2002). Kamangar et al. (2009) demonstrated that low intake of fresh fruits and vegetables increase the risk of esophageal squamous cell carcinoma (ESCC). Similarly, a reduced risk of prostate cancer was observed with dietary intake of plant foods including fruit and vegetable which are rich sources of carotenoids (Stacewicz-Sapuntzakis et al., 2008). The apparent protective effect of these foods may be due to their antioxidant potential (Weisburger, 1999) which act as an anti-carcinogen (Potter and Steinmetz, 1996) and prevent nitrosamine formations (Birt, 1986).

2.2.2 Lifestyle related factors: Tobacco, other smoke, and alcohol

Smoking or smokeless tobacco is an established risk factor of several cancers (Sasco et al., 2004; Boffetta et al., 2008). Several studies in NPC endemic areas have reported for a strong contribution of tobacco habits and NPC carcinogenesis. Tobacco smoking was found to have a 3.29 fold (95% CI: 0.68–15.9) risk of NPC in the Taiwan, China (Lin et al., 1979). Similarly, in its endemic population in Shanghai (Yuan et al., 2000), Yangjiang (Zou et al., 2000), and Wuhan (Ji et al., 2011) region of China, Thailand (Ekburanawat et al., 2010), Malaysia (Armstrong et al., 1983; Armstrong et al., 2000) and Philippines (West et al., 1993) tobacco smoking was found to be highly associated with the risk of NPC.

Furthermore, studies revealed that the association of tobacco smoking and NPC risk is dose-dependent. Yu et al. (1990) showed a moderate association of tobacco products with NPC, however a lifetime exposure of 30+ pack-year equivalents conferred a 2-fold increased risk in Guangdong, China. Vaughan et al. (1996) reported that that current smokers with a history of more than 60 pack-years have the 6.5 fold risk (95% CI: 2.0–21.3) of NPC in the Unites States. Similarly, in Taiwan (Hsu et al., 2009) a longer and greater cigarette smoking habit (≥30 pack-years of cumulative cigarette smoking) have a higher risk for NPC, compared with those of <30 pack-years (OR=3.0; 95% CI: 1.3–7.2). However, other studies conducted by Li et al. (1985), Sriamporn et al. (1992), Zheng et al. (1994b) and Zou et al. (2000) do not found risk of NPC with the habits of tobacco smoking.

NPC includes two separate entities: the differentiated NPC, associated with tobacco smoking like other cancers of head and neck, and the undifferentiated NPC, upon which tobacco smoking has little or no influence. Polesel et al. (2011) showed that statistically significant elevated OR were associated with increasing smoking intensity (OR for ⩾15cigarettes/day=5.40; 95% CI: 1.34-21.76) and duration of the habit (OR for ⩾32years=4.48; 95% CI: 1.11-18.04) for differentiated NPC. He also reported that the combination of tobacco smoking and alcohol drinking accounted for 57% of differentiated NPCs, whereas it accounted for only 14% of undifferentiated carcinomas. Cigarette smoking and snuff (tobacco powder with additives) intake were significantly associated with differentiated NPC but not with undifferentiated carcinoma (UCNT), which is the major histological type of NPC in North African populations (Feng et al., 2009). Moreover, the association of NPC risk with cigarettes was stronger for nonkeratinizing carcinoma than for keratinizing squamous cell carcinoma (KSCC) (Ji et al., 2011).

Some studies have suggested that smoke from other sources like wood fire burning in house without window or chimney (Clifford, 1972; Zheng et al., 1994c; Guo et al., 2009; Lakhanpal et al., 2015), burning incense or anti-mosquito coils (West et al., 1993) might contribute to NPC development (Chang and Adami, 2006) while others do not find such association (Geser et al., 1978; Yu et al., 1986; Yu et al., 1988; Chen et al., 1990). (Xie et al., 2014) observed an increased NPC risk associated with daily burning in women (OR=2.49, 95% CI: 1.33-4.66) but not in men. The OR for daily burning with poor ventilation was 2.08 (95% CI: 1.02, 4.24), while that with good ventilation was 1.35 (95% CI: 0.92, 1.98). Feng et al. (2009) also reported that domestic cooking fumes intake by using kanoun (compact charcoal oven) during childhood increased NPC risk, whereas exposure during adulthood had less effect.

Most case-control studies in endemic area demonstrated that alcohol consumption is not associated with NPC (Chelleng et al., 2000; Yuan et al., 2000; Ji et al., 2011; Polesel et al., 2011) and few studies showed a positive relation (Nam et al., 1992; Vaughan et al., 1996; Ruan et al., 2010; Lakhanpal et al., 2015). However, Chen et al. (2009) in a meta-analysis of 14 case–control studies from 5 countries reported that the pooled odds ratio between the highest and lowest alcohol consumption groups was 1.33 (95% CI = 1.09–1.62). Similarly, a recent meta-analysis conducted by Marron et al. (2010) demonstrated that high volume of alcohol consumption is significantly correlated with an increase risk of NPC, while low dosage consumption presents a protective effect.

2.2.3 Viral factor: Epstein Barr virus infection

Epstein Barr Virus infection is widespread all over the word, and persists latently over 95% of the adult population worldwide (Epstein et al., 1964). In the early 70s Henle et al. (1970) using indirect immune fluorescence technique detected higher

anti-EBV antibody titers in NPC patients than in controls. Later, Wolf et al. (1973) also established the presence of EBV genome in NPC cells. Due to its involvement in NPC development, IARC has been classified EBV as a group I carcinogen (IARC 1997). Seroepidemiological studies from different parts of the world demonstrated elevated levels of antibodies especially IgG and IgA titers against viral capsid antigen (*VCA*) and early antigen (EA), IgG to EBV nuclear antigens 1 and 2 (*EBNA 1* and *EBNA 2*) (Sawaki et al., 1975; Lin et al., 1977; de-The et al., 1978; Cevenini et al., 1986; Bogger-Goren et al., 1987; Kumar et al., 2001). Besides these studies conducted by Saemundsen et al. (1982),Chen et al. (1987), Cheng et al. (1980) and reported high level of neutralizing anybodies against EBV specific DNase in the sera of NPC patients. Neel et al. (1983) further showed that the anti-Epstein-Barr virus (EBV) profile of elevated antibody titers directed against *VCA* and *EA* was seen in 85% of the patients with WHO types 2 and 3 tumors but in only 16% of the patients with WHO type 1 tumor. Though the role of EBV in the causation of NPC is not well understood, Pearson et al. (1983); Hadar et al. (1986); Chen et al. (1989), and Cai et al. (2014) reported that the anti-VCA antibodies is the most specific for this disease and of the greatest diagnostic value when used alone or in combination with the anti-EA test and/or EBV specific DNase.

zur Hausen et al. (1970); Nonoyama et al. (1973); Wolf et al. (1973); Desgranges et al. (1975); Lanier et al. (1981) also showed the involvement of EBV by detecting EBV DNA in biopsies of NPC patients. Klein et al. (1974) reported the evidence for the presence of EBV DNA and nuclear antigen (NA) in malignant epithelial cells from patients with poorly differentiated carcinoma of the nasopharynx. Studies conducted by Fahraeus et al. (1988); Young et al. (1988); Hu et al. (1991a) reported the expression of EBV encoded proteins in nasopharyngeal carcinoma.

Besides, the studies reported above ChanLo (2002); Kondo et al. (2004); Lin et al. (2004); Hassen et al. (2011) suggested that serum/plasma EBV-DNA is promising molecular tumor marker for monitoring NPC. Although, EBV is detected in virtually in all NPC cases, regardless of their geographical origins studies showed that the undifferentiated carcinomas but not squamous cell carcinomas of the nasopharynx are regularly associated with the virus (Niedobitek et al., 1991; Pathmanathan et al., 1995a; Nicholls et al., 1997).

Not only the presence but also the clonal expansion of EBV in tumor tissues is necessary to associate EBV with tumorigenesis. (Pathmanathan et al., 1995b) and (Raab-Traub and Flynn, 1986) suggested that EBV-associated NPC tumors are clonal expansions of a single EBV-infected progenitor cell by detection homogeneous repetitions of variable repeat sequences at EBV termini in carcinoma of the nasopharyngeal samples.

Considerable research is carried out in different parts of the world to determine if the international pattern of NPC is associated with specific tumor related strains of the virus. Based on the sequence variation of the viral EBNA genes (*ENNA2*, *EBNA3A*, *EBNA3B*, and *EBNA3C*) the virus is divided into two subtypes (Dambaugh et al., 1984; Sample et al., 1990). The two subtypes showed variation in geographical distribution. Young et al. (1987) determined that both the strains are widespread in Africa and New Guinea region, however, EBV1 was predominant in Caucasian and Asian populations including those showing a high frequency of NPC (Chang et al., 2009). The B95.8 strain of EBV was the first human herpes virus to have its genome completely cloned and sequenced (Baer et al., 1984). Later, with the development in molecular techniques extensive research has been conducted to determine the strain variations in a wider range of loci. Particularly, variation in the LMP-1 sequence has

led to identification of certain EBV strains associated with NPC tumor. Studies conducted in EBV of NPC tumors from China (Hu et al., 1991b; Chen et al., 1992; Miller et al., 1994), Malaysia (Tan et al., 2003), Alaska natives (Edwards et al., 1999), and some U.S. Caucasians (Abdel-Hamid et al., 1992), has detected a loss of a *XhoI* restriction site in the N-terminus. Among others a 30bp deletion in C-terminus has also been detected (Cheung et al., 1998; Plaza et al., 2003; Tan et al., 2003; Dardari et al., 2006). In addition Hu et al. (1993); Li et al. (1996) and Tiwawech et al. (2008) reported that the 30bp deletion seems to enhance the transforming potential of *LMP1 in vitro*, and had a significantly higher frequency in advanced NPC patients (stage III and IV), suggesting a link with development and progression of this malignancy.

Limited research has been conducted that assesses the relationship between EBV and sporadic Indian NPC and the role of serum EBV DNA/RNA or protein and/or serum anti-VCA/EA antibodies titre in NPC detection. And till date there is no report for strains involved in NPC carcinogenesis in Indian population. However, study conducted by Rathaur et al. (1999) reported that geographically A type EBV was far more prevalent in Western India, while in Eastern India particularly Assam, all five cases were positive for B type EBV. Thus, a significant variation in the type of EBV infection was observed in nasopharyngeal carcinoma in different ethnic populations in India. Krishna et al. (2004) detected EBNA-1 in the serum and corresponding tissues of NPC patients suggesting that the serum EBV DNA originates from NPC and also indicates the benefit of circulating viral DNA as an early marker in the diagnosis of NPC. He further suggested that serum DNA-PCR methods can be extrapolated to follow-up studies involving tumor regression or to assess the response to various therapies.

2.3 Genetic risk factors for NPC

The role of genetic and environmental factors in cancer development is now clearly understood from numerous epidemiological studies. Variation in immune related, cell cycle control genes, DNA repair and metabolic genes etc. have been reported to be associated with cancers (Oyama et al., 1997; Przygodzki et al., 1998; Romanowicz et al., 2010). Such variation may be inherited or spontaneous and may result into hereditary (2%) or sporadic cancers (98%) (Roukos, 2009). Some of the important variations that play important roles in NPC carcinogenesis may include:

2.3.1 Metabolic and DNA repair genes

Several epidemiological studies have provided sufficient evidence for the role of environmental carcinogens, particularly those present in dietary (salted fish, smoked meat) and lifestyle factors (tobacco and alcohol habits) including smoke from wood-fire burning and other occupational exposure to chemical (Jia and Qin, 2012). Phase I (*CYPs*) and Phase II (*GSTs*) enzymes activate and detoxify these carcinogens before eliminating from the body (Xue and Warshawsky, 2005; Shukla et al., 2013). These compounds causing DNA damage will lead to gene mutations, chromosomal instability and initiating carcinogenesis (Mondal and Ghosh, 2013), but are often repair by the host DNA repair mechanism (Berwick and Vineis, 2000; Metsola et al., 2005). However, inter-individual genetic variation may alter enzymatic activity thereby increasing susceptibility to cancer risk (Rodriguez-Antona and Ingelman-Sundberg, 2006; Sterpone et al., 2010; Tamaki et al., 2011). Some metabolic and repair genes were examined for the association with NPC and often have given in conflicting results.

Several members of the phase I (*CYPs*) superfamily have been investigated. Studies conducted by Hildesheim et al. (1995); Hildesheim et al. (1997) in the

Taiwanese population indicated that *rs2031920* (*RsaI*) and *rs6413432* (*DraI*) variants at *CYP2E1* gene were higher among cases compare to controls and that Rsa I digestion (c2 allele) have an increased risk of nasopharyngeal carcinoma (RR = 2.6; 95% CI = 1.2-5.7). Moreover this effect was limited to non-smokers (RR = 9.3; 95% CI = 2.7-32) and was not affected by alcohol consumption. However, Kongruttanachok et al. (2001) and Guo et al. (2010) found no association in Thais and Chinese population. Moreover, Jia et al. (2009) systematically conducted two independent studies (a family-based association study and a case–control study) in the Cantonese Chinese to investigate the association *CYP2E1* genetic variants and NPC risk using the haplotype-tagging single-nucleotide polymorphism approach. In the family based study involving 2499 individuals from 546 nuclear, *rs9418990, rs915908, rs8192780, rs1536826, rs3827688* and one *haPlotyPe h2 (CGTGTTAA)* were revealed to be significantly associated with the NPC (P = 0.045–0.003 and P = 0.003, respectively). Further, the case–control study which includes 755 cases and 755 controls also observed similar results in younger individuals (<46 years of age) with a history of cigarette smoking, with ORs of specific genotypes ranging from 1.88 to 2.99. This study provides evidence for associations between genetic variants of *CYP2E1* in the susceptibility of NPC risk and suggests that their interaction with environmental factors awaits further investigations. Two independent studies in Han Chinese population from Guangdong and Guangxi Provinces of southern China reported variants of *CYP2E1*- rs2031920 and rs6413432, *GSTP1*-rs947894, *MPO*-rs2333227 and *NQO1*-rs1800566 have no association with NPC risk (Guo et al., 2010). In addition studies conducted in Thai population (Tiwawech et al., 2006) reported individual with mutant alleles (*1B and *4C) had an increased risk for NPC (OR=2.37, 95% CI=1.27-4.46); while no association was reported *CYP2A13* (Jiang et al., 2004) and *CYP1A1* (m1:m2 alleles) (Cheng et al.,

2003) in Cantonese and Taiwanese populations. Beside the above reports studies were also conducted in phase II and other metabolic genes. Association of *GSTM1* and *GSTT1* null genotypes with NPC had shown conflicting results. Studies conducted in US (Nazar-Stewart et al., 1999), China (Deng et al., 2004; Jiang et al., 2011), Tunisia (Bendjemana et al., 2006) reported a significant association of GSTM1 null genotype and NPC risk. However, such association was not found in Taiwan (Cheng et al., 2003). Similarly, Guo et al. (2008) reported that null genotypes of *GSTM1* and *GSTT1* are not associated with NPC in Han Chinese population. But individuals who carried *GSTM1/GSTT1*-double null genotype had a higher risk for nasopharyngeal carcinoma in the male population (odds ratio, 1.76; 95% confidence interval, 1.04-2.97; P = 0.03). Tiwawech et al. (2005) suggested that *GSTM1* polymorphism may be associated with NPC susceptibility in Thais, especially for *GSTM1* null genotype carriers of age higher than 45 years. *GSTT1* null genotype was also reported to have a positive association with NPC risk (Deng et al., 2004; Jiang et al., 2011), while other studies denied such an association (Cheng et al., 2003; Bendjemana et al., 2006). No significant association was observed for variants of GSTP1 or NAT2 (slow or fast acetylator) (Cheng et al., 2003; Jiang et al., 2011) in the contribution to NPC risk.

Variations in Arg194Trp, Arg280His, and Arg399Gln codons of *XRCC1* gene have been extensively studies for their associations with NPC susceptibility. Study conducted in Cantonese population (Cao et al., 2006) showed that the *XRCC1* Trp194Trp variant genotype is associated with a reduced risk of developing NPC which was in contrast to the Sichuan population (Yang et al., 2007) where it was a risk factors for NPC and in North African population where it was not associated with NPC risk (Laantri et al., 2011). The Arg280His was found to be associated with a reduced risk of NPC in the Taiwanese population (Cho et al., 2003). However, other studies conducted

in Arg280His, and Arg399Gln codons did not report association with NPC risk (Cho et al., 2003; Yang et al., 2007; Laantri et al., 2011). Studies were also conducted in other repair genes. *XPD* codon Lys751Gln allele was associated with a borderline decrease of NPC (OR = 0.600, 95% CI: 0.361-1.000) while no association was reported with *XRCC3* Thr241Me (Yang et al., 2007). Cho et al. (2003) reported that polymorphisms of the DNA repair genes *hOGG1* Ser326Cys are associated with an altered risk of NPC in the Taiwanese population, however it is likely to play a role in North Africans population (Laantri et al., 2011). The polymorphism of *ERCC1* 8092 C > A might be a contributing factor in the development of NPC in Chinese population (Yang et al., 2009). While two rare haplotypes (ATTA and GTTG) for rs17511668, rs794001, rs2252352, and rs2271395 of the showed *N4BP2* gene showed significant differences between case and control groups in southern Chinese, conferring risk and protective effects to NPC, respectively (Zheng et al., 2007). A candidate gene association study was conducted among the Cantonese population in Southern China (Qin et al., 2011) utilizing a two-stage study design. In the discovery stage, 676 tagging SNPs covering 88 DNA repair genes were genotyped in 755 cases and 755 matched controls where 11 SNPs with P (trend) < 0.01 were identified. Seven of these SNPs were located within 3 genes, *RAD51L1*, *BRCA2*, and *TP53BP1*. In the validation stage, these 11 SNPs were genotyped in a separate Cantonese population o 1568 cases and 1297 controls. Two of the SNPs (rs927220 and rs11158728), both in *RAD51L1*, remained strongly associated with NPC. The SNP rs927220 had a significant P (combined) of 5.55×10^{-5}, with OR = 1.20 (95% CI = 1.10-1.30), Bonferroni corrected P = 0.0381. The other SNP (rs11158728), which is in strong linkage disequilibrium with rs927220 ($r^2 = 0.7$), had a significant P (combined) of 2.0×10^{-4}, Bonferroni corrected P = 0.1372. These findings support the involvement of genes related to DNA damage and repair processes, in

particular *RAD51L1*, in the development of NPC. A multiple centre case-control with 1052 cases and 1168 controls was performed in Eastern and Southern Chinese population (Zheng et al., 2011) to assess the association between NBS1 polymorphisms and NPC risk. Significant difference was observed in genotype frequencies at the rs1805794 C/G site between cases and controls (P trend < 0.0001). The C allele was found to increases the risk for invasive disease or metastatic disease, compared with G allele. Moreover, CNE-2 cells (NPC cell line) transfected with the mutant with C allele at the polymorphic site had significantly higher migration levels than those transfected with pcDNA-*NBS1*-185E (8360GG) (P = 0.024). These findings suggest that E185Q polymorphism in *NBS1* may be a genetic modifier for the occurrence and aggression of NPC.

2.3.2 Newly identified susceptible loci in *GWAS*

GWAS have been conducted for NPC to identified common variants linked to NPC susceptibility. The first study was conducted in Malaysian Chinese population (Ng et al., 2009). It was a two-stage design where more than 500,000 tag single-nucleotide polymorphisms (SNPs) were genotype in an initial sample set of 111 unrelated NPC patients and 260 controls. The top 200 SNPs showing the smallest P-values, where further evaluated using a replication sample set that consisted of 168 cases and 252 controls. The combined analysis of the two sets of samples found an SNP in intron 3 of the *ITGA9* (integrin-alpha 9) gene, rs2212020, to be strongly associated with NPC ($P=8.27 \times 10^{-7}$, OR=2.24, 95% CI=1.59-3.15). The gene is located at 3p21 which is commonly deleted in NPC cells. Subsequent genotyping of additional 19 tag SNPs within a 40-kb linkage disequilibrium (LD) block surrounding this landmark SNP showed that SNP rs189897 have the strongest association with a P-value of 6.85 x 10(-

8) (OR=3.18, 95% CI=1.94-5.21), suggesting that a genetic variation(s) in *ITGA9* may influence susceptibility to NPC in the Malaysian Chinese population.

Another GWAS was conducted within the Taiwanese population to identify the common genetic variants linked to NPC susceptibility (Tse et al., 2009). In the initial phage 480,365 single-nucleotide polymorphisms (SNPs) were genotype in n 277 NPC patients and 285 healthy controls. Twelve statistically significant SNPs were identified and mapped to chromosome 6p21.3. Associations were further replicated in two independent sets of case-control samples. Two of the most significant SNPs (rs2517713 and rs2975042; Pcombined= 3.9×10^{-20} and 1.6×10^{-19}, respectively) were located in the HLA-A gene. Moreover, significant associations between NPC and two genes were detected: specifically, gamma amino butyric acid b receptor 1 (*GABBR1*) (rs29232; Pcombined= 8.97×10^{-17}) and HLA-F (rs3129055 and rs9258122; Pcombined= 7.36×10^{-11} and 3.33×10^{-10}, respectively). Notably, the association of rs29232 remained significant (residual $P < 5 \times 10^{-4}$) after adjustment for age, gender, and HLA-related SNPs. Furthermore, higher *GABA* (B) receptor 1 expression levels can be found in the tumor cells in comparison to the adjacent epithelial cells ($P< 0.001$) in NPC biopsies, implying a biological role of GABBR1 in NPC carcinogenesis. It was the first GWAS report of NPC showing that multiple loci (HLA-A, HLA-F, and GABBR1) within chromosome 6p21.3 are associated with NPC. Although some of these relationships may be attributed to linkage disequilibrium between the loci, the findings clearly provide a fresh direction for the study of NPC development.

Recently, a genome-wide association study was performed in southern Chinese descent with 1,583 NPC cases and 1,894 controls using 464,328 autosomal SNPs in the initial phase of genotyping (Bei et al., 2010). The top 49 SNPs were further genotyped in 3,507 cases and 3,063 controls of southern Chinese descent from

Guangdong and Guangxi. Seven supportive SNPs were further confirmed by transmission disequilibrium test analysis in 279 trios from Guangdong. The study identified three new susceptibility loci, *TNFRSF19* on 13q12 (rs9510787, Pcombined=$1.53x10^{-9}$, OR=1.20), *MDS1-EVI1* on 3q26 (rs6774494, Pcombined=$1.34x10^{-8}$, OR=0.84) and the *CDKN2A-CDKN2B* gene cluster on 9p21 (rs1412829, Pcombined=$4.84x10^{-7}$, OR=0.78). Furthermore, the role of *HLA* was confirmed by revealing independent associations at rs2860580 (Pcombined=$4.88x10^{-67}$, OR=0.58), rs2894207 (Pcombined=$3.42x10^{-33}$, OR=0.61) and rs28421666 (Pcombined=$2.49x10^{-18}$, OR=0.67). These studies provide new insights into the pathogenesis of NPC by highlighting the involvement of pathways related to *TNFRSF19* and *MDS1-EVI1* in addition to *HLA* molecules.

The findings of the GWAS have not been established in other populations. However, a recent study in Thailand (Fachiroh et al., 2012) investigate the involvement of variants at rs2860580 (*HLA-A*), rs2894207 (*HLA-B/C*), rs1412829 (*CDNK2A/B*), rs1572072 and rs9510787 (*TNFRS19*), and rs6774494 (*MDS1-EVI1*) and another three variants rs2736098 and rs402710 (*TERT*) and rs578776 (CHRNA3). Four out of six genetic variants (rs2860580, rs1412829, rs1572072 and rs6774494) implicated in the recent NPC GWAS were associated with NPC risk (P trend $\leq$ 0.03), as well as two variants (rs402710 and rs2736098) on the TERT locus at 5p15.33 (p=0.004 and p=0.04, respectively suggesting that this locus is involved in NPC susceptibility, representing a novel finding in NPC epidemiology.

2.3.4 Mitochondrial DNA copy number variation in human cancers

Mutations in mitochondrial DNA have been reported to be involved in numerous diseases including cancers, diabetes, aging and neurodegenerative diseases (Chinnery and Samuels, 1999; Wallace, 1999; Wallace, 2010). However, several

studies have been conducted using real time qPCR that analysed the increased or decreased mtDNA content in human body fluid, circulating cells, and tissues to find their association with diseases including cancers (Yu, 2011; Malik and Czajka, 2013).

Mambo et al. (2005) and Fan et al. (2009) showed that mtDNA content was reduced in 80-82% of the breast tumors compare with the normal ones, and suggested that mtDNA content can be used as a molecular diagnostic tool to help identify genetic abnormalities in breast cancer tumors. Studies were also conducted to confirm whether the alterations in mtDNA are related to the clinic-pathological parameters and patient prognosis in breast cancer. Tumors with somatic mtDNA alterations in the D-Loop region have significantly lower mtDNA content and it decreased from grades 0 and I to grade II tumors, but increased from grade II to grade III tumors (Bai et al., 2011). Similarly, Xia et al. (2009) reported the content of mtDNA in stage I breast cancer patients was significantly lower than in other stages (overall $P = 0.023$). Further, reduced mtDNA was found often in post menopausal cancer group ($P = 0.024$) and there were no difference in mtDNA content, in regards to age ($P = 0.564$), lymph node involvement ($P = 0.673$), estrogen receptor (ER) ($P = 0.877$), progesterone receptor (PR) ($P = 0.763$), and Her-2/neu protein expression ($P = 0.335$). On the other hand, occurrence of mtDNA D-loop mutations or reduced copy number was associated with an older onset age (>or=50 years old) as well as a higher histological grade and tumors that lacked expressions of estrogen receptor and progesterone receptor. Moreover, patients with mtDNA d-loop mutation or reduced mtDNA content were found to have significantly poorer disease-free survival rate (Tseng et al., 2006; Yu et al., 2007). Further, the level of MtDNA in breast cancer tissue correlated with patient response to anthracycline chemotherapy, the disease-free survival of patients with higher mtDNA content breast cancer was significantly lower than that of patients with lower mtDNA

content breast cancer (Hsu et al., 2010) whereas in acute lymphoblastic lymphoma (ALL), reduced blood mtDNA after treatment was found to confer increased susceptibility to chemotherapy which can be a clue to the good prognosis of childhood ALL (Kwok et al., 2011).

Decrease mtDNA content was also detected in 34 of 37 renal carcinoma tissues as compared with control kidney (Meierhofer et al., 2004). Similarly, Purdue et al. (2012) reported Renal cell carcinoma (RCC) patients had significantly lower mtDNA copy number compared to the controls. Further, the lowest quartile of mtDNA copy number was associated with a 60% increase in RCC risk relative to the highest quartile (OR = 1.6, 95% CI = 1.1-2.2; P trend = 0.009). In gastric cancer mtDNA depletion was found to be association with clinic-pathologic features. mtDNA depletion was observed significantly in the ulcerated, infiltrating (Borrmann's type III) and diffusely thick (Borrmann's type IV) types of gastric carcinomas (P = 0.018) (Wu et al., 2005).

In non-small cell lung cancer (NSCLC), low mtDNA copy number (P = 0.089) and low degree of oxidative mtDNA damage (P = 0.036) were found to associate with tumor progression. Moreover, mtDNA copy number was significantly related to the degree of oxidative mtDNA damage (P = 0.031). After chemotherapy, the mtDNA copy number and oxidative mtDNA damage were found to be lower in advanced NSCLC. This finding suggests that a decrease in the content of mtDNA may result in a decrease of mitochondrial density in cancer cells, which leads to a decrease of endogenous ROS production and reduction of ROS-triggered DNA damage to achieve immortalization (Lin et al., 2008).

Jiang et al. (2006) analysis the cytochrome c oxidase (Cox) I and Cox II genes to measure changes in mtDNA content in pre-treatment and post-treatment salivary

rinses obtained from 76 patients undergoing surgical resection for primary head and neck squamous cell carcinoma. Further, the relationship between changes in mtDNA content and postoperative radiation therapy, smoking exposure, alcohol intake, and other clinical characteristics were also examined. It was reported that salivary mtDNA content is decreased in never smokers and in response to radiation therapy after primary surgical resection. A recent study conducted by Mondal et al. (2013) found an increased risk of oral squamous cell carcinoma (OSCC) with decreased mtDNA content. Moreover, the association between mtDNA copy number and OSCC risk was also found to be evident among tobacco - betel quid chewers rather than tobacco - betel quid non chewers.

Lee et al. (2004) examined effects of the D-loop mutations on the copy number of mtDNA in human hepatocellular carcinoma (HCC) tissues. They found that reduction in mtDNA copy number was highly associated with the occurrence of point mutations near the replication origin of the heavy-strand of mtDNA, suggesting an important event during the early phase of liver carcinogenesis. In another study conducted by Yin et al. (2004) non-tumor liver of HCC patients with alcohol drinking habits have reduced mtDNA content and higher level of somatic mutation in mtDNA (4977 bp-deleted) as compared with non-alcohol patients. This study further reported that decreased mtDNA content, reduction in expression level of the peroxisome proliferator-activated receptor gamma coactivator-1, while an upregulation of the mitochondrial single-strand DNA-binding protein indicating that the regulation of mitochondria biogenesis is disturbed in HCC. Yamada et al. (2006) showed that reduction in mtDNA copy number is significantly correlated with tumour size ($P = 0.014$) and cirrhosis ($P = 0.048$) and patients with a low mtDNA copy number tended to show shorter 5-year survival rates than patients with a high mtDNA copy number.

Recently, Zhao et al. (2011) found that that mtDNA content in peripheral blood leukocytes is significantly associated with HCC, compared to individuals with high mtDNA content, those with low mtDNA content had a significantly increased risk of HCC when health controls, chronic liver disease (CLD) controls or combined controls were used as reference. Additionally, significant association was only evident in younger individuals, male individuals, ever-smokers, and never-drinkers. These above studies demonstrate that decreased mtDNA is associated with various types of cancers. However, other studies showing increased mtDNA content in cancer have also been reported.

Increased mtDNA content was also associated with lung cancer (Bonner et al., 2009; Hosgood et al., 2010) and thyroid cancer (Mambo et al., 2005). High mtDNA level in saliva was found to be associated with increased risk of head and neck cancer (Kim et al., 2004; Jiang et al., 2005). Recent study conducted by Lin et al. (2010) evaluated the roles of mitochondrial DNA alterations in esophageal squamous cell carcinoma (ESCC), emphasising the changes in the copy number and D310 variants of mtDNA. Esophageal muscles, noncancerous esophageal mucosa, cancerous esophageal squamous cell carcinoma nests, and metastatic lymph nodes of 72 patients were included in the study. The D310 variants were found to decrease from 2.2 to 1.7 and 1.5, respectively, in noncancerous esophageal mucosa to cancerous esophageal squamous cell carcinoma nests and metastatic lymph nodes. While the mtDNA copy number was increased from 0.159 to 0.192 and 0.206, respectively, ($P = 0.024$), especially in cigarette smokers ($P = 0.014$) and heavy wine drinkers ($P = 0.005$). This study demonstrated that somatic D310 mutations and increase in the copy number of mitochondrial DNA are of clinical importance in esophageal squamous cell carcinoma. Increased mtDNA content was associated with a significantly increased risk of breast

cancer and showed an inverse association with several important endogenous oxidants and antioxidants in blood (Shen et al., 2010). A prospective study in Finnish population showed elevated mtDNA content of peripheral white blood cell as a risk factor for non-Hodgkin lymphoma (Lan et al., 2008). MtDNA copy number was found to increased during endometrial cancer development and was a correlated with mtDNA instability in endometrial cancer cells (Wang et al., 2005). In ovarian cancer, mtDNA content in tumour cell was significantly higher than that in normal ovary. The average mtDNA copy number in pathological low-grade tumours was over two-fold higher than that in high-grade carcinomas and type I carcinomas also had a significantly higher mtDNA copy number than in type II carcinomas (Wang et al., 2006). Mizumachi et al. (2008) reported that an increased variance of mtDNA content in prostate cancer cells and in prostate cancer cell lines, compared to normal prostate cells. Cell-free mtDNA levels were greater in the serum of patients with testicular cancer (Ellinger et al., 2009). Higher mtDNA was also reported in the in leukocytes of patients with colorectal cancer (Qu et al., 2011). Moreover, in children with acute lymphoblastic leukaemia (ALL), there was an increased level of mtDNA in leukaemia cells obtained from cerebrospinal fluid (Egan et al., 2010).