

Head and neck squamous cell carcinoma – an overview

*Jayalalitha Sathiyamoorthy, Natarajan Sudhakar

Dept. of Biotechnology, Dr.M.G.R Educational and Research Institute (University), Maduravoyal, Chennai-95.

*Corresponding Author: E-Mail: jayalalitha.vit@gmail.com, Phone: 9677136340

ABSTRACT

Head and Neck Squamous cell carcinoma (HNSCC) is a group of malignant diseases arising from the mucosa of upper aero digestive tract. With a multifactorial etiology, HNSCC results in progressive interactions, which contributes to genetic susceptibility. The prevalence rate of HNSCC is higher in the Western population compared to non - Caucasian patients (including Indians) and it has gradually become a major public health issue due to rapidly changing demographics and life style factors. Genome – wide association studies (GWAS) showed on a large case – control cohorts have helped in mapping various genes and also that are involved in the regulation of immunity with HNSCC Susceptibility. Various Genes are involved with the mitochondrial oxidative stress and extracellular matrix regulation also play a very important role in HNSCC Pathogenesis. Gene-Gene interactions and correlations with the environmental conditions (smoking and alcohol consumption) have been showed significant covariates in HNSCC pathology. In this review, we have provided on the underlying molecular and as well as genetic mechanism in HNSCC worldwide which also in turn highlight the HNSCC – associated candidate genes and their potential role in causing disease pathogenesis.

KEY WORDS: HNSCC, Epidemiology, genes, DNA Methylation, DNA Repair.

1. INTRODUCTION

HNSCC is 6th most common cause of cancer in the world, with the annual incidence rate of over 6,00,000 cases per year and accounts for 3,50,000 persons deaths per year (Ferlay, 2010). Head and neck carcinoma refers to various malignancies arising in the mucosal surfaces of the oral cavity, pharynx and larynx, out of which 90% are Squamous cell carcinomas (Curado, 2009). With multifactorial etiology, HNSCC results in progressive interactions between various factors such as carcinogenic effects or viral infections, and a small fraction of familial cases in nature which implies that genetic polymorphism also contributes to HNSCC Susceptibility (Sturgis, 2007). The relative risk of developing HNSCC in individuals exposed to carcinogenic agents like tobacco varies from person to person, which also depends on the genetic factors like DNA Repair, and DNA methylation processes. When combined with these two factors seems to have high adverse synergistic effects in HNSCC patients (Spitz, 1994; Kruman, 2012). In other developing countries, various factors like betel, Human Papillomavirus infection types 16 and 18 are considered to be associated risk factors for HNSCC which plays a very important role in affected individuals and also act as potent carcinogen leading to malignant transformation (Szymanska, 2010; Stadler, 2008). The prevalence of HNSCC varies widely across different ethnic group worldwide. It is estimated that by the year 2020, atleast 15 million people will be affected by HNSCC globally (Nair, 1991). The prevalence of HNSCC is relatively higher in western population, and increasingly become a public health issue among the non-caucasian population (including Indians) due to the rapid changing demographics, senescence and life style factors (Paterson, 1996). HNSCC, being a complex disease, is attributed to multiple genes with different magnitudes of effect. Along with the genetic variants; environmental factors such as age, smoking, gender also play a very important role in the disease pathogenesis (Daly, 2003).

Gene - Gene and gene - environment interactions are the major hallmarks of HNSCC Susceptibility. In this review, we provide an overview on the epidemiology of HNSCC and its candidate risk factors. Briefly we focus on the epidemiology of this disease, its associated risk factors and also to provide some insights on the role of some major genes associated with HNSCC and their involvement and interactions with other genes in different populations.

1.1. HNSCC Phenotype: Phenotypically HNSCC is classified based on special clinical features like tumor size, extent of metastasis, and other indicators in the patient prognosis, these together are known as TNM staging system. TNM Classification are based on American Joint Committee on Cancer (AJCC) guidelines and patient prognosis is based on the stage in which the cancer is presented. Patients with stage I tumors usually have a 90% survival rate, whereas patients with stage II tumors have approximately a 70% survival rate. Unfortunately patients with head and neck cancers often present to the surgical oncologist with late stage tumors (e.g. III/IV), and therefore have a worse prognosis than early stage (e.g. I/II) patients also, in two-thirds of HNSCC patients have local lymph node tumor positivity leading to poor survival (Sobin, 2002).

1.2. Grading the HNSCC Phenotype: HNSCC being a complex disease which requires a uniform definition to understand the disease mechanism comprehensively, predictive risk and progression, identification of common risk factors and also for comparison across different studies. The tumors are classified into different categories. While Broder's grading system is widely used, other grading systems were introduced for simpler and rapid diagnosis and classification of HNSCC. The TNM Classification based on five – stage clinical scale provides a very simpler

demarcation of various stages of progressive HNSCC. Hence, it is widely used, as it requires relatively lesser time and expertise when compared to other systems' (Greene, 2002; Goyanna, 1951).

2. EPIDEMIOLOGY OF HNSCC

2.1. Epidemiological studies in non-Indian population: Epidemiological studies in large population cohorts have provided prevalence data in different population's worldwide. The Investigation of occupational and environmental causes of respiratory cancers (ICARE Study) from France reported a 7 years cumulative incidence of HNSCC in which age > 45 years was strongly associated with disease prevalence (Loredana Radoi, 2013).

2.2. Epidemiological studies in Indian Population: It is estimated that by the year 2020, there would be 120 million people (12.0% of the population) female and male population in India which may lead to an increased prevalence of HNSCC. Thus, showed an increased proportion of gender population, limited treatment options and lack of appropriate modalities of intervention to prevent disease progression, HNSCC would likely become a major public health problem. While, there was no major difference in the disease prevalence between North and South Indian populations, the subtle differences could be attributed to ethnic differences, study design and methodology, clinical parameters and the definition of disease status.

3. RISK FACTORS FOR HNSCC

Based on various epidemiological studies, several risk factors have been identified with the altered risk of HNSCC. These include age, race, smoking, alcohol consumption, dietary factors and genetic predisposition.

3.1. Age: Age was shown to be a consistent risk factor for HNSCC in various epidemiological studies. Subjects aged between 36 – 45 years showed a higher risk of developing advanced HNSCC when compared to other age groups (Martin-Granizo, 1997). Nearly 98% of the individuals aged more than 40 years old are affected by HNSCC in Europe. However studies showed that adverse conditions are seen in young individuals due to delay in diagnosis (Mehanna, 2010).

3.2. Ethnicity: The prevalence of HNSCC varied greatly among different ethnic population. It has been speculated that susceptibility to HNSCC from tobacco and alcohol use may vary by race and ethnicity. Among them; Western caucasians have a high rate of affected population of HNSCC due to smoking and alcohol rather than genetic factors, which may play a high incidence in some ethnic and racial groups (Warnakulasuriya, 1999).

3.3. Tobacco and alcohol consumption: Many population-based studies have demonstrated the association of smoking and alcohol intake individuals showed synergistic increased risk factor of HNSCC, which is approximately greater than 10 folds when compared to non - smoker and non-alcoholic individuals regardless of age (Gillison, 2000). In the Indian context, smoking as a risk factor was assessed in terms of smokers and non-smokers, and light and heavy smokers. Moreover, notable modifiable risk factors seem to enhance the carcinogenic effect in every individual. In addition, epidemiologic studies also showed a strong association between smoking and alcoholic drinking which lead to oral carcinoma. However, the risk factor of cancer decreases soon after a smoker quits, while precancerous conditions often diminish after a person stops using tobacco (Hashibe, 2007). Subjects aged between 46 – 55 years showed a greater risk of developing HNSCC when compared to other age groups (Martin-Granizo, 1997).

SNPs in these genes could play an alternative mechanism that modulates the effects of cigarette smoking. Even though alcohol and smoking are known to be major risk factors, only a fraction of smokers and alcohol consumers develop HNSCC, suggesting that genetic susceptibility and interactions between genetic and environmental factors also play very important role in the etiology of HNSCC (Marian, 2010; Solomon, 2008).

Various Genetic polymorphisms of alcohol-metabolizing enzymes, includes alcohol dehydrogenases (ADH) which also metabolizes alcohol into acetaldehyde, with a different ability to generate carcinogen acetaldehyde, may determine individual susceptibility to head and neck cancer. Acetaldehyde can form adducts with DNA, interfering with DNA synthesis and repair mechanism (Supic, 2011).

“Fast-metabolizing” ADHs genotype showed that there is increased HNSCC risk. By contrast, in other studies “fast-metabolizing” ADHs genotype was found to be associated with decreased risk factor of HNSCC (Seitz, 2007; Schwartz, 2001). Therefore, the mechanism by which smoking and alcohol causes increased risk for HNSCC and the role of alcohol and tobacco-related polymorphisms have not been fully elucidated.

3.4. Dietary factors: Among the other factors, dietary factors has been associated as a major risk factor for HNSCC of (Lucent forte, 2009). The importance of diet and nutrition in head and neck carcinogenesis has been indicated in several epidemiological studies, but only a few studies explored the association between dietary patterns and HNSCC (De Stefani, 2005). Increased intake of fruits and vegetable consumption was also found to be recently associated with the reduced occurrence of HNSCC (Freedman, 2008). However, the relative association of HNSCC and dietary factors are still confounding and the mechanism is yet to be known completely.

3.5. HPV influence in HNSCC: Human papilloma virus (HPV) infections also play a very significant part in the etiology of HNSCC. Epidemiological studies showed that nearly 25% cases of HNSCC are associated with HPV and Molecular evidence also has showed a causative role for causing HPV, primarily type of 16 and 18 in the patients,

who are affected with HNSCC, particularly those arising in the base of the tongue and tonsils (Peters, 2005). In addition there is increasing evidence that HPV-associated HNSCC carcinomas showed distinct clinical and pathological tumor entity from alcohol and smoking-associated HNSCCs with regard to risk factors, tumor biology and progression (Ragin, 2007).

Presence of TP53 mutations in HPV infected tumors confers a high risk of recurrence in causing this disease. However, it has been recently reported that it is unlikely that HPV infection play significant role in tongue carcinogenesis in young HNSCC patients (Klussmann, 2001). The major causative role of HPV infection in the etiology, as well as in prognosis of head and neck cancers varies and it also depends on head and neck cancer subtypes and different anatomic tumor sites. From various point of view, it is clearly shown that HPV typing together with other molecular markers may help in defining a particular group of tumors with regard to prognosis and response to anti-cancer therapies.

3.6. HIV infection and HNSCC: The relation between HIV infection and HPV - related HNSCC is considered as the most complex disease. Most HNSCC are frequently seen in HPV Positive individuals than HPV negative people. One of the most common type of HNSCC in patients with correlation of HIV infections are Kaposi Sarcoma and Non-Hodgkin's lymphoma. However, the possible risk factors showed carcinogenic effects among these patients apart from tobacco and alcohol exposure, including immunosuppression, opportunistic infections, and high-risk HPV subtypes (Kabeya, 2012; Powles, 2004).

Studies have shown that approximately 2 to 3 fold increase in the incidence of Squamous cell carcinoma of the head and neck. Further investigations are required to explore the pathogenesis, biology and management in HIV-positive patients affected with head and neck carcinomas (Frisch, 2001; Warnakulasuriya, 1999).

3.7. Exposure to UV Light: There have been conflicting reports on association of ultraviolet light or visible light in HNSCC. Ultraviolet (UV) light exposure may cause chronic irritation to the lining of the mouth, particularly in the lip cancer which mainly caused by UV irradiation due to increased risk factor of mucosal lining of the lips to the sun (Powles, 2004). In addition to it cancer found to be common oral malignancy and also responsible for causing more than 40% oral carcinoma (Perea-Milla Lopez, 2003).

High incidence rates for lip cancers are predominantly seen in Western Caucasian populations. The variations in the incidence of lip cancer in the western populations attributes to various differences in the rural and urban populations with the respect to exposure to UV (Sugerman, 2002). This supports the hypothesis of a causal association between sun exposure and lip cancer, but further research is needed.

3.8. Genetic predisposition: Genetic predisposition also plays an important role in the etiology and development of head and neck Squamous cell carcinoma and was initially indicated by case - control association studies (Jeffries, 1999). A higher concordance of HNSCC was reported among the monozygotic and dizygotic twins and also showed a higher disease risk among the first degree relatives of HNSCC probands provided further evidence that the genetic factors might play a key role in HNSCC pathogenesis (Warnakulasuriya, 2009; Foulkes, 1996).

3.9. DNA methylating factors in HNSCC: Frequent aberrant DNA hypermethylation within gene promoters showed hallmark in various human malignancies, including HNSCC. DNA methylation has been considered as important factor in head and neck carcinogenesis (Brown, 2001). There is increase in the number of genes that are found to be inactivated by DNA Methylation in HNSCC and multiple genes are involved in cell cycle control (p14, p15, p16 and p53), DNA damage repair (MGMT, hMLH1, and ATM), apoptosis (DAPK, RASSF1A, and RAR β), Wnt signalling (APC, RUNX3, WIF1, E-cad and DCC) SFRP family genes, TCF21 (Gonzalez-Ramirez, 2011).

3.10. DNA methylation genes involved in HNSCC:

3.10.1. p16, p15, p14: Various cell cycle regulatory genes have been extensively studied and promoter hyper methylation is common seen in HNSCC, however there is no significant correlation with clinic pathological characteristics or prognosis is observed. Loss of p16, p15 and p14 is frequently noticed in HNSCC and also found to be correlated with different stages of tumor, lymph nodes metastasis which is more prominently is seen in poorly differentiated HNSCC (Ha, 2006).

3.10.2. hMLH1: Involved in DNA Repair mechanism in which the promoter methylation in hMLH1 gene is considered as one of the common event in HNSCC and thereby resulting in high frequency at the early stage or at the later stage of HNSCC. However, it was correlated with poor survival rate in HNSCC (Steinmann, 2009).

3.10.3. MGMT: MGMT gene is located on the chromosomal region 10q26 encoding MGMT, a DNA repair enzyme in which it removes adducts caused by various alkylating agents. MGMT gene has been associated with different stages of HNSCC and is association between poor survival rate and prognosis in HNSCC patients (Zuo, 2009).

3.10.4. DAPK: DAPK play a very important role in apoptotic pathway where by helps in regulation also commonly methylated in HNSCC. However, reduced expression of DAP-kinase enzyme is associated with loss of apoptosis, cell immortality (Inbal, 1997).

3.10.5. RASSF1A: RASSF1A is considered as one of the most important tumor suppressor genes, belonging to RAS association domain family member 1 A, which plays an important role in silencing including HNSCC. However,

RASSF1A is also involved in maintaining genomic stability and also is highly mutated in poorly differentiated HNSCC when compared to moderately differentiated and well differentiated HNSCC (Hogg, 2002).

3.10.6. APC: APC gene plays a major role in Wnt Signaling mechanism in binding and degrading of beta – catenine. However, hypermethylation of APC gene promoter are seen in HNSCC (Chen, 2007).

3.10.7. WIF1: WIF1 gene involved in the Wnt signaling mechanism and methylated in HNSCC whereas the methylation status is been correlated with lymph node and metastasis in nasopharyngeal carcinoma (Fendri, 2010).

3.10.8. E-CADHERIN: Calcium Dependant cell adhesion molecule is located on chromosome 16q^{22.1} which plays a very important role in wnt signaling mechanism which interacts with β - and α -catenine. E-Cadherin is 120 kDa transmembrane glycoprotein which is also responsible for cell – cell adhesion. However, hypermethylation of E-cad is also associated with different stages of tumor leading to poor differentiation in HNSCC. However the patients with E-cad promoter methylation showed lower rates of local recurrences and also had better disease-specific survival and outcome (Supic, 2009).

3.10.9. SFRP (Secreted Frizzled – related Protein 1): SFRP involves in inhibition of Wnt signaling pathways. Different Promoter genes are present such as SFRP-2, SFRP-4, SFRP-5 genes which showed methylation in HNSCC, whereas SRFP-1 was demethylated in oral cancer and also been associated with various tumor grade in HNSCC (Pannone, 2010).

3.11. DNA repair genes: DNA repair mechanism is one of the fundamental processes in maintaining genomic stability in cancer. Several strategies have been undertaken to identify the precise genomic regions involved in HNSCC pathogenesis. However, various genetic alterations associated with HNSCC are numerous and involved in various pathways.

3.11.1. XRCC1: XRCC1 play a very important role in Base Excision Repair Pathway and gene is located at the chromosomal region 19q13.2 which possesses 17 exons and encodes nearly 633 amino acids long proteins (Cappelli, 2002). Various mutations have been noticed in XRCC1 gene which also has the potentiality in developing Cancer and there are major factor that XRCC1 is not only affected by point mutations but also by various other Single Nucleotide polymorphism (Goode, 2002). Two common polymorphism of XRCC1 have been noted that leading to various amino acid substitutions which are located in the exon 10 (G to A, Arg399Gln) and in exon 6 (C to T, Arg194Trp). However, XRCC1 is studied in various cancers including HNSCC and XRCC1 gene also plays a major component in several different DNA damage recovery pathways (Tuimala, 2002).

3.11.2. XRCC3: XRCC 3 gene located in the chromosomal region 14q32.3 and XRCC 3 protein participates in DNA double stranded break/recombination repair mechanism, is a XRCC 3 member of family of Rad 51 related proteins that participate in homologous recombination in order to maintain chromosomal instability and repair DNA damage. However, various studies have been carried on XRCC 3 and found that Thr241Met plays a major role in causing cancer (Tebbs, 1995).

3.11.3. ERCC2: ERCC2 gene located on the Chromosomal region 19q13.3 which encodes ERCC2 protein and also serves as one of the genetic complementation groups that plays as important barrier for NER Pathway. ERCC 2 protein plays a vital role and acts as ATP dependant helicase which helps in joining the transcription factor complex (Friedberg, 2001). ERCC2 also help in unwinding of DNA molecule allowing the transcription process and in turn the promoter permits the NER to access the lesions (Affatato, 2004).

4. CONCLUSION

The complexities associated with the etiology of HNSCC provide a challenging problem in understanding the molecular mechanism of pathogenesis and its subsequent management. However, genomic studies are based on GWAS and facilitated a better understanding of the molecular genetic basis of HNSCC.

Future studies are being done in order to determine the causative role and significance of these epigenetic alterations which may provide important clues into the mechanism and contribution of the specific events and also help to establish a sequence of methylation events during tumor development associated with different stages of head and neck carcinogenesis.

Additional studies are necessary in order to confirm findings and also to identify the genetic or epigenetic mechanisms responsible for the altered gene expression. However, currently, there is no specific molecular marker available in order to predict the mechanism of HNSCC.

In addition, identification of new molecular markers with biological relevance can also help in the development of more efficient therapeutic strategies against a specific population of patients who do not show any satisfactory response to conventional treatments. Hence, these studies are considered as important aspect if these are to be further explored in frequent DNA methylation detected in HNSCC and also with tumor progression association and survival indicates that DNA methylation plays an important role in head and neck carcinogenesis and may be a useful diagnostic marker or potential therapeutic targets for HNSCC in near future.

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REFERENCES

- Affatato A.A, Wolfe K.J, Lopez M.S, Hallberg C, Ammenheuser M.M, and Abdel-Rahman S.Z, Effect of XPD/ERCC2 polymorphisms on chromosome aberration frequencies in smokers and on sensitivity to the mutagenic tobacco-specific nitrosamine NNK, *Environ. Mol. Mutagen.*, 44, 2004, 65–73.
- Brown LM, Gridley G, Diehl SR, Family cancer history and susceptibility to oral carcinoma in Puerto Rico, *Cancer*, 92, 2001, 2102–2108.
- Cappelli E, Taylor R, Cevasco M, Abbondandolo A, Caldecott K, and Frosina G, Involvement of XRCC1 and DNA ligase III in DNA base excision repair, *J Biol Chem.*, 272, 1997, 23970-23975.
- Chen K, Sawhney R, Khan M, Benninger M.S, Hou Z, Sethi S, Methylation of multiple genes as diagnostic and therapeutic markers in primary head and neck squamous cell carcinoma, *Arch Otolaryngol Head Neck Surg.*, 133, 2007, 1131-1138.
- Curado M, Hashibe M, Recent changes in the epidemiology of head and neck cancer, *Curr Opin Oncol.*, 21, 2009, 194-200.
- Daly B, Watt RG, Batchelor P, Treasure ET, Oral Cancer Prevention in Essential Dental Public Health, 1st ed., Oxford, UK, Oxford University Press, 2003.
- De Stefani E, Boffetta P, Ronco A.L, Correa P, Oreggia F, Deneo Pellegrini H, Mendilaharsu M, Leiva J, Dietary patterns and risk of cancer of the oral cavity and pharynx in Uruguay, *Nutr. Cancer*, 51, 2005, 132–139.
- Fendri A, Khabir A, Hadri-Guiga B, Sellami-Boudawara T, Daoud J, Frikha M, Ghorbel A, Gargouri A, Mokdad-Gargouri R, Epigenetic alteration of the Wnt inhibitory factor-1 promoter is common and occurs in advanced stage of Tunisi-an nasopharyngeal carcinoma, *Cancer Invest.*, 28 (9), 2010, 896-903.
- Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM, Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008, *Int J Cancer*, 127(12), 2010, 2893 - 2917.
- Foulkes WD, Brunet JS, Sieh W, Familial risks of squamous cell carcinoma of the head and neck: retrospective case-control study, *BMJ*, 313, 1996, 716-721.
- Freedman N, Park Y, Subar A, Hollenbeck A, Leitzmann M, Schatzkin A, Fruit and vegetable intake and head and neck cancer risk in a large United States prospective cohort study, *Int J Cancer*, 122, 2008, 2330-2336.
- Friedberg E.C, How nucleotide excision repair protects against cancer, *Nat. Rev. Cancer*, 1, 2001, 22–33.
- Frisch M, Biggar RJ, Engels EA, Goedert JJ, AIDS-Cancer Match Registry Study Group. Association of cancer with AIDS-related immunosuppression in adults, *JAMA*, 285, 2001, 1736-45.
- Gillison M.L, Koch W.M, Capone R.B, Spafford M, Westra W.H, Wu L, Evidence for a causal association between human papillomavirus and a sub-set of head and neck cancers, *J Natl Cancer Inst.*, 92, 2000, 709-720.
- Gonzalez-Ramirez I, Garcia-Cuellar C, Sanchez-Perez Y, Granados-Garcia M, DNA methylation in oral Squamous cell carcinoma: molecular mechanisms and clinical implications, *Oral Dis.*, 17 (8), 2011, 771–778.
- Goode EL, Ulrich CM and Potter JD, Polymorphisms in DNA repair genes and associations with cancer risk, *Cancer Epidemiol Biomarkers Prev.*, 12, 2002, 1513-1530.
- Goyanna R, Torres Et, and Broders AC, Histological grading of malignant tumors; Broder's method, *Hospital (Rio J)*, 39, 1951, 791-818.
- Greene FL, Page DL, Fleming ID, Fritz AG, Balch CM, Haller DG, American Joint Committee on Cancer, *Cancer Staging Manual*, Springer, New York, 2002.
- Ha P.K, Califano J.A, Promoter methylation and inactivation of tumour suppressor genes in oral squamous-cell carcinoma, *Lancet Oncol.*, 7, 2006, 77-82.
- Hashibe M, Brennan P, Benhamou S, Castellsaque X, Chen C, Curado MP, Alcohol drinking in never users of tobacco, cigarette smoking in never drinkers, and the risk of head and neck cancer: pooled analysis in the International Head and Neck Cancer Epidemiology Consortium, *J Natl Cancer Inst.*, 99, 2007, 777-89.

Hogg R.P, Honorio S, Martinez A, Frequent 3p allele loss and epigenetic inactivation of the RASSF1A tumour suppressor gene from region 321 in head and neck Squamous cell carcinoma, *Eur J Cancer*, 38, 2002, 1585-92.

Inbal b, Cohen O, Polak-Charcon S, Kopolovic J, Vadai E, Eisenbach L, Kimchi A, DAP kinase links the control of apoptosis to metastasis, *Nature*, 390, 1997, 180-184.

Jeffries S, Eeles R, Goldgar D, The role of genetic factors in predisposition to Squamous cell cancer of the head and neck, *Br J Cancer*, 79, 1999, 865.

Kabeya M, Furuta R, Kawabata K, Takahashi S, Ishikawa Y, Prevalence of human papillomavirus in mobile tongue cancer with particular reference to young patients, *Cancer Sci.*, 103, 2012, 161-168.

Klussmann J.P, Weissenborn S, Fuchs P.G, Human papillomavirus infection as a risk factor for squamous-cell carcinoma of the head and neck, *N Engl J Med.*, 345, 2001, 376-81.

Kruman II, Henderson GI, Bergeson SE, DNA damage and neuro-toxicity of chronic alcohol abuse, *Exp Biol Med.*, 237, 2012, 740–747.

Loredana Radoi, Sophie Paget-Bailly, Florence Guida, Diane Cyr, Gwenn Menvielle, Annie Schmaus, Matthieu Carton, Sylvie Cenee, Marie Sanchez, Anne-Valérie Guizard, Brigitte Tretarre, Isabelle Stucker and Daniele Luce, Family history of cancer, personal history of medical conditions and risk of oral cavity cancer in France: the ICARE study, *BMC*, 13, 2013, 560.

Lucenteforte E, Garavello W, Bosetti C, La Vecchia C, Dietary factors and oral and pharyngeal cancer risk, *Oral Oncol.*, 45, 2009, 461–467.

Marian M, Roberts S, Esophageal and Head and Neck Cancer: Clinical Nutrition for Oncology Patients, (1st edition), UK, Jones and Bartlett India Private Limited, 2010.

Martin-Granizo R, Rodriguez-Campo F, Naval L, Diaz Gonzalez FJ, Squamous cell carcinoma of the oral cavity in patients younger than 40 years, *Otolaryngology Head Neck Surg.*, 117, 1997, 268-75.

Mehanna H, Paleri V, West C, Nutting C, Head and neck cancer—Part 1: epidemiology, presentation, and prevention, *BMJ*, 341, 2010, 4684.

Nair MK, Sankaranarayanan R, Epidemiologic leads to cancer control in India, *Cancer Causes Control*, 2 (4), 1991, 263-265.

Pannone G, Bufo P, Santoro A, Franco R, Aquino G, Longo F, Botti G, Serpi- co R, Cafarelli B, Abbruzzese A, Caraglia M, Papagerakis S, Lo Muzio L, WNT pathway in oral cancer: epigenetic inactivation of WNT-inhibitors, *Oncol Rep.*, 24 (4), 2010, 1035-1041.

Paterson IC, Eveson JW, Prime SS, Molecular changes in oral cancer may reflect aetiology and ethnic origin, *Eur J Cancer B Oral Oncol.*, 32, 1996, 150–53.

Perea-Milla Lopez E, Minarro-Del Moral RM, Martinez Garcia C, Zanetti R, Rosso S, Serrano S, Aneiros JF, Jimenez-Puente A, Redondo M, Lifestyles, environmental and phenotypic factors associated with lip cancer: a case–control study in southern Spain, *Br J Cancer*, 88, 2003, 1702–1707.

Peters E, Mc Clean M, Liu M, Eisen E, Mueller N, Kelsey K.T, The ADH1C polymorphism modifies the risk of Squamous cell carcinoma of the head and neck associated with alcohol and tobacco use, *Cancer Epidemiol Biomarkers Prev.*, 14, 2005, 476-482.

Powles T, Powles J, Nelson M, Head and neck cancer in patients with human immunodeficiency virus-1 infection: incidence, outcome and association with Epstein-Barr virus, *Journal of Laryngology and Otology*, 118, 2004, 207–212.

Ragin C.C, Taioli E, Survival of squamous cell carcinoma of the head and neck in relation to human papilloma virus infection: review and meta-analysis, *Int J Cancer*, 121, 2007, 1813-1820.

Schwartz S, Doody D, Fitzgibbons E, Ricks S, Porter P, Chen C, Oral squamous cell cancer risk in relation to alcohol consumption and alcohol dehydro-genase-3 genotypes, *Cancer Epidemiol Biomarkers Prev.*, 10, 2001, 1137- 1144.

Seitz H.K, Stickel F, Molecular mechanisms of alcohol-mediated carcino-genesis, *Nat Rev Cancer*, 7 (8), 2007, 599-612.

Sobin LH and Wittekind CTNM, Classification of Malignant Tumors, 6th ed., John Wiley and Sons, New York, 2002.

Solomon P, Selvam G, Shanmugam G, Polymorphism in ADH and MTHFR genes in oral squamous cell carcinoma of Indians, *Oral Diseases*, 14, 2008, 633-639.

Spitz MR, Epidemiology and risk factors for head and neck cancer, *Seminars in Oncology*, 21, 1994, 281-288.

Stadler ME, Patel MR, Couch ME and Hayes DN, Molecular biology of head and neck cancer: risks and pathway, *Hematol Oncol Clin North Am.*, 22, 2008, 1099-1124.

Steinmann K, Sandner A, Schagdarsurengin U, Dammann R.H, Frequent promoter hypermethylation of tumor-related genes in head and neck squamous cell carcinoma, *Oncol Rep.*, 22(6), 2009, 1519-26.

Sturgis EM, Cinciripini PM, Trends in head and neck cancer incidence in relation to smoking prevalence: an emerging epidemic of human papillomavirus-associated cancers?, *Cancer*, 110, 2007, 1429-35.

Sugerman P, Savage N, Oral cancer in Australia: 1983– 1996, *Aust Dent J.*, 47(1), 2002, 45–56.

Supic G, Jovic N, Kozomara R, Zeljic K, Magic Z, Interaction between the MTHFR C677T polymorphism and alcohol--impact on oral cancer risk and multiple DNA methylation of tumor-related genes, *J Dent Res.*, 90, 2011, 65-70.

Supic G, Kozomara R, Brankovic-Magic M, Jovic N, Magic Z, Gene hypermethylation in tumor tissue of advanced oral Squamous cell carcinoma patients, *Oral Oncol.*, 45, 2009, 1051-1057.

Szymańska K, levi Je, menezes A, TP53 and EGFR mutations in combination with lifestyle risk factors in tumours of the upper aerodigestive tract from South America, *Carcinogenesis*, 31, 2010, 1054-59.

Tebbs R.S, Zhao Y, Tucker J.D, Scheerer J.B, Siciliano M.J, Hwang M, Correction of chromosomal instability and sensitivity to diverse mutagens by a cloned cDNA of the XRCC3 DNA repair gene, *Proc. Natl. Acad. Sci. USA*, 92, 1995, 6348–6354.

Tuimala J, Szekely G, Gundy S, Hirvonen A, and Norppa H, Genetic polymorphisms of DNA repair and xenobiotic-metabolizing enzymes: role in mutagen sensitivity, *Carcinogenesis*, 23, 2002, 1003–1008.

Warnakulasuriya K A S, Johnson N W, Linklater K M, Bell J, Cancer of mouth, pharynx and nasopharynx in Asians and Chinese immigrants resident in Thames regions, *Eur J Cancer Oral Oncol.*, 35, 1999, 471–475.

Warnakulasuriya K.A.A.S, Johnson N.W, Linklater K.M, Bell J, Cancer of mouth, pharynx and nasopharynx in Asians and Chinese immigrants resident in Thames regions, *Eur J Cancer Oral Oncol.*, 35, 1999, 471–475.

Warnakulasuriya S, Global epidemiology of oral and oropharyngeal cancer, *Oral Oncol.*, 45(4), 2009, 309–16.

Zuo C, Zhang H, Spencer H.J, Vural E, Suen J.Y, Schichman S.A, Smoller B.R, Kokoska M.S, Fan C.Y, Increased microsatellite instability and epigenetic inactivation of the hMLH1 gene in head and neck Squamous cell carcinoma, *Otolaryngol Head Neck Surg.*, 141(4), 2009, 484- 490.