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RESEARCH ARTICLE

EFFECTS OF SALIVARY E-CADHERIN (CDH1-160) GENE PROMOTER POLYMORPHISM ON THE RISK OF DEVELOPMENT OF ORAL CANCER IN INDIAN ORIGIN

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ABSTRACT

Background and Objectives: Annually more than 1 million new cases of oral cancer are detected in the Indian subcontinent of which 92-95% are Oral squamous cell carcinomas (OSCC).A wide spectrum of chromosomal, genetic and molecular alterations is associated with both pathogenesis and malignant transformation of OSMF and oral leukoplakia, yet majority remains unclear.A correlation of E-cadherin-160C/A with cancer susceptibility was described in a premalignant diseases.In this context this study aims at investigating the association between polymorphism in the CDH1-160 promoter region in patients with Oral leukoplakia and Oral sub-mucous fibrosis (OSMF) and in healthy control group of patients.

Materials and Methods: The study comprised of 90 samples, of which 30 belonged to OSMF, 30 Leukoplakia and 30 controls with tobacco related habits but without any trace of oral potentially malignant disorders. A detailed history of the subjects was recorded following which clinical examination and histopathological confirmation was done. Genomic DNA isolation was done from saliva samples and PCR amplification were done to assess polymorphism of E-cadherin gene.

Results: 14 and 3 patients from OSMF showed C/A and A/A polymorphism respectively. 9 and 4 patients showed C/A and A/A polymorphism in leukoplakia subjects. Notably 13 (43%) of habitual chewers also showed polymorphism.

Conclusion: Within the limitations of the present study it can be concluded that there is a correlation of E-cadherin gene polymorphism with OSMF & leukoplakia.

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INTRODUCTION

Oral cancer is a highly lethal and disfiguring disease which imposes a considerable problem worldwide (Nather et al., 2002) and patients with oral cancer commonly seek treatment at the advanced stage of the disease, thereby diminishing the chances of therapeutic success (Angadi, 2010). The overall poor living quality and genetic susceptibility of the growing population in Southern Asia may increase the disease process by acting cumulatively which lead to numerous molecular and biochemical changes that may result in oral precancerous lesions which will further develop into malignancy (Das, 2002). Tobacco in the form of chewing, or smoking and alcohol consumption are the major risk factors for oral potentially malignant disorders (Hashibe, 2000; Hashibe, 2000 and Hashibe, 2002) and the prevalence is seen high among men (Neville, 2002). In addition to this, the inherited genetic susceptibility may also play an important role in oral tumorigenesis (Wu, 2002). The use of a variety of molecular and biochemical techniques provides abundant information

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regarding pre neoplastic changes and to detect malignant change at an early stage. Therefore, the application of these biochemical and molecular methods in the more accurate detection of potential oral lesions is of great importance. These molecular markers will not only facilitate early malignant transformation, but also act as prognostic indicators for treatment outcomes. Considering the sampling procedures, saliva has the advantages of being readily accessible, non-invasive, less time consuming, causes less anxiety to patients (Wong, 2006), and therefore less complex than blood. The exfoliated cells in oral cavity allows saliva to be the first choice of screening and the identification for the potential biomarkers of OSCC (Zimmermann, 2007). Epithelial or E-cadherin (E-cad) is a 97-kDa transmembrane glycoprotein which is encoded by the E-cadherin gene (CDH1) located in chromosome 16q22.1, is the main adhesion protein of the epithelia (Takeichi, 1991) and is generally known to have role in both in invasion and tumor suppression. Reduction of E-cadherin expression has been associated with a lack of cohesiveness, a higher malignant potential and invasiveness in a variety of epithelial neoplasms including oral cancer (Sung Woon and Mitsuyoshi, 2012).

Polymorphisms within gene promoter regions can have profound effects on the transcriptional efficiency of the genes and one such polymorphisms that affect transcription is a C/A SNP160 base-pairs (bp) upstream of the transcriptional start site of CDH1 (Shin, 2014). However resources to validate the association of the CDH1-160 gene polymorphism with potentially premalignant conditions like OSMF and leukoplakia are few. In this context this study aims at investigating the association between polymorphism in the E-cadherin/ CDH1-160 promoter region in salivary samples of patients with oral leukoplakia and OSMF with the control group of patients having tobacco related oral habits without any oral potentially malignant disorders.

PATIENT AND METHODS

Sample collection: Study population includes a total of 90 samples of which 30 comprised of OSMF subjects, 30 of oral leukoplakia and 30 were controls with tobacco related habits, without any trace of oral potentially malignant disorders. Prior to start of the study, Ethical Clearance was obtained from Institutional Review Board. An informed written consent was obtained from all the participants before collection of saliva sample. Patients reporting to Out-patient Department of Oral Medicine, SDM College of Dental Sciences and Hospital, Dharwad, who reported with tobacco and betel quid chewing habits were included. A detailed history was recorded and was screened for the presence of OSMF and oral leukoplakia and histopathologic confirmation was done for clinically evident Oral Potentially Malignant Disorders. Control group included Patients who had tobacco related oral habits without any oral potentially malignant disorders. Patients who have been previously treated for OSMF and leukoplakia, pregnant woman and patients unwilling to participate in the study were excluded. Standardization was done by collecting 2 ml of unstimulated saliva samples from each patients by the spit method in a sterile container which was then transferred to calibrated test tube from 3 individual groups (1-OSMF group, 2-oral leukoplakia group and 3- control group) to determine the final sample size and to standardize on the method of DNA extraction. Genomic DNA was isolated from the samples using standard Saliva DNA isolation kit (NORGEN- Biotek corporation; saliva DNA isolation kit-product #RU45400), after standardizing the protocol to the laboratory conditions.

PCR Reaction

The designed primer for E- Cadherin is; F-GCCCCGACTTGTCTCTCTA and R-GGCCACA GCCAATCAGCA. 30µl PCR reaction mixture was prepared which had 10 µM forward and reverse primer each; about 100ng of DNA made up to volume with nuclease free water. Total product length is of 447 base pairs. Agarose gel image of PCR products were obtained. (Figure1)

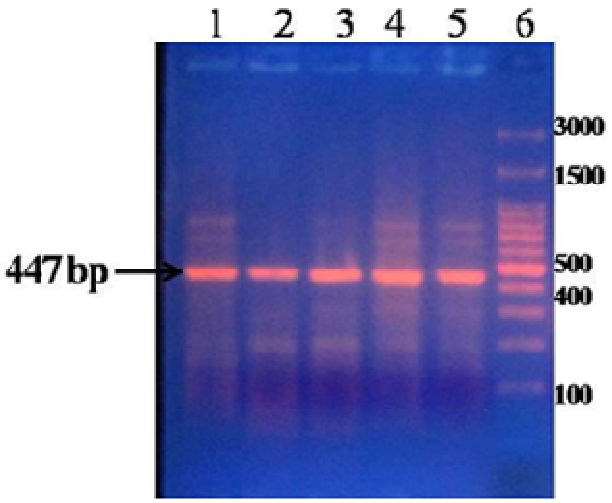


Figure 1. Agarose gel image of PCR products; line 6 shows DNA ladder; Showing 447bp PCR amplification product

Restriction Digestion

All PCR products (30µl) were incubated at 60°C for 30 minutes and digested with 10 units of BST *EII* restriction endonuclease enzyme. The restriction enzyme digestion was subjected to agarose gel electrophoresis. 2.5% agarose was prepared in 1X TAE (tris acetate EDTA) buffer (1L). To this 5µl of Ethidium Bromide was added. 15µl of restriction enzyme digest product was mixed with 5µl of loading dye Bromophenol blue. The electrophoresis was conducted at a constant voltage of 100V for 60minutes. The observed bands were analyzed under the UV Transilluminator (Figure 2).

Statistical Analysis

The collected information of the data was entered into Microsoft excel and then subjected to statistical analysis using SPSS 11 software. The polymorphic nature of E-Cadherin gene in subjects with OSMF, leukoplakia and comparison between the association of E-Cadherin gene polymorphism in OSMF group, leukoplakia group and controls were analyzed using Chi-square test. P value < 0.05 was considered to be significant.

RESULTS

The OSMF group comprised of 28(93.3%) males and 2(6.66%) females and all 30 (100%) patients were male in leukoplakia group. The age of the patients in the present study ranged between 20- 80 years. The mean age in case group was 41.41yrs and in control group was 47.30yrs. Majority of the

OSMF, 13 (43%) patients were in the age group of 21-30 yrs whereas 23(76.66%) in the leukoplakia group belonged to age group between 41-70yrs. 14 (46.66%) and 3(10%) patients from OSMF showed C/A and A/A polymorphism respectively. 9(30%) and 4(13.33%) patients showed C/A and A/A polymorphism in leukoplakia subjects. Notably 12(40%) and 1(1%) of control patients showed C/A and A/A polymorphism (Table1).

Table 1. Comparison of cases and controls with Polymorphism

	OSMF	%	Leukoplakia	%	Controls	%
C/C	13	43.33	17	56.66	17	56.66
C/A	14	46.66	9	30.00	12	40.00
A/A	3	10.00	4	13.33	1	3.33
Total	30	100	30	100	30	100
Chi square=3.746						
p= 0.442						

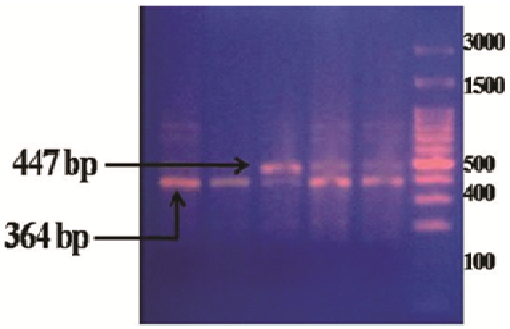
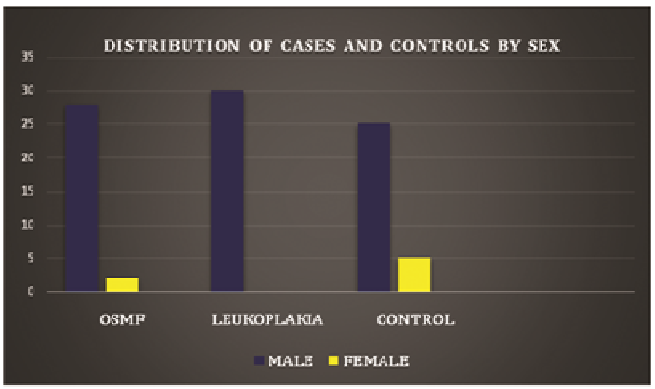
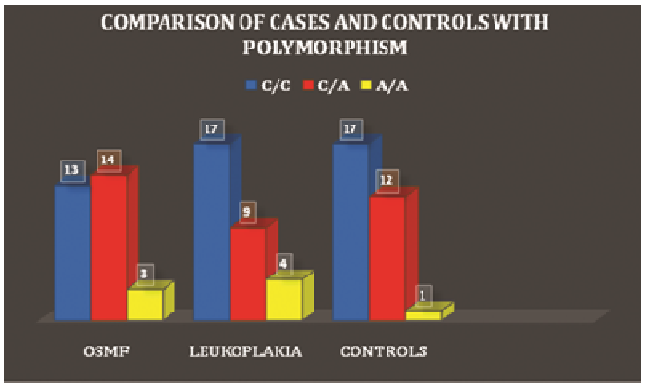


Figure 2. Figure showing Agarose Gel Image of Restriction Digested PCR products with BSE *II* enzyme. Line 6 – DNA ladder; 364 bp- showing normal restriction digested product/C/C; 447 bp- showing A/A genotype; 4 and 5 line- showing C/A genotype among cases



Graph 1. Distribution of cases and controls by sex



Graph 2. Comparison of cases and controls with polymorphism

In the present study subjects who consumed tobacco related products for 0-5yrs, 9(36%) of the patients had C/A polymorphism suggesting that patients with habits for a less span of time also show polymorphism. Also controls chewing tobacco with betel quid habits showed 6(37.7%) C/A and 1(6.2%) A/A polymorphism. In the present study we found that the polymorphism was more evident in the advanced stages of Oral submucous fibrosis than in the earlier stages. 6(20%) C/A polymorphism was seen in subjects with speckled leukoplakia and also 3(10%) C/A and A/A polymorphism each in homogenous leukoplakia. However there was no significant association seen between habits and frequency of habits with the nature of polymorphism among cases (OSMF & leukoplakia) and control group.

DISCUSSION

Oral cancer is defined as a malignant neoplasm on the lip or in the mouth and is more common in men than in women (Robert Roskoski, 2007). Being frequently associated with an addiction to chewing betel quid mixed with tobacco, which contains areca nut-based carcinogens, it is one of the major emerging health problems worldwide with variable incidences and severities depending on the region; its frequency is particularly high in South Asian countries, including India (Robert Roskoski, 2007). Owing to increase in trend of tobacco related oral cancer secondary to tobacco related habits, it is mostly preceded by the development of potentially malignant disorders. The changes could present as oral leukoplakia, oral erythroplakia, nicotine stomatitis, tobacco pouch keratosis, and oral submucous fibrosis. Extensive research spanning over a period of several decades has stated the presence of numerous biochemical changes at the onset of oral cancer. However, much variability has been observed in these biochemical parameters, which could be attributed to the differing lifestyles, genetic make-ups and distinct geographic regions of origin of the patients. Thus, identifying a common set of biomarkers will be important to the diagnostic precision (Moore, 2010). Examination of various bodily fluids, such as saliva and plasma, can provide valuable information regarding premalignant oral lesions. Saliva being the most easy of all biological fluids to collect and process, maybe a very good target for the search of new biomarkers of cancer[45] and therefore for the present study we collected 2ml of unstimulated saliva samples from each patients by the spit method in a sterile container which was then transferred to calibrated test tube from 3 individual groups (1-OSMF group, 2-oral leukoplakia group and 3- control group).

In the present study, OSMF group comprised of 28(93.3%) males and 2(6.66%) females and all 30 (100%) patients were male in leukoplakia group. Control group comprised of 25(83.3%) males and 5(16.6%) females. Male predominance in our study reflects, increased frequency of tobacco related habits in males than females in our society. All the female subjects in the present study with OSMF and controls were addicted to areca nut or betel quid, none showed addiction to gutkha. These findings are in accordance with the observations in the study conducted by Hazarey et al, (2007). Predominantly in this study, 13 (43%) of the OSMF patients were in the age group of 21-30 yrs, this could be attributed to the changing lifestyle of the younger generation, falling prey to adverse habits unknowingly or due to peer group influence; whereas 23(76.66%) male patients in the leukoplakia group belonged to age group between 41- 70yrs as leukoplakia is known to affect

men over 40yrs of age. Again in controls majority of them belonged to less than 30yrs of age. This may be due to the fact that various extrinsic and intrinsic etiological factors are more prevalent in today's younger population. The present study was first of its kind to assess the polymorphic nature of E-Cadherin -160 gene from the saliva samples of OSMF, leukoplakia patients and healthy subjects. It was reported that SNPs in the E-cadherin (CDH1) gene promoter region are responsible for interindividual variations in the production of E-cadherin and, in turn, leading to individual susceptibility to cancer (Kiemeney, 2006). In the present study, 14 (46.66%) and 3(10%) patients from OSMF showed C/A and A/A polymorphism respectively. 9(30%) and 4(13.33%) patients showed C/A and A/A polymorphism in leukoplakia subjects which means subjects with C/A heterozygote in the E-Cadherin -160 region are at a higher risk of developing OSMF and leukoplakia and therefore can have an increased susceptibility of malignant transformation.

This can be co-related by the study performed by Clague J et al (2010) who identified a significant trendbetween increasing oral premalignant lesions (OPL) risk and the increasing number of unfavorable genotypes, andreinforced the belief that the development of OPL is a polygenic process and a combinedanalysis of multiple factors may have a greater ability to characterize populations at highestrisk (Jessica Clague, 2010). Taking into account the current study population of 60 cases and 30 controls, the values indicate significant polymorphism in the OPL group. It is observed that the distribution frequency of the E-cadherin -160 gene C/A polymorphism differed between patients with cases (OSMF &leukoplakia) and the control group, indicating that E-cadherin -160 gene C/A polymorphism can serve as an important prognostic marker to assess malignant transformation. In an attempt to relate the type of adverse chewing habits and polymorphism, it is observed that majority of patients who had habits of chewing gutkha, betel quid and panmasla have only CC genotypes. The possible explanation for this could be that the commercially available gutkha products are concentrated; freeze dried and have higher dry weight concentration of pathology causing irritants in comparison to the traditionally prepared products like pan masala (Murti, 1999; Ranganathan, 2004). The results from current study imply that in subjects, chewing tobacco along with betel quid, 13(40.6%) had C/A and 5(15.6%) had A/A polymorphism.

However, since p value > 0.05, there is no statistically significant association found between the type of tobacco related habits and the polymorphism. In the present study subjects who consumed tobacco related products for duration of 0-5yrs, 9(36%) of the patients had C/A polymorphism suggesting that patients with habits for a less span of time also show polymorphism. This is in accordance with the results of Shukla D et al (2012), where it was assumed that the genotypes C and GSTM1 null play an important role in individual differences in susceptibility to OSCC, especially to the lowest tobacco dose level. The results of this study revealed that genetically predisposed betel quid/tobacco chewers are much more susceptible to environmental and life-style risk factors (Deepika Shukla, 2012). In a subject group of patients with chewing habits for 6-10yrs duration, 6(46.2%) had C/A polymorphism and 2(15.4%) had A/A polymorphism. Also, in a group of >15yrs duration, 6(37.5%) had C/A and 5(31.2) had A/A polymorphism respectively. The present study also reveals that, as the span of chewing habits increased above 15 years, the severity of the disease also increased with maximum number of cases having grade III, grade IV OSMF and speckled leukoplakia among subjects which is in accordance with the study conducted by Reddy V et al(2010), where the authors have co-related the clinical grading to various habit factors, and have also stated that as the duration of consuming habits increased above 10 years the severity of the disease also increased with maximum number of cases observed in grade II and grade III OSMF (Vanaja Reddy, 2011).

However in the present study there was no significant statistical association with the duration of the habits and nature of polymorphism. According to many authors, Intrinsic susceptibility and exposure to carcinogens can act in concert to modify cancer risk (Li, 1994; Harris, 1991; Ponder, 1990). Genetic instability, either spontaneous or mutagen induced, has been considered as a predisposing factor for malignant transformation. In response to environmental exposures, genetic damage accumulates more quickly in individuals with genetic susceptibility to DNA damage than in those without such instability but with a similar exposure. Consequently, individuals with genetic instability might be at a greater risk for developing cancer (Cloos, 1991). Remarkably, the present study showed that in controls who were chewing tobacco, gutkha along with betel quid habits also showed 12(40%) C/A and 1(3.33%) A/A polymorphism. This can be again co-related with the study performed by Wu X et al (2002) who believed that even though tobacco smoking and chewing are established risk factors for Oral potentially malignant disorders, it is not very clear why only a fraction of individuals who use tobacco or alcohol develop these lesions or subsequently develop oral cancer. Substantial evidence suggests that the carcinogenic process is determined by the interaction between exposure to exogenous carcinogens and inherent genetic susceptibility. However, little is known about the genetic basis for susceptibility to premalignancy (Xifeng Wu, 2002). It can also be said that an individual's exposure to tobacco carcinogens may therefore be altered by sequence variation in genes coding for these enzymes. However there have been a relatively small number of epidemiological studies that have examined the impact of genetic determinants on host susceptibility to this oral disease in Indian populations (Deepika Shukla, 2012). In present study, polymorphism was seen in subjects with following grades of Oral submucous fibrosis. C/A polymorphism was noted in grades S2M2 (50%), S2M4 (57.1%) and A/A polymorphism was seen in grades S2M2 (16.7%), S4M2 (50%). Thus suggesting that polymorphism is evident at the later stages of oral submucous fibrosis. Initial grades of OSMF showed only C/C genotype whereas the polymorphism was more evident in the advanced stages of OSMF. Also, 6(20%) C/A and 1(3.3%) polymorphism was seen in subjects with speckled leukoplakia and also 3(10%) C/A and A/A polymorphism each in homogenous leukoplakia, thereby showing increased frequency of polymorphism in speckled leukoplakia subjects. Out of 5.1 million tobacco attributable deaths in the world, more than 1 million are in South East Asia Region (SEAR) countries. Therefore reducing the use of tobacco is one of the best buys along with harmful use of alcohol for preventing non-communicable diseases.[29] (NCD). Tobacco in the form of chewing, smoking and alcohol consumption are major risk factors for Oral potentially malignant disorders. In addition, the inherited genetic susceptibility may also play an important role in oral tumorigenesis (Wu, 2002). Therefore, Knowledge of the

prevalence and distribution of common genetic susceptibility factors and the ability to identify susceptible individuals or subgroups will have considerable preventive implications, in particular if more data are collected to show that people with certain "at risk" genotypes are more susceptible to low levels of exposure. The loss of E-cadherin function during tumor progression can be caused by various genetic or epigenetic mechanisms. Therefore, E-cadherin could be used as a novel biomarker to identify Oral potentially malignant disorders at increased risk for malignant transformation, which may provide opportunities for prophylactic intervention in high risk patient groups. Hence we can use these biomolecules as biomarkers for further research on the microinvasion of oral cancers and to develop early diagnosis, and better treatment modalities and increase patient survival. However further research is required to understand the possible role of E-Cadherin -160 gene in malignant transformation of OSMF with a larger sample size and a long term follow up of these patients has to be done in order to determine the significance of E-Cadherin as a prognostic marker.

Conclusion

Oral submucous fibrosis (OSMF) and leukoplakia is a well-recognized potentially malignant disorder of the oral mucosa. Various studies had been conducted so far in order to identify the important aspects in malignant transformation of OSMF and leukoplakia. As the risk of malignant transformation is higher in OSMF and leukoplakia, the need to study the genetic markers such as E-cadherin gene is necessary to predict the outcome of the disease and to establish prognostic markers.

The present study was an attempt to assess the polymorphic nature of E-cadherin-160 gene in subjects with OSMF and leukoplakia and to compare the same in healthy group of controls having tobacco related oral habits without any oral potentially malignant disorders. It was evident in the study that the distribution of C/A polymorphism was higher in OSMF and leukoplakia patients than controls. However, there was no significant correlation between the type of habits, duration of habits with the nature of polymorphism of E- cadherin gene. Remarkably, our study showed that in controls who were chewing tobacco, gutkha along with betel quid habits also showed 12(40%) C/A and 1(3.33%) A/A polymorphism. This can be attributed to the fact that inherited genetic susceptibility may also play an important role in oral tumorigenesis. Therefore, Knowledge of the prevalence and distribution of common genetic susceptibility factors and the ability to identify susceptible individuals or subgroups will have substantial preventive implications, in particular if more data are collected to show that people with certain "at risk" genotypes are more susceptible to low levels of exposure. Within the limitations of the present study it can be concluded that there is a correlation of E-cadherin gene polymorphism with OSMF & leukoplakia. However further research is required to understand the possible role of E-Cadherin -160 gene in malignant transformation of OSMF and leukoplakia with a larger sample size and a long term follow up of these patients has to be done in order to determine the significance of E-Cadherin as a prognostic marker.

REFERENCES

Angadi PV, Rekha KP 2010. Oral submucous fibrosis: a Clinicopathologic review of 205 cases in Indians. *Oral Maxillofac Surg.* 2010 Sep; 14 (3):133-42.

Cloos, J., Spitz, M. R, Schantz, S. P., Hsu, T. C., Zhang, Z. F., Tobi, H., Braakhuis, B. J., and Snow, G. B. 1996. Genetic susceptibility to head and neck squamous cell carcinoma. J. Natl. Cancer Inst. (Bethesda), *88:* 530–535, 1996

Das, B.R., Nagpal, J.K. 2002. Understanding the biology of oral cancer. Med SciMonit 2002; 8 (11):RA258-267.

Deepika Shukla, 2012. Genetic polymorphism of drug metabolizing enzymes (GSTM1 and CYP1A1) as risk factors for oral premalignant lesions and oral cancer; Biomed Pap Med FacUnivPalacky Olomouc Czech Repub. 2012; 156:XX.

Harris, C.C. 1991. Chemical and physical carcinogenesis: advances and perspectives for the 1990s. Cancer Res 1991:51(18 Suppl):5023s 4s.

Hashibe, M., Mathew, B., Kuruvilla, B., Thomas, G., Sankaranarayanan, R., Parkin, M. and Zhang, Z.F. 2000 Chewing tobacco, alcohol, and the risk of erythroplakia. Cancer Epidemiol. Biomarkers Prev., 9, 639–645.

Hashibe, M., Sankaranarayanan, R., Thomas, G., Kuruvilla, B., Mathew, B., Somanathan, T., Parkin, D.M. and Zhang, Z.F. 2000. Alcohol drinking, body mass index and the risk of oral leukoplakia in an Indian population. Int. J. Cancer, 88, 129–134.

Hashibe, M., Sankaranarayanan, R., Thomas, G., Kuruvilla, B., Mathew, B., Somanathan, T., Parkin, D.M. and Zhang, Z.F. 2002. Body mass index, tobacco chewing, alcohol drinking and the risk of oral submucous fibrosis in Kerala, India. Cancer Causes Control, 13, 55–64.

Jessica Clague, et al 2010. Genetic Variation in MicroRNA Genes and Risk of Oral Premalignant Lesions; MolCarcinog. 2010 February; 49(2): 183–189. doi:10.1002/mc.20588.

Kiemeney, L.A., van Houwelingen, K.P., Bogaerts, M., et al. 2006. Polymorphisms in the E-cadherin (CDH1) gene promoter and the risk of bladder cancer. Eur J Cancer 2006; 42: 3219–3227.

Li, FP, Montesano, R 1994. Interactions of cancer susceptibility genes and environmental carcinogens. American Association for Cancer Research (AACR)—International Agency for Research on Cancer (IARC) Joint Conference. Cancer Res 1994:54:4243-7.

Moore, M.A., Ariyaratne, Y., Badar, F., Bhurgri, Y., Datta, K., Mathew, A., et al. 2010. Cancer epidemiology in South Asia – past, present and future. Asian Pac J Cancer Prev. 2010; 11(Suppl 2): 49–66.

Murti P, Gupta P, Bhonsle R, Daftary D, Mehta F, Pindborg J. 1990. Effect on the incidence of oral submucous fibrosis of intervention in the areca nut chewing habit. Journal of Oral Pathology & Medicine. 1990; 19(2): 99-100.

Nather A, Sami A, Hefler L et al. 2002. Serum levels of squamous cell carcinoma antigen as a predictor of inguino-femoral lymph node metastasis in patients with vulvar cancer. J Reprod Med.2002; 47: 718–20

Neville BW, Day TA. Oral Cancer and Precancerous Lesions. CA Cancer J Clin 2002. 52: 195-215. doi:10.3322/canjclin.52.4.195.

Ponder BA 1990. Inherited predisposition to cancer. Trends Genet 1990; 6:213-8.

Ranganathan K, Uma Devi M, Joshua E, Kirankumar K, Saraswathi TR 2004. Oral submucous fibrosis: a case control study in Chennai South India.J Oral Pathol Med.2004; 33: 274–7.

Robert Roskoski 2007 Jr. Vascular endothelial growth factor (VEGF) signaling in tumor progression. Critical Reviews in Oncology/Hematology 2007; 62: 179–213.

Shin Y, Kim IJ, Et Al 2004. The E-Cadherin _347g Ga Promoter Polymorphism and Its Effect on Transcriptional Regulation. Carcinogenesis 2004; 25: 895–899.

Sung Woon, Mitsuyoshi et al 2007; Expression of E-cadherin, P-cadherin and N-cadherin in oral squamous cell carcinoma: Correlation with the clinico-pathologic features and patient outcome: Journal of Cranio-Maxillofacial Surgery 35, 1–9

Takeichi M 1991. Cadherin Cell Adhesion Receptors as a Morphogenetic Regulator. Science 1991; 251: 1451–1455.

Thakur JS, Garg R, Narain JP, Menabde N. 2011. Tobacco use: a major risk factor for non-communicable diseases in South-East Asia region. *Indian J Public Health.* 2011 Jul-Sep; 55(3):155-60. doi: 10.4103/0019-557X.89943.

V. K. Hazarey, D. M. Erlewad, K. A. Mundhe, S. N. Ughade 2007. Oral submucous fibrosis: study of 1000 cases from central India: *J Oral Pathol Med*. 2007; 36: 12–7.

Vanaja Reddy, P.V.Wanjari, Naveen Reddy Banda, Prashanti Reddy 2011. Oral Submucous Fibrosis: Correlation of Clinical Grading to various habit factors. *International journal of dental clinics*. 2011; 3(1): 21-4.

Wong DT. Salivary diagnostics powered by nanotechnologies, proteomics and genomics. J Am Dent Assoc. 2006. 137:313–321. [PubMed: 16570464]

Wu, X., Lippman, S.M., Lee, J.J., Zhu, Y., Wei, Q.V, Thomas, M., Hong, W.K. and Spitz, M.R. 2002. Chromosome instability in lymphocytes: a potential indicator of predisposition to oral premalignant lesions. Cancer Res., 62, 2813–2818.

Wu,X., Lippman, S.M., Lee, J.J., Zhu, Y., Wei,Q.V., Thomas, M., Hong,W.K. and Spitz, M.R. 2002. Chromosome instability in lymphocytes: a potential indicator of predisposition to oral premalignant lesions. Cancer Res., 62, 2813–2818.

Xifeng Wu et al 2002. Chromosome Instability in Lymphocytes: A Potential Indicator of Predisposition to Oral Premalignant Lesions; chromosome instability and oral premalignant lesions; cancer research 62, 2813–2818, May 15, 2002

Zimmermann, B.G., Park, N.J., Wong, D.T. 2007. Genomic targets in saliva. *Ann NY AcadSci*; 1098: 184–91.

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