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# Clinical Profile and Role of VEGF-C (+936c/T) Polymorphism in Prognosis and Management of Oral Squamous Cell Carcinoma

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**Conflicts of Interest:** Nil.

**Abstract**

**Aims:** Angiogenesis and lymph angiogenesis are necessary step in tumour growth and metastasis. Vascular endothelial growth factor (VEGF-c) is a major mediator of oral squamous cell carcinoma angiogenesis and lymphangiogenesis. Therefore, we investigated the association of one polymorphism (+936 C/T) in the VEGF-c gene with oral squamous cell cancer risk and prognostic characteristics of the tumour in a case-control study.

**Experimental Design:** We examined one polymorphism in the VEGF –c gene (+936C/T) in 75 oral squamous cell cancer cases and 75 controls from Kanpur, Uttar Pradesh, India and adjacent areas together with geographically selected controls**.**

**Results:** None of the polymorphism or any halotype was significantly associated with either oral squamous cell cancers. Our study suggests that the +936C/T polymorphism is unlikely to be associated with oral squamous cell cancer. We also analysed the cases for genotypes or halotypes that associated with tumour characteristics. The genotypes and halotypes were not related with other tumour characteristics such as regional and distant metastasis, stage at diagnosis.

**Conclusion:** In our study, the most common site for oral squamous cell carcinoma (OSCC) was buccal mucosa associated with tobacco chewing. Although +936 C/T polymorphisms studied in the VEGF-c gene was not found to influence susceptibility to oral squamous cell cancer significantly in our study but some of the VEGF-c genotypes and halotypes may influence angiogenesis and lymph angiogenesis.

**Keywords:** Polymorphism, VEGF-c, Oral squamous cell carcinoma, Indian population

## Introduction

Angiogenesis and lymph angiogenesis is an important step in the development of cancer and is necessary for primary tumour growth, invasiveness, and metastasis.[1]Vascular endothelial growth factor (VEGF-c) is believed to be important for process of initiation of angiogenesis and lymph angiogenesis and is major mediator of oral squamous cell cancer angiogenesis and lymphangiogenesis.[2]Over expression of VEGF-c has been shown in various cancer.[3]Several polymorphisms in the VEGF-c gene have been reported to affect the expression of the gene. The 2578CC, 2549del/del, 1154GG, and 634CC have been shown associated with a higher VEGF-c production,[4-7] whereas the +936 T allele

has been shown to correlate with lower VEGF plasma levels.[8,9] In addition, the 634G/C polymorphism is located within a potential binding site of the MZFI transcription factor[10] and the +936C/T polymorphism leads to loss of a potential AP-4 binding site.[8,9]Recent studies have shown that some VEGF-c polymorphism are associated with the development of cancer. The risk of prostate cancer and less advanced melanomas are decreased in association with the 154 AA genotype. [11, 12] and the +936C/T polymorphism associated with a decreased risk of oral squamous cell carcinoma. VEGF-c (+936C/T) has been shown to be of prognostic importance in oral squamous cell cancer. These data suggest that the polymorphism involved in angiogenic and lymphangiogenic pathway may play an important role in the progression or aggressiveness of the tumour, including oral squamous cell cancer. In present study, we investigated the relationship between genetic polymorphism in the VEGF-c gene and development of oral squamous cell carcinoma in patients from Kanpur and adjacent areas. VEGF-c polymorphisms carriers who chewed any preparation which contain betel nut only or with other constituents like tobacco have14.5-24.2 fold risk of cancer compared to VEGF-c wild type carrier who did not chew these kind of preparation.[15]Polymorphisms were located in the 3' untranslated region at position +936,according to numbering used by Renner et al.[Figure 1].[8] This polymorphism was selected for further analysis to evaluate the possible influence on the risk of oral squamous cell cancer and the prognostic characteristics of the tumour.

The translation starting site is marked by the ATG codon. The polymorphisms within the box are in the complete linkage. The +936C/T polymorphism was studied in Kanpur, India for oral squamous cell cancer cases and controls.

**Materials and Methods**

Totally, 75 oral squamous cell cancer cases together with ethnically and geographically selected controls were used in study. The study populations consisted of North Indian were incident cases collected during the years 2013 to 2015 according to criteria described earlier through the outpatient and indoor patient surgery department clinics (LLR Hospital, Kanpur, India ) and Cancer clinics (J.K.Cancer Institute Kanpur ,India).About 90% of patients approved participation in the study. The controls were recruited to earlier studies with comparable participation rate. These case together with controls were analysed at Department of Biochemistry, GSVM Medical College Kanpur, India .Samples (n=75) were collected in hospital-based manner from untreated patients referred to the Department of Surgery for newly diagnosed oral squamous cell cancer, the controls were selected from the hospital staff nurse and volunteers who were healthy. After DNA digestion, the coded samples were divided on the plates by randomly mixing cases and controls.

**Polymerase chain reaction (PCR) amplification**

The primer sequence for the polymorphism of VEGF-c gene (+936C/T) was retrieved from the Ensemble database. Primers were designed for the regions around the polymorphic sites/or for the entire coding regions with the help of the primer blast tool of the NCBI or Gene tool software. Primers were custom synthesized by Micelles Life Sciences, Lucknow, India.

**Restriction fragment length polymorphism (RFLP) analysis**

The +936C/T polymorphism was analysed in the sample sets using RFLP analysis.PCR products were digested with restriction endonucleases, using the buffers and temperatures recommended by the manufacturers. The digested PCR products were resolved on a 10%

polyacrylamide gel and stained with ethidium bromide for visualization under ultraviolet light.

**Statistical analysis**

Genotype data of control samples for polymorphism were analysed for fitness in the Hardy-Weinberg equilibrium using the online calculator.[16] Chi-square test was used to compare genotype data between cases and controls using Vassar stats online calculator[17]adopting dominant, recessive, codominant, and additive models. P<0.05 was considered statistically significant.

The differences in the genotype and halotype frequencies of the studied polymorphism in the oral squamous cell cancer cases and controls were compared for statistical significance by the Yates corrected Chi-square test .Odds ratio and 95% confidence intervals were calculated for associations of genotypic polymorphism between oral squamous cell cancer cases and healthy controls.

**Results**

We examined the effect of polymorphism in the VEGF-c gene on oral squamous cell cancer development [Figure 1].The polymorphism +936C/T were examined in oral squamous cell cancer samples and controls using the RFLP assay. The genotype distribution of studied polymorphism followed the Hardy-Weinberg equilibrium in every sample set. The genotype and allele distributions among the oral squamous cell cancer cases and control subjects are shown in Table 6.The number of samples analysed for polymorphism was not exactly equal because of unsuccessful amplification of a few samples. No differences in the allele or genotype frequencies between the oral squamous cell cancer cases and controls were detected in the population. The lack of association remained when the data were stratified by age (data not shown).Halotypes were created using genotyping data of the polymorphism +936C/T. No significant differences in halotype frequencies between the oral squamous cell cancer cases and controls were detected. There were no indications that the polymorphism would have any effect on the other tumor characteristics. The addition of the information of the +936C/T polymorphism data did not change the results (data not shown).

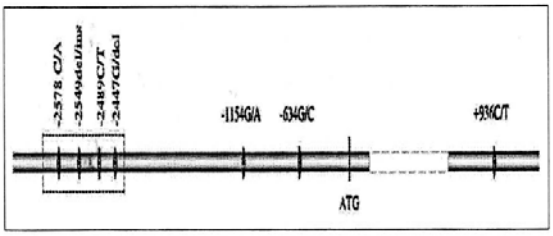


Figure 1: Structure of the vascular endothelial growth factor gene indicating the polymorphism included in the present study

**Discussion**

Functional polymorphisms, which have an effect on the regulation of gene expression, can contribute to the differences between individuals in susceptibility to and severity of a disease. The effect may be seen by a polymorphism alone, or in combination with other polymorphisms. Several studies have shown that polymorphisms in the promoter as well as in the 5' and 3' untranslated regions of the VEGF-c gene are associated with the production of the VEGF-c protein.[4-10]We analysed the +936C/T polymorphism using RFLP assay. Here, we did a case control study of 75 patients with oral squamous cell carcinoma. We observed no differences in the allele or genotype frequencies between oral squamous cell cancer cases and control groups, (odds ratio 1.11; 95% confidence interval=0.74).In our study we did not find any significant association between gene polymorphism (+936C/T) and increased risk of oral carcinoma.

The results of **Liu XL et al (2013)** met analysis provided evidence that the VEGF +936C/T polymorphism was not associated with overall cancer risk in Asians.

Our study is unique since it has been done for the first time in Indian sub-population on gene polymorphism

(+936C/T) associated with OSCC, and no other study has been published till date. In a future study, increasing specimen number, changing geographical area and taking more OSCC risk factors into account in the analysis might precisely validate these findings.

TABLE 1: Showing age and gender distribution of patients with tumor staging

| Age Interval (in years) | Male | Female | Total |
|---|---|---|---|
| 21-30 | 09 | 00 | 09 |
| 31-40 | 10 | 02 | 12 |
| 41-50 | 11 | 06 | 17 |
| 51-60 | 16 | 03 | 19 |
| 61-70 | 11 | 04 | 15 |
| > 70 | 03 | 00 | 03 |
| Total Patients: | 60 | 15 | 75 |

TABLE 2: Showing no of patients with primary site of carcinoma

| Primary site | No. of Patients ( n = 75 ) | Percentage |
|---|---|---|
| Buccal mucosa | 36 | 48 |
| Lip | 6 | 8 |
| Tongue | 14 | 18.67 |
| Alveolus | 17 | 22.67 |
| Hard palate | 1 | 1.33 |
| Floor of mouth | 1 | 1.33 |
| Total | 75 | 100 |

(n= no of patients)

TABLE 3: Showing no of patients with tumour staging

| T staging | No. of Patients ( n = 75 ) | Percentage |
|---|---|---|
| T1 | 15 | 20 |
| T2 | 23 | 30.7 |
| T3 | 29 | 38.7 |
| T4 | 08 | 10.6 |
| Total | 75 | 100 |

(n= no of patients)

TABLE 4: Showing no of patients with lymph node status

| Lymph node status | No. of Patients ( n = 75 ) | Percentage |
|---|---|---|
| Present | 34 | 45.3 |
| Absent | 41 | 54.7 |
| Total | 75 | 100 |

(n= no of patients)

TABLE 5: Showing no of patients with tumour histological grading

| Histopathological grading | No. of Patients ( n = 75 ) | Percentage |
|---|---|---|
| Well | 46 | 61.3 |
| Moderate | 23 | 30.7 |
| Poorly | 06 | 08 |
| Total | 75 | 100 |

In our study, the mean age of the cases at diagnosis was 49.68 years which was similar to that reported by **Shenoi R et al (2015)** which was 49.73 in his study of 295 patients. However our mean age observed was comparatively lower than **Brocic M et al (2009**) and **Pires FR et al (2013)** which reported the mean age of 60.6 years and 62.3 years respectively.

Majority of patients in our study were males and M: F ratio was 4:1 which was similar to the study conducted by **Shenoi R et al** who reported ratio of 4.12:1**.**

In our study, the most common primary site of carcinoma was buccal mucosa followed by alveolus. This could be explained as tobacco and betel nut quid is kept in gingivobuccal sulcus so the site suffers maximum insult. **Shenoi R et al was** found mandibular alveolus was the most frequent site followed by buccal mucosa.

In our study most of the patient presented in T3 stage and similar results were reported by **Oliveria et al (2015**), they reported 55.6% cases in T3/T4 stage.

Maximum patient (54.7%) presented to us was not having lymph node metastasis and similar results were observed by **Yanase M et al (2014)**.

In our study, most of OSCC histologically diagnosed as well differentiated tumours or moderately differentiated tumours and similar results were reported in the study of **Andisheh-Tadbir A et al (2008).**

**Table 6: Allelic and genotype distribution (+936 C/T) of VEGF-c gene in oral squamous cell cancer cases and controls**

(+936 C/T) polymorphism

| | 2*2 Table | | | | 3*2 Table | | | |
|---|---|---|---|---|---|---|---|---|
| Groups (%) | CC | CT/TT | P-value | OR(95% CI | CC | CT | TT | P-value |
| Controls (n=75) | 43 | 32 | Ref | | 43 | 29 | 3 | Ref |
| OSCC (n=75) | 41 | 34 | 0.74 | 1.11 (0.584-2.124) | 41 | 27 | 7 | 0.42 |

Ref: It is treated as reference to compare between cases and control .VEGF-c: Vascular endothelium growth factor OSCC-oral squamous cell carcinoma OR: Odd ratio CI: confidence interval

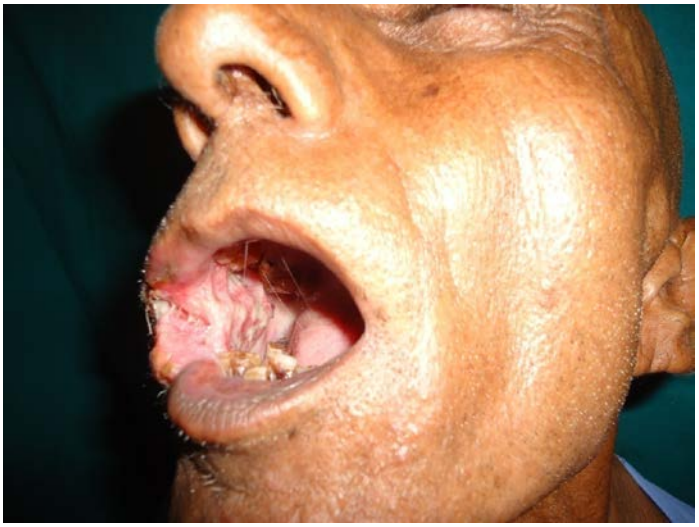

Figure 2 showing growth in buccal mucosa

**Conclusions**

In our study the most common site for OSCC was buccal mucosa associated with tobacco chewing. The present study investigated the association of VEGF-c gene (+936C/T) polymorphism with susceptibility to oral squamous cell carcinoma (OSCC). For 936C/T genotypes analysis, the CC genotypes were high, whereas CT, TT genotypes were considered to be low activity genotypes. Our results suggest that there is no significant difference in cases and controls in the distribution of high and low activity genotypes (2*2 Table) (p=0.74), as well as when applying (3*2 Table) (p=0.42). In our study, we find that +936 C/T polymorphisms studied in the VEGF-c gene was not found to influence susceptibility to oral squamous cell cancer significantly.

**References**

[1]. Ferrara N, Gerber HP, LeCouterJ.The biology of VEGF and its receptors.Nat Med 2003;9:669-76

[2]. Brocic M, KozomaraR,CerovicS,JovicN,Markovic S, SrboljubS.Clinical significance of vascular endothelial growth factor expression in patients with carcinoma of mouth floor and tongue.Vojnosanit Pregl 2009;66(6):440-448.

[3]. Nakamura M, Abe Y, Tokunaga T. Pathological significance of vascular endothelial growth factor A isoform expression in human cancer. PatholInt 2002;52:331-9.

[4]. Yang B, Cross DF, Ollerenshaw M, Millward BA, DemaineAG.Polymorphisms of the vascular endothelial growth factor and susceptibility to diabetic microvascular complications in patient with Type 1 diabetes mellitus.J Diabetes Complications 2003;17:1-6

[5]. Mohammadi M, Ollier WE, Hucthinson IV.A fuctional association study of VEGF gene promoter polymorphisms with VEGF expression by stimulated cells. Hum Immunol 2003;64:s125

[6]. Shahbazi M, Fryer AA,PravicaV,BroganIJ,Ramsay HM, HutchinsionIV,etal.Vascular endothelial growth factor gene polymorphisms are associated with acute renal allograft rejection. J Am SocNephrol 2002;13:260-4

[7]. Awata T,Inoue K, KuriharaS,Ohkubo T, Watanabe M, Inukai K, et al.A common polymorphism in the 5'-untranslated region of the VEGF gene associated with diabetic retinopathy in Type 2 diabetes.Diabetes 2002;51:1635-9.

[8]. Renner W, Kotschan S, Hoffmann C,Obermayer-PietschB,Pilger E.A common 936 C/T mutation in the gene for vascular endothelial growth factor is associated

with vascular endothelial growth factor plasma levels.JVasc Res 2000:37:443-8.

[9]. Krippl P, Laangsenlehner U Renner W, Yazdani-Biuki B, Wolf G,Wascher TC, et al.A common 936C/T gene polymorphism of vascular endothelial growth factor is associated with decreased risk of cancer.

[10]. Watson CJ, Webb NJ, Bottomely MJ, BrechleyPE.Identification of polymorphisms within the vascular endothelial growth factor (VEGF) gene: Correlation with variation in VEGF protein production.Cytokine2000;12:1232-5.

[11].HowellWM,BatemanAC,TurnerSJ,CollinsA,Theaker JM.Influence of vascular endothelial growth factor single nucleotide polymorphisms on tumor development in cutaneous malignant melanoma.GenesImmun 2002;3:229-32.

[12]. McCarron SL,EdwardsS,EvansPR,GibbsR,Deamaley DP, DoweA,etal.Influence of cytokine gene polymorphisms on the development of prostate cancer.Cancer Res2002;62:3369-72.

[13]. Brogan IJ, Khan N, Issac K, Hutchinson JA, Pravica V, HutshinsionIV.Novel polymorphism in the promoter and 5' UTR regions of the human vascular endothelial growth factor gene.HumImmunol 1999,60:1245-9.

[14]. Wason CJ, Webb NJ, Bottomley MJ, BrenchleyPE.Identification of polymorphism within vascular endothelial growth factor (VEGF) gene:Correlation with variation in VEGF protein production.Cytokine 2000;12:1232-5.

[15]. Chien M, Liu YU and Lin CW:Impact of VEGF-c gene polymorphisms and environmental factors on oral cancer susceptibility in Taiwan.Plos one.2013.8(4):e60283

[16]. Hardy-Weinberg equilibrium in genetic association studies:An empirical evaluation of reporting, deviations, and power.Availablefrom:http://www.ihg.gsf.de/cgi-bin/hw/hwal.pl.

[17]. Online calculator.Available from: http://faculty.vassar.edu/vassarstats..html.