International Journal of Pharmacy and Pharmaceutical Sciences

Print ISSN: 2656-0097 | Online ISSN: 0975-1491 Vol 16, Issue 2, 2024

Original Article

RESISTIN GENE POLYMORPHISMS: POTENTIAL BIOMARKER FOR ORAL SQUAMOUS CELL CARCINOMA

DIVYA TANDON

Molecular and AMP, Human Genetics Lab, University of Lucknow, Lucknow, Uttar Pradesh, India
***Corresponding author: Divya Tandon; *Email: d.tandon84@gmail.com**

Received: 26 Sep 2023, Revised and Accepted: 04 Jan 2023

ABSTRACT

Objective: The objective of the work is to study the association between Resistin Gene polymorphisms and susceptibility of Oral Squamous Cell carcinoma.

Methods: In the present study, we scrutinize the connection between four genetic polymorphisms present in RETN gene with the susceptibility, progression, and clinical outcome of OSCC among 200 OSCC patients and 200 healthy controls**.**

Results: The results of the study reveal that among 260 smokers, the risk of developing OSCC is significantly more among the subjects having history of using betel quid in comparison to those who are not habitual users of betel quid**.**

Conclusion: The study reveals that patients with OSCC exhibiting G/A heterozygous genotype of RETN rs3219175 polymorphism have lesser risk for developing high-grade tumor compared to the patients with G/G homozygotes in North Indian population.

Keywords: Oral cancer, Resistin, Single nucleotide polymorphisms, RETN, Prognosis

© 2024 The Authors. Published by Innovare Academic Sciences Pvt Ltd. This is an open access article under the CC BY license (https://creativecommons.org/licenses/by/4.0/)
DOI: https://dx.doi.org/10.22159/ijpps.2024v16i2.49493. Journal homepage: https://innovareacademics.in/journals/index.php/ijpps

INTRODUCTION

Squamous cell carcinoma of oral cavity (OSCC) can be known or defined as the cancer of mouth, tongue, vermillion borders of lip, palate or any part of the oral cavity [1]. Pindborg *et al.* (1997) clearly defined OSCC as "An epithelial tumor with squamous differentiation that developed keratin and/or had intercellular bridges" [2]. Despite the fact that OSCC is becoming a serious problem around the globe, it has a predominant presence in developing countries like India. In spite of the advancements in treatment modalities, the five-year survival rate has reduced to only 50% in the past 50 y [3]. Several reasons pose a significant barrier in improving the survival of Oral Squamous Cell Carcinoma (OSCC) patients; some of these reasons are poverty, illiteracy, advanced stage at the time of presentation, lack of access to good healthcare and poor infrastructure, late referral and less public awareness about the disease. Various factors attribute to the occurrence of Oral Squamous Cell Carcinoma (OSCC), and some of them are tobacco consumption, alcohol intake, betel quid chewing, chronic inflammation, viral infections like Human Papilloma Virus (HPV) and chronic exposure to environmental carcinogens [4, 5]. But all the patients with exposure to these etiological factors do not develop Oral Squamous Cell Carcinoma (OSCC); the most appropriate explanation of this variability is the presence of genetic variations, somatic mutations, and epigenetic regulation. The most commonly occurring genetic variation is Single Nucleotide Polymorphisms (SNPs); these are DNA sequence variations that directly impact gene expression, gene function, and transcription, thereby affecting disease susceptibility among individuals.

SNP studies on diverse populations emphasize the association of these genetic variants with the predisposition and vulnerability to Oral Squamous Cell Carcinoma (OSCC) [6, 7]. For example, the genes involved in PI3K/AKT pathway [9], IL-8 [8], miR [1] genes, TNF-α etc., are found to be associated with Oral Squamous Cell Carcinoma (OSCC). These studies have proved that the identification of gene variants can be an important aspect for the early-stage diagnosis of Oral Squamous Cell Carcinoma (OSCC).

Resistin is a 12.5kDa cysteine enriched polypeptide, which exerts the main function of upregulation of proinflammatory cytokines and helps in increasing the population of regulatory T cells [10, 11]. RETN (Resistin) gene encodes resistin and is located on chromosome 9 (19p13.2). Resistin is increased in type 2 diabetes mellitus because the hormone released suppresses insulin ability, which in turn increases glucose uptake in adipose cells [12, 13]. Resistin gene is found to be associated with pathogenesis of several types of cancers. The serum levels of Resistin are found to be associated with colorectal cancer and breast cancer [12, 13]. Similarly, the resistin level in plasma is linked with the markers of inflammation and coronary artery calcification. Coronary artery calcification is the prime measure of coronary atherosclerosis [14-16]. Several number of SNPs are recognized in the promoter and 3'UTR region of RETN (Resistin) gene [17]. Studies have revealed that genetic polymorphisms of RETN gene result in increasing the risk emanating from varied conditions such as metabolic disorders cancers like those of breast and colon [6, 17-19]. Further, a functional genetic variant of the RETN gene, i.e.,. rs186513 is found to be linked with type 2 diabetes mellitus [20]. A study done on Chinese population discovered the role of RETN (Resistin) SNPs (Single Nucleotide Polymorphisms) in lung cancer [21]. Another study on Taiwanese population correlates RETN SNPs with that of Oral Squamous Cell Carcinoma (OSCC) [22]. However, no study has revealed the correlation between Oral Squamous Cell Carcinoma (OSCC) and RETN (Resistin) gene polymorphisms in Indian population. The present study aims to study the association of genetic variants of RETN gene with the demographic and clinicopathological parameters, susceptibility and prognosis among the North Indian population.

MATERIALS AND METHODS

Selection of case and control subjects and sample collection

In aggregate, 200 clinically confirmed OSCC patients from King George Medical College were recruited for the study. The human ethical committee, KGMU Lucknow, scrutinized and approved this study (1252/R. Cell-19) The participation of patients and their family members was voluntary and the information was obtained with their approval and consent. The cases and controls were selected on the basis of some criteria. The inclusion criteria for selecting the cases were: OSCC patients with clinically and histopathologically established diagnosis, Patients aged above 18 y.

The inclusion criteria for selection of control samples were: Patients who were never diagnosed with any type of cancer or irregular test findings, Patients aged over 18 y Criteria of excluding cases and controls were: Patients distressed from some kind of systemic disorders such as cardiac abnormalities, diabetes mellitus, breathing disorders, Alzheimer's disease and defect in renal system, Subjects suffering from any type of infectious diseases like HIV infection or carcinoma of breast or lungs, leukemia, lymphoma or any other malignancies, Patients having precancerous lesions or dysplastic diseases of mouth including Submucus Fibrosis, Leukoplakia, Erythroplakia, smokers' palate or Verrucous carcinoma, Patients suffering from some kind of malnutrition, Lactating women, Patients who did not readily give consent.

All the subjects were requested to complete a questionnaire that would provide details about their demographic, habitual and clinical details. Information on the cancer subtype and disease stage were obtained from the hospital medical records.

Using venipuncture method, blood samples were collected from the subjects recruited for the study. After collecting the blood samples, these were immediately transferred to EDTA vials. The vacutainers were stored at 4 °C for subsequent processing. For this study, we took a total of 100 tissue samples from different cancer patients and 30 control tissues sections were cut from the peripheral areas of patients suffering from oral cancer. After gathering all the samples, one part of the tissue was placed in formal saline and the other in RNA (Thermo Scientific) and preserved at −20 °C before processing.

Isolation of DNA from both the groups

After taking blood samples from the subjects, DNA was isolated using a blood mini kit (Qiagen, Germany). Following all the instructions given by the manufacturer, the steps were performed. With the help of UV-vis spectrophotometer, DNA was quantified after secluding it from blood. Further, the quality of DNA samples was checked by performing agarose gel electrophoresis and then stored at −20°C.

Histopathological Grading of OSCC tissue samples

To carry out the histopathological grading of cases, 50 mg of tissue samples were taken from oral cancer patients and stored immediately in formal saline for further histopathological processing. After this, tissue sections were imbedded in paraffin wax, the tissue blocks were made. Later, the sections were microtomed and placed with utmost care on the glass slides for performing staining protocol with hematoxylin and eosin, subsequent to which the sections were graded with regard to their cell differentiation.

Determination of genotypes

Four genetic variants of the RETN gene were selected, and these were rs3745367, rs7408174, rs1862513, and rs3219175 with minor allele frequencies>5%. Primers and probes designed for all the four variants were:

- "CTCCGACTGTCCCCACCTTATCCAC[A/G]GCTCCAAACCCAA" for rs3745367.
- "TTTTACCACAAAAAGGCCCGTTGTA[C/T]TGGAAACAAAGAA" for rs7408174.
- "CCTGACCAGTCTCTGGACATGAAGA[C/G]GGAGGCCCTGTTG" for rs1862513.
- "CTCCAGCCCTTACTGTCTGCTCAGG[A/G]GCTTCCTCTTGGC" for rs3219175.

These SNPs were previously studied in relation to breast cancer, rheumatoid arthritis, and lung cancer [21, 23, 24]. The correlation of SNPs with lung cancer is the reason for their selection in this study, as both lung carcinoma and oral cancer have tobacco as the major etiological factor. So, we genotyped these genetic variants with commercially accessible TaqMan SNP genotyping assay method of Applied Biosystems, Warrington, UK, and the procedure followed was based on manufacturer's protocol [25, 26].

Statistical analysis

Firstly, it was checked that the genotypic distribution of every genetic variant satisfy the Hardy-Weinberg equilibrium and this was established by the Chi-square analysis. The demographic details of both the groups, i.e., cases and controls, were compared and the p-value were calculated to define the significance. The correlation between genotypes, risk of development of Oral Squamous Cell Carcinoma (OSCC), and clinicopathological parameters were calculated with the use of Odds Ratio and 95% Confidence Interval obtained from age and gender-adjusted multiple logistic regression models. The data was analyzed using SPSS software.

RESULTS

Demographic features of OSCC patients and matched controls

This study incorporates 200 Oral Squamous Cell Carcinoma (OSCC) patients and 200 matched healthy subjects or controls. The distribution of both the cases and controls is adjusted according to age and sex. However, there was a significant difference in the etiological parameters like areca nut intake, alcohol use history, cigarette smoking between both the groups, as shown in table 1.

Table 1: Categorization of cases and controls by demographics and clinical characteristics

Variables	Control [n=200]	Cases [n=200]	P value
Age-<50 y	96(48%)	97(48.5%)	0.556
>50 y	104(52%)	103(51.5%)	
Gender-Male	117(58.5%)	132(66%)	0.122
Female	83(41.5%)	68(34%)	
Betel quid Chewing-No	166(83%)	42(21%)	P<0.001*
Yes	34(17%)	158(79%)	
Cigarette Smoking-No	96(48%)	24(12%)	P<0.001*
Yes	104(52%)	176(88%)	
Alcohol-No	156(78%)	93(46.5%)	P<0.001*
Yes	44(22%)	107(53.5%)	
Tumor location-Buccal mucosa		129(64.5%)	
Tongue		60(30%)	
Upper and lower gingiva		11(5.5%)	
Size of Tumor-T1-T2		160(80%)	
T3-T4		40(20%)	
Lymph node involvement-N0-N1		148(74%)	
N2-N3		52(26%)	
TNM Stage		163(81.5%)	
1-2			
3-4		37(18.5%)	
Grade of Tumor-G1		105(52.5%)	
G2-G3		95(47.5%)	

Selection of SNPs

For our analysis, we picked three RETN SNPs (rs7408174, rs1862513, and rs3219175) from a 2kb region upstream of the RETN gene and one SNP out of the RETN intron(rs3745367). All three SNPs had minor allele frequencies greater than 5%. These multiple variants have all been related to the different types of cancer.

Histopathological and clinicopathological characteristics of OSCC cases

All the OSCC patients were graded clinicopathologically and histopathologically under various categories to keep an estimate about the aggressiveness and prognosis of the patient. The patient samples were dissected histopathologically to find out the grade of cancer they were experiencing, i.e., well-differentiated, moderately differentiated, or poorly differentiated. The pattern of development was recognized under various stages, and the tumor distribution was either in the peri-oral or distant lymph nodes. Most of the patients recruited were in the early stages of OSCC. The distant metastasis is seen in several patients (table 1).

The arrangement of genotypic and allelic frequency among cases and controls

To further study the association of all these 4 SNPs with the probability of OSCC, we genotyped controls and OSCC patients. Table *2* presents the distributions of genotype in both the groups and the relationship between OSCC and RETN genetic variants. In controls, we found that all the genotype frequencies followed Hardy-Weinberg equilibrium. In both the cases and controls, the subjects having rs3745367, rs7408174, rs1862513, and rs3219175 SNPs were found to be homozygous for GG, TT, GG and GG genotypes, respectively. After performing logistic regression analysis, we found that both heterozygous and homozygous mutant genotype of rs3219175 increases the risk of developing OSCC in comparison to controls. Further, the combined effect of mutant genotypes has a significant association with risk of OSCC. Besides this, we found that C allele of rs7408174 and A allele of rs3219175 increase the risk of developing OSCC in comparison to the reference allele. Contrary to this, we found that in rs 1862513, C allele is showing a protective effect against the development of OSCC in comparison to the reference allele.

Table 2: Association of RETN gene variants in OSCC patients and controls

Variables	Controls [200]	Cases [200]	Odds ratio	P value
rs 3745367				
GG	76	72	Ref	
GA	91	96	1.114(0.7233-1.714)	0.6251
AA	33	32	1.0(0.5711-1.835)	0.9376
GA+AA	124	128	1.090(0.72559-1.636)	0.6787
G allele	212	184	Ref	
A allele	224	172	0.8847(0.6685-1.171)	0.3913
rs 7408174				
TT	99	106	Ref	
TC	82	78	0.8884(0.5874-1.344)	0.5750
CC	19	16	0.7865(0.3830-1.615)	0.5122
TC+CC	101	94	0.8692(0.5871-1.287)	0.4838
T allele	219	180	Ref	
C allele	181	220	1.479(1.119-1.954)	0.0058
rs 1862513				
GG	73	75	Ref	
GC	94	96	0.9940(0.6467-1.528)	0.9783
CC	33	29	0.8554(0.4722-1.549)	0.6060
GC+CC	127	125	0.95800.6383-1.438()	0.8359
G allele	216	185	Ref	
C allele	184	215	0.73(0.555-0.968)	0.029
rs 3219175				
GG	138	115	Ref	
GA	56	74	1.586(1.035-2.429)	0.0402
AA	6	11	2.2(0.7891-6.133)	0.1235
GA+AA	62	85	1.645(1.091-2.480)	0.0171
G allele	225	172	Ref	
A allele	175	228	1.704(1.288-2.254)	0.0002

Correlation between the integrated impact of genetic variants of RETN gene and Areca nut use habit with the OSCC patients who smoke

Tobacco consumption, either in smoking or smokeless form and areca nut use are the primary factors that result in the occurrence of OSCC (27-28). Table 3 demonstrates the impact of the integrated effect of tobacco use (either smoking or smokeless) and areca nut chewing with RETN genotypes in relation to the spread of OSCC. Among the 260 smokers (100 healthy controls and 160 OSCC patients combined), we found that patients with either one form of A allele of rs3745367, one type of C allele of rs7408174, one type of C allele of rs1862513, and one type of A allele of rs3219175 with betel quid chewing have 7.000 fold, 4.447 fold, 5.345 fold, and 4.764 fold more risk to acquire OSCC than smokers with no background of betel quid chewing. Smokers with at least one A-allele of rs3745367, one C-allele of rs74081741, one C-allele of rs1862513 or one A-allele or betel quid eating habit have 8.217 fold, 9.900 fold, 7.714 fold, and 13.01 fold higher risk, respectively, in developing OSCC than smokers of wild homozygous genotypes. These findings of our study implied that RETN gene polymorphisms had a strong correlation with susceptibility of OSCC in smokers or subjects habitual to betel quid.

Correlation between the rs3219175 genetic variant of RETN gene and clinical parameters in OSCC patients above 50 y of age

Subsequent to this, we compared the association between rs3219175 genetic variant of RETN gene and clinical parameters in OSCC patients who are more than 50 y of age (table 4). After investigation, we analyzed that patients with G/A genotype are more likely to develop less aggressive/high-grade tumor in comparison to patients with G/G genotype. In addition, there is no significant difference in relation to clinical stage, size of tumor, and lymph node involvement.

Table 3: Cumulative risk of developing OSCC on 260 smokers with RETN gene polymorphisms from betel quid chewing

Variables	Controls [100]	Cases [160]	Odds	P value
rs 3745367				
GG genotypes and no betelquid	28	8	Ref	
GA or AA genotypes or betelquid chewing	23	54	8.217(3.258-20.73)	P<0.0001
GA or AA genotypes with betelquid chewing	49	98	7.000(2.969-16.50)	P<0.0001
rs 7408174				
TT genotypes and no betelquid	33	12	Ref	
TC or CC genotypes or betelquid chewing	20	72	9.900(4.334-22.61)	P<0.0001
TC or CC genotypes with betelquid chewing	47	76	4.447(2.091-9.455)	P<0.0001
rs 1862513				
GG genotypes and no betelquid	24	8		
GC or CC genotypes or betelquid chewing	21	54	7.714(2.996-19.87)	P<0.0001
GC or CC genotypes with betelquid chewing	55	98	5.345(2.249-12.71)	P<0.0001
rs 3219175				
GG genotypes and no betelquid	46	16	Ref	
GA or AA genotypes or betelquid chewing	19	86	13.01(6.113-27.70)	P<0.0001
GA or AA genotypes with betelquid chewing	35	58	4.764(2.349-9.661)	0.0771

Table 4: Correlation between RETN genetic variant rs3219175 and clinical parameters in patients above 50 y of age and suffering from OSCC

rs3219175	Tumour size <=T2	>T2	OR (95% CI)	AOR (95% CI)
GG	94(58.8)	29(72.5)	1.000 (reference)	1.000 (reference)
GA	56(35)	8(20)	0.463(0.198-1.083)	0.455(0.193-1.072)
AA	10(6.3)	3(7.5)	0.972(0.251-3.773)	0.917(0.231-3.644)
	Clinical Stage			
rs3219175	I+II	III+IV		
GG	97(59.5)	26(70.3)	1.000 (reference)	1.000 (reference)
GA	54(33.1)	10(27)	0.691(0.310-1.540)	0.649(0.288-1.463)
AA	12(7.4)	1(2.7)	0.311(0.039-2.502)	0.329(0.040-2.675)
	Lymph Node			
rs3219175	N0-N1	N3-N4		
GG	89(60.1)	34(65.4)	1.000 (reference)	1.000 (reference)
GA	48(32.4)	16(30.8)	0.873(0.438-1.740)	0.845(0.421-1.698)
AA	11(7.4)	2(3.8)	0.476(0.100-2.259)	0.516(0.107-2.483)
	Clinical Grade			
rs3219175	Grade I	Grade II		
GG	56(53.3)	67(70.5)	1.000 (reference)	1.000 (reference)
GA	41(39)	23(24.2)	0.469(0.252-0.873)*	0.472(0.2520.885)*
AA	8(7.6)	5(5.3)	0.522(0.162-1.687)	0.85(0.176-1.914)

Multivariate statistical analysis adjusted with effects of arecanutuse, smoking, and alcohol consumption. *Indicated p value significant, p<0.05.

RETN haplotype association with OSCC susceptibility

Lastly, we studied the haplotype frequencies of RETN gene to evaluate the integrated effect of all the four genetic variants on the risk of developing OSCC (table 5). For this, we checked the most frequently found haplotype in control patients, i.e., G/T/G/G and considered it for reference. Finally, after conducting the adjusted multivariate analysis, we found that none of the haplotype showed a statistically significant association with the threat of developing OSCC.

Table 5: Comparison of RETN haplotype frequencies in OSCC patients and controls

Haplotypes	Controls [n=400]	Cases [n=400]	Odds ratio	P value
GTGG	112(28%)	105(26.3%)	Ref	Ref
GCGG	84(21%)	91(22.8%)	1.156 (0.7757-1.722)	0.5422
ATCA	54(13.5%)	62(15.5%)	1.225 (0.7795-1.924)	0.4212
ATGG	41(10.3%)	49(12.3%)	1.275 (0.7786-2.087)	0.3339
ATCG	32(8%)	31(7.8%)	1.033 (0.5895-1.811)	0.9088
GTCG	32(8%)	30(7.5%)	1 (0.5684-1.759)	1
GTCA	11(2.8%)	13(3.3%)	1.261 (0.5409-2.938)	0.591
ACCA	14(3.5%)	8(2%)	0.6095 (0.2456-1.512)	0.2818
GCCG	10(2.5%)	6(1.5%)	0.64 (0.2247-1.823)	0.4001
ACGG	6(1.5%)	2(0.5%)	0.3556 (0.07017-1.802)	0.1933
ACCG	2(0.5%)	2(0.5%)	1.067 (0.1475-7.714)	0.949
GCCA	1(0.3%)	0(0%)	0.3555 (0.01431-8.829)	0.334
ATGA	0(0%)	0(0%)		
GCGA	1(0.3%)	1(0.3%)	1.067 (0.06583-17.28)	0.9638

DISCUSSION

OSCC is one of the most fatal types of cancer and has recorded high morbidity and mortality rates across the globe [27, 28]. In the clinical treatment of OSCC, neither traditional chemotherapy, radiotherapy or modern targeted therapy is efficacious. Deeper understanding of the genetic aspect of disease and signaling mechanisms might pave the path for better and early diagnosis and treatment of OSCC.

Resistin is a secretory polypeptide rich in cysteine, expressed in adipocytes, and belong to a family of proteins called as resistin like molecules. Studies revealed its strong correlation with obesity, diabetes, inflammation and different type of cancers [29-31]. The analysis has also shown that the elevated serum resistin levels are correlated with pathogensis of cancer cachexia in patients suffering from lung cancer [32]. In OSCC patients, a related kind of serum resistin increase is also observed [33]. RETN polymorphisms were investigated in multiple forms of tumours; however, experiments with OSCC are less common. To the best of our knowledge, this is the first-ever study that revealed the distribution of rs3745367, rs7408174, rs1862515 and rs3219175 SNPs and their association with susceptibility of OSCC.

In this study, we checked the association between genotypes of RETN gene polymorphisms and OSCC, and observed that rs3219175 of RETN gene has a considerable association with the incidence of OSCC. The results definitely indicated a significant correlation of GA genotype of rs3219175 with less aggressive form of oral cancer as compared to GG genotype. On the contrary, RETN polymorphisms at rs7408174, rs1862513, and rs3745367 were found to be insignificantly associated with the risk of oral cancer than those in controls. Similar study conducted on the Taiwanese population found no association between these genotypic distributions of RETN gene polymorphisms and OSCC [22]. Importantly, a previous study showed that this *RETN* SNP (rs3219175) affect the response to interferon-based anti-hepatitis C virus therapy in a positive way [34]. Thus, our results provided intuitions about the development of targeted therapy for oral cancer among patients having specific SNP.

The exposure to betel quid and tobacco carcinogens can result in the commencement of oral cancer. The exposure to carcinogens released by tobacco smoke and betel quid chewing for a long time results in augmenting chronic inflammatory reactions in oral tissues, which consequently leads to genetic alterations and finally causes oral carcinogenesis [7-9]. Keeping this in mind, we checked the collective impact of RETN SNPs and these environmental carcinogens. The results of this study depict a collaborative effect of areca nut use habit and smoking tobacco with four genetic variants of RETN gene (rs3745367, rs7408174, rs1862513 and rs3219175) on the risk of developing OSCC.

Moreover, we checked the association of rs3219175 genetic variant of RETN gene with clinical estimation of patients approximately 50 y of age or above for estimating the prognosis of disease. Subsequently, we discovered with regard to OSCC cases that patients above 50 y of age and possessing rs3219175 G/A and A/A genotype had a considerably lower risk of developing more aggressive tumors than those carrying G/G genotype. A previous study revealed that the presence of the polymorphic allele of RETN rs3219175 had marked effects on plasma resistin in patients with type 2 diabetes (35). Another study reported that rs3219175 SNPs were significantly associated with log-resistin levels in Malaysian population [36-38].

In the human genome, the linkage equilibrium is obvious, and may be used as a genetic marker to identify neighboring variants that may be useful in disease detection and recovery. The impact of different haplotypes of four RETN genetic variants (i.e. rs3745367, rs7408174, rs1862513 and rs3219175) in association to the risk of triggering OSCC was assessed in this analysis. Further, we found that neither of the RETN haplotypes raises the chance of OSCC, which is contrary to the findings of the study on Taiwanese population where ATGG haplotypes significantly associate with the risk of developing oral cancer [2].

Therefore, it can be concluded that the association of 1 RETN polymorphic variant rs3219175is related to the susceptibility of OSCC. This is the first study that examines the North Indian population for identifying the relationship between polymorphism of the RETN gene and the risk of OSCC. However, broader demographic trials are required to affirm the role of RETN polymorphism in the likelihood of causing oral cancer.

Since this is a case-control study, some degree of selection bias and recall bias exists. But we have tried our best on selection of patients and quality of our data to reduce the biasness.

Although a more elaborated study on large sample size is needed taking our study as base to further study the function of Resistin gene as biomarker for OSCC (Oral Squamous Cell Carcinoma).

CONCLUSION

OSCC is the most prominent oral malignant tumor that is characterized with high morbidity and mortality rates. For the clinical treatment of oral cancer, neither conventional chemotherapy nor molecular targeted therapy is found to be effective. The increasing number of genetic studies might help in planning a proper strategy for the timely treatment of OSCC. In this study, we observed that rs3219175, the polymorphic variant of Resistin gene, augments the risk of OSCC. Therefore, the findings imply that RETN gene polymorphism has a strong correlation with susceptibility of OSCC among smokers or subjects habitual to betel quid. The study showed that OSCC patients older than 50 y and bearing the heterozygous mutant genotype rs3219175 G/A have a substantially lower chance of developing high-grade tumours. Thus, resistin gene can be used as a biomarker not only for early diagnosis of oral cancer but also for providing novel scope regarding the development of new treatment modalities for OSCC. To confirm the role of resistin gene as biomarker for oral cancer risk, there is a requirement of further studies among different populations and other types of cancer.

ACKNOWLEDGEMENT

The author would like to thank Indian Council of Medical Research (ICMR), University Grant Commission (UGC), New Delhi, India and Centre of Excellence, Higher Education, Government of Uttar Pradesh, Lucknow. DST-FIST-PURSE is duly acknowledged for Central Instrumentation Facility. DT acknowledges UGC for postdoctoral fellowship and Molecular and Human Genetics Laboratory, Department of Zoology, University of Lucknow. SK acknowledges Department of Health Research-Multidisciplinary Research Unit (DHR-MRU), King George's Medical University, Lucknow for the completion of this study.

FUNDING

Nil

AUTHORS CONTRIBUTIONS

The study is designed, planned, executed and compiled by Divya Tandon.

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

REFERENCES

1. Tandon D, Dewangan J, Srivastava S, Garg VK, Rath SK. miRNA genetic variants: as potential diagnostic biomarkers for oral cancer. Pathol Res Pract. 2018;214(2):281-9. doi: 10.1016/j.prp.2017.10.002, PMID 29103762.
2. Pindborg J, Reichart P, Smith C, Waal I. Histological typing of cancer and precancer of the oral mucosa, WHO international histological classification of tumors. 2nd ed; 1997.
3. NIDCR. Oral cancer 5 y survival rate by race, gender, and stage of diagnosis, available. In: https://www.nidcr.nih.gov/research/data-statistics/oral-cancer/survival-rates.
4. Ajila V, Shetty H, Babu S, Shetty V, Hegde S. Human papilloma virus associated squamous cell carcinoma of the head and neck. J Sex Transm Dis. 2015;2015:791024. doi: 10.1155/2015/791024, PMID 26483987.
5. Nowak F, Soria JC, Calvo F. Tumour molecular profiling for deciding therapy-the French initiative. Nat Rev Clin Oncol. 2012;9(8):479-86. doi: 10.1038/nrclinonc.2012.42, PMID 22777058.
6. Das S, Mukherjee S, Choudhury S, Bose A, Roy S. Antioxidant and cytotoxic activities of areca catechu seed extract in swiss albino mice using EAC cell line in different culture medium. Int J Curr Pharm Sci. 2020;12(2):20-7. doi: 10.22159/ijcpr.2020v12i2.37481.

7. Sumintarti MR, Hajrah Yusuf ASt. Oral hairy leukoplakia manifestations related to CD4 count in HIV/AIDS patients at Dr Wahidin Sudirohusodo Hospital. Int J Appl Pharm. 2019;11(4):57-9. doi: 10.22159/ijap.2019. v11s4.35292.
8. Tsai HT, Hsin CH, Hsieh YH, Tang CH, Yang SF, Lin CW. Impact of interleukin-18 polymorphisms-607A/C and 137G/C on oral cancer occurrence and clinical progression. PLOS ONE. 2013;8(12):e83572. doi: 10.1371/journal.pone.0083572, PMID 24349532.
9. Shah S, Mishra G, Kalia K. Single nucleotide polymorphism rs17849071 G/T in the PIK3CA gene is inversely associated with oral cancer. Oral Cancer. 2018;2(3-4):83-9. doi: 10.1007/s41548-018-0012-1.
10. Bokarewa M, Nagaev I, Dahlberg L, Smith U, Tarkowski A. Resistin, an adipokine with potent proinflammatory properties. J Immunol. 2005;174(9):5789-95. doi: 10.4049/jimmunol.174.9.5789, PMID 15843582.
11. Steppan CM, Bailey ST, Bhat S, Brown EJ, Banerjee RR, Wright CM. The hormone resistin links obesity to diabetes. Nature. 2001;409(6818):307-12. doi: 10.1038/35053000, PMID 11201732.
12. Shuldiner AR, Yang R, Gong DW. Resistin, obesity, and insulin resistance-the emerging role of the adipocyte as an endocrine organ. N Engl J Med. 2001;345(18):1345-6. doi: 10.1056/NEJM200111013451814, PMID 11794158.
13. John BJ, Irukulla S, Abulafi AM, Kumar D, Mendall MA. Systematic review: adipose tissue, obesity and gastrointestinal diseases. Aliment Pharmacol Ther. 2006;23(11):1511-23. doi: 10.1111/j.1365-2036.2006.02915.x, PMID 16696799.
14. Danese E, Montagnana M, Minicozzi AM, Bonafini S, Ruzzenente O, Gelati M. The role of resistin in colorectal cancer. Clin Chim Acta. 2012;413(7-8):760-4. doi: 10.1016/j.cca.2012.01.019, PMID 22296675.
15. Gonullu G, Kahraman H, Bedir A, Bektas A, Yucel I. Association between adiponectin, resistin, insulin resistance, and colorectal tumors. Int J Colorectal Dis. 2010;25(2):205-12. doi: 10.1007/s00384-009-0828-6, PMID 19888587.
16. Reilly MP, Lehrke M, Wolfe ML, Rohatgi A, Lazar MA, Rader DJ. Resistin is an inflammatory marker of atherosclerosis in humans. Circulation. 2005;111(7):932-9. doi: 10.1161/01.CIR.0000155620.10387.43, PMID 15710760.
17. Kumar S, Gupta V, Srivastava N, Gupta V, Mishra S, Mishra S. Resistin 420C/G gene polymorphism on circulating resistin, metabolic risk factors and insulin resistance in adult women. Immunol Lett. 2014;162:287-91. doi: 10.1016/j.imlet.2014.07.009, PMID 25088792.
18. Steppan CM, Brown EJ, Wright CM, Bhat S, Banerjee RR, Dai CY. A family of tissue-specific resistin-like molecules. Proc Natl Acad Sci USA. 2001;98(2):502-6. doi: 10.1073/pnas.98.2.502, PMID 11209052.
19. Vallega KA, Liu NN, Myers JS, Yu K, Sang QXA. Elevated resistin gene expression in African American estrogen and progesterone receptor-negative breast cancer. Plos One. 2016;11(6):e0157741. doi: 10.1371/journal.pone.0157741, PMID 27314854.
20. Chung CM, Lin TH, Chen JW, Leu HB, Yin WH, Ho HY. Common quantitative trait locus downstream of RETN gene identified by genome-wide association study is associated with risk of type 2 diabetes mellitus in Han Chinese: a Mendelian randomization effect. Diabetes Metab Res Rev. 2014;30(3):232-40. doi: 10.1002/dmrr.2481, PMID 24123702.
21. Hu WW, Tang CH, Sun Y, Lu TT, Jiang P, Wu YM. Correlation between resistin gene polymorphism and clinical aspects of lung cancer. Medicine. 2017;96(52):e9485. doi: 10.1097/MD.0000000000009485, PMID 29384942.
22. Yang WH, Wang SJ, Chang YS, Su CM, Yang SF, Tang CH. Association of resistin gene polymorphisms with oral squamous cell carcinoma progression and development. BioMed Res Int. 2018;2018:9531315. doi: 10.1155/2018/9531315, PMID 30406149.
23. Vallega KA, Liu NN, Myers JS, Yu K, Sang QXA. Elevated resistin gene expression in African American estrogen and progesterone receptor-negative breast cancer. PLOS ONE. 2016;11(6):e0157741. doi: 10.1371/journal.pone.0157741, PMID 27314854.
24. Wang L, Tang CH, Lu T, Sun YI, Xu G, Huang CC. Resistin polymorphisms are associated with rheumatoid arthritis susceptibility in Chinese Han subjects. Medicine. 2018;97(12):e0177. doi: 10.1097/MD.0000000000010177, PMID 29561430.
25. Lin YJ, Ho TJ, Lin TH, Hsu WY, Huang SM, Liao CC. P-coumaric acid regulates exon 12 splicing of the ATP7B gene by modulating hnRNP A1 protein expressions. Biomedicine. 2015;5(2):10. doi: 10.7603/s40681-015-0010-0, PMID 26048696.
26. Li TC, Li CI, Liao LN, Liu CS, Yang CW, Lin CH. Associations of EDNRA and EDN1 polymorphisms with carotid intima-media thickness through interactions with gender, regular exercise, and obesity in subjects in Taiwan: Taichung Community Health Study (TCHS). Biol Med. 2015;5(2):8. doi: 10.7603/s40681-015-0008-7, PMID 26040574.
27. Chang MC, Chiang CP, Lin CL, Lee JJ, Hahn LJ, Jeng JH. Cell-mediated immunity and head and neck cancer: with special emphasis on betel quid chewing habit. Oral Oncol. 2005;41(8):757-75. doi: 10.1016/j.oraloncology.2005.01.007, PMID 16109353.
28. Chen YJ, Chang JTC, Liao CT, Wang HM, Yen TC, Chiu CC. Head and neck cancer in the betel quid chewing area: recent advances in molecular carcinogenesis. Cancer Sci. 2008;99(8):1507-14. doi: 10.1111/j.1349-7006.2008.00863.x, PMID 18754860.
29. Tsai CH, Tsai HC, Huang HN, Hung CH, Hsu CJ, Fong YC. Resistin promotes tumor metastasis by down-regulation of miR-519d through the AMPK/p38 signaling pathway in human chondrosarcoma cells. Oncotarget. 2015;6(1):258-70. doi: 10.18632/oncotarget.2724, PMID 25404641.
30. Su CM, Huang CY, Tang CH. Characteristics of resistin in rheumatoid arthritis angiogenesis. Biomark Med. 2016;10(6):651-60. doi: 10.2217/bmm.15.125, PMID 26867862.
31. Su CM, Tang CH, Chi MJ, Lin CY, Fong YC, Liu YC. Resistin facilitates VEGF-C-associated lymphangiogenesis by inhibiting miR-186 in human chondrosarcoma cells. Biochem Pharmacol. 2018;154:234-42. doi: 10.1016/j.bcp.2018.05.001, PMID 29730230.
32. Demiray G, Degirmencioglu S, Ugurlu E, Yaren A. Effects of serum leptin and resistin levels on cancer cachexia in patients with advanced-stage non–small cell lung cancer. Clin Med Insights Oncol. 2017;11:1179554917690144. doi: 10.1177/1179554917690144, PMID 28469508.
33. Wu CC, Chu HW, Hsu CW, Chang KP, Liu HP. Saliva proteome profiling reveals potential salivary biomarkers for detection of oral cavity squamous cell carcinoma. Proteomics. 2015;15(19):3394-404. doi: 10.1002/pmic.201500157, PMID 26205615.
34. Chang ML, Liang KH, Ku CL, Lo CC, Cheng YT, Hsu CM. Resistin reinforces interferon lambda-3 to eliminate the hepatitis C virus with fine-tuning from RETN single-nucleotide polymorphisms. Sci Rep. 2016;6:30799. doi: 10.1038/srep30799, PMID 27477870.
35. Onuma H, Tabara Y, Kawamura R, Tanaka T, Ohashi J, Nishida W. A at single nucleotide polymorphism-358 is required for G at 420 to confer the highest plasma resistin in the general Japanese population. PLOS ONE. 2010;5(3):e9718. doi: 10.1371/journal.pone.0009718, PMID 20300528.
36. Apalasamy YD, Rampal S, Salim A, Moy FM, Su TT, Majid HA. Polymorphisms of the resistin gene and their association with obesity and resistin levels in Malaysian Malays. Biochem Genet. 2015;53(4-6):120-31. doi: 10.1007/s10528-015-9678-9, PMID 25991560.
37. Anjum R, Hussain M, Kaur M. Expression of cyclinD1 in varying grades of oral squamous cell carcinoma: an immunohistochemical study. Asian J Pharm Clin Res. 2023;16(6):100-2.
38. Sathiyamoorthy J, Shyamsundar V, Shanmugam S, Mani JG, Hari R. Immunohistochemical expression of c-kit in oral squamous cell carcinoma patients in south Indian Populations. Asian J Pharm Clin Res. 2018;11(10):247-50. doi: 10.22159/ajpcr.2018.v11i10.27443.