RESEARCH ARTICLE

Association of Poly (ADP-Ribose) Polymerase 1 Variants with Oral Squamous Cell Carcinoma Susceptibility in a South Indian Population

Sukumaran Anil[1], PB Gopikrishnan[2], Ashik Bin Basheer[3], BG Vidyullatha[4], Yahya A Alogaibi[5], Elna P Chalisserry[6], Fawad Javed[7], MHN Dalati[8], Sajith Vellappally[9], Mohamed Ibrahim Hashem[9], Darshan Devang Divakar[9]*

Abstract

Background: Oral cancers account for approximately 2% of all cancers diagnosed each year; however, the vast majority (80%) of the affected individuals are smokers whose risk of developing a lesion is five to nine times greater than that of non-smokers. Tobacco smoke contains numerous carcinogens that cause DNA damage, including oxidative lesions that are removed effectively by the base-excision repair (BER) pathway, in which poly (ADP-ribose) polymerase 1 (PARP-1), plays key roles. Genetic variations in the genes encoding DNA repair enzymes may alter their functions. Several studies reported mixed effects on the association between PARP-1 variants and the risk of cancer development. Till now no reported studies have investigated the association between PARP-1 variants and oral squamous cell carcinoma (OSCC) risk in an Indian population. Materials and Methods: In the present case control study 100 OSCC patients and 100 matched controls were genotyped using PARP1 single nucleotide peptides (SNP's) rs1136410 and rs3219090 using TaqMan assays. Results: The results indicated significantly higher risk with PARP1 rs1136410 minor allele "C" (OR=1.909; p=0.02942; CI, 1.060-3.439). SNP rs1136410 also showed significantly increased risk in patients with smoking habit at C/C genotype and at minor allele C. Conclusions: The PAPR-1 Ala762Val polymorphism may play a role in progression of OSCC. Larger studies with a greater number of samples are needed to verify these findings.

Keywords: Oral squamous cell carcinoma - poly (ADP-Ribose) polymerase 1 - risk factor

Asian Pac J Cancer Prev, **17 (8)**, 4107-4111

Introduction

Cancer is a class of diseases in which a group of cells display new properties such as hyperactive growth and division, protection against programmed cell death, loss of respect for normal tissue boundaries, and the ability to become established in diverse environments (Aly, 2012). Every gene involved in cancer progression often has at least one or multiple roles, during the course of normal growth and development of an embryo (Hanahan et al., 2000). Tumour initiation and progression rely on the interactions among the cancer cells and framework of the surrounding tissue and the microenvironment which influence tumor growth and progression (Gupta et al., 2006; Hainaut et al., 2013; Hanahan et al., 2011).

Oral cancers account for approximately 2% of all cancers diagnosed each year; however, the vast majority (80%) of the affected individuals are smokers whose risk of developing an oral cancer in this population is five to nine times greater than that of non-smokers (Napier et al., 2008; Rai et al., 2015). The main risk factor for oral cancer is exposure to exogenous carcinogens such as tobacco smoke and alcohol (Lacko et al., 2014; Rai et al., 2015; Shishodia et al., 2015). Annually, it is estimated that 127,459 deaths are caused from oral cavity cancer worldwide, of which 96,720 occur in developing countries (De Camargo Cancela et al., 2010). Oral squamous cell carcinoma (OSCC) is a multi-causal disease with close

*[1]Department of Preventive Dental Sciences, College of Dentistry, Prince Sattam Bin Abdulaziz University, Al-Kharj, [2]Department of Conservative Dentistry and Endodontist, [3]Department of Oral and Maxillofacial Pathology, PSM College of Dental Sciences and Research, Trichur, Kerala, India, [4]Department of Oral and Maxillofacial Surgery and Diagnostic sciences, King Saud Bin Abdulaziz University for Health Sciences, Riyadh, [5]Department of Orthodontics, Faculty of Dentistry, King Abdulaziz University, Jeddah, [6]Department of Maxillofacial Surgery & Diagnostic Sciences, College of Dentistry, College of Dentistry, Jazan University, Jazan, KSA, [7]Department of General Dentistry, Eastman Institute for Oral Health, University of Rochester, NY, USA, [8]Consultant Orthodontist and Restorative Dentist, Springs Dental Care, New Road Side, Horsforth, Leeds, UK, [9]Dental Biomaterials Research Chair, Dental Health Department, College of Applied Medical Sciences, King Saud University, Riyadh, KSA *For correspondence: darshandevang@gmail.com*

interrelationships among etiologic factors (Da Silva et al., 2011). Risk factors include lifestyle habits (tobacco exposure and alcohol consumption), dietary factors, occupational activity, socioeconomic status, exposure to external agents, and genetic susceptibility. Tobacco is considered as one of the major factors leading to oral cancer (Petti, 2009). There are approximately 1.1 million smokers worldwide. The prevalence of smoking among the Indian population ranges from 2.4 to 52.9% with a median of 17.5% (Petti, 2009). Male adolescents are more attracted by tobacco smoke. The prevalence of OSCC in cigarette smokers is 4–7 times greater than in non-smokers. Furthermore, there is a synergistic effect between tobacco and alcohol in relation to the duration and frequency of exposure and tumor development (Da Silva et al., 2011). Carcinogenic agents in tobacco may directly cause mutations in DNA but also suppress DNA repair enzymes (the critical component against human cancer). Brennan et al., (Brennan et al., 1995) presented evidence linking cigarette smoking to specific p53 mutations but the exact mechanism of carcinogenesis is not always clear (Brennan et al., 1995). Genetic susceptibility (predisposition) to OSCC is also a major risk factor especially in young patients and based on inherited differences in the efficiencies of metabolizing carcinogens, DNA repair and cell cycle control, alone or in combination(Wu et al., 2004).

One of the most important DNA repair systems is the base excision repair (BER) (Mohammad Alanazi et al., 2013). Mutations in the DNA repair systems' genes can lead to genomic instability which in turn causes the development of many types of cancer, including Oral squamous cell carcinoma (Feinberg et al., 2004). Poly (ADP-Ribose) Polymerase 1 (PARP-1) is one of the most identified and well characterized BER system's proteins that catalyzes the poly (ADP-ribosylation) of various proteins involved in many cellular processes including; DNA damage repair, differentiation, proliferation, and cell death (Jagtap et al., 2005). Mutations of PARP-1 gene are found to be implicated in the carcinogenesis (Mohammed Alanazi et al., 2013). The aim of this study was to assess the association between different PARP-1 polymorphisms and increased risk of OSCC in South Indian population and to determine common variants in South Indian OSCC patients and allele frequencies of each variant in South Indian OSCC patients and normal individuals.

Materials and Methods

The OSCC samples were collected from patients undergoing a routine biopsy procedure, as part of their medical treatment and/or diagnosis by qualified surgeon at KVG, Dental College and Hospital, Karnataka, India, after obtaining the necessary approval from the institutional review board (IRB # 15/1818, KVG, Dental College). All participating subjects signed an informed written consent in order to be included in this study, and their clinical data were recorded.

The genomic DNA was purified from whole blood samples using QI Aamp DNA Blood mini kit (Qiagen) following the manufacturer's instructions. After extraction, the nucleic acids concentrations was quantitated using NanoDrop 8000 (Thermo Fisher Scientific, Epsom, UK).

Genotyping

All blood samples were genotyped using pre-designed TaqMan allelic discrimination genotyping assays (Applied Biosystems, Foster City, USA). SNPs rs1136410 and rs3219090 in PARP1 were selected based on other population studies. The two SNPs were genotyped by ABI Quant studio 7 RT-PCR system. The PCR amplification in 10 µl reactions contains 20 ng of genomic DNA, 0.28 µl of 40x SNP Genotyping Assay Mix and 5.8 µl of 2x TaqMan Universal PCR Master Mix.

Statistical analysis

The statistical software IBM SPSS version 22 (IBM SPSS, Chicago, IL, USA) and Microsoft Excel® was used to perform all statistical analyses. Case-control and other genetic comparisons were performed using the chi-square test (χ^2), odds ratios (OR), and 95% confidence intervals (CI). p-value less than 0.05 was considered statistically significant.

Results

The demographic and clinical characteristics of the OSCC patients are shown in (Table 1). The demographic characteristics of the control population were similar to those of the patients. Among the OSCC patients, 68 (68%) were males and 32 (32%) were females and 66% of this population were above 50 years of age.

Both the polymorphisms are following Hardy-Weinberg Equilibrium (HWE). The homozygous wild-type allele was used as a reference to calculate the odds of acquiring OSCC cancer in comparison with the other two genotypes. The genotype frequencies of the analyzed SNPs along with the resulting odds ratio and significance levels are shown in (Table 2). Of the two SNPs tested, only PARP1 SNP rs1136410 showed significant association with OSCC patients.

In the present study, we found significant association with only PARP1 SNP rs1136410 genotypes between OSCC cancer cases and matched healthy controls. The frequencies of rs1136410 (T > C) genotypes in OSCC cancer cases were 72 (0.72), 21 (0.21), and 7 (0.07), respectively, whereas in healthy controls the frequencies

Table 1. Distribution of OSCC Patients According to Demographic Data

	No.	%
Gender		
Male	68	68
Female	32	32
Age		
>50	66	66
<50	34	34
Smoking status		
Ever	72	72
Never	28	28
Alcohol		
Yes	64	64
No	36	36

Table 2. Genotype Frequencies of PARP1 Gene Polymorphisms in OSCC and Controls

Genotype	OSCC	Controls	OR	(95% CI)	c2 Value	P*- Value
rs1136410						
TT	72 (0.72)	82(0.82)	Ref			
TC	21 (0.21)	16 (0.16)	1.495	0.725-3.081	1.19	0.27433
CC	7 (0.07)	2 (0.02)	3.986	0.802-19.803	3.28	0.07026
T	165(0.825)	180(0.90)	Ref			
C	35(0.175)	20(0.10)	1.909	1.060-3.439	4.74	0.02942
rs3219090						
TT	18 (0.18)	20 (0.20)	Ref			
TC	38 (0.38)	39 (0.39)	1.083	0.497-2.357	0.04	0.84145
CC	44 (0.44)	41 (0.41)	1.192	0.554-2.565	0.2	0.65229
T	74(0.37)	79(0.395)	Ref			
C	126(0.63)	121(0.605)	1.112	0.743-1.664	0.26	0.60697

Table 3. Genotype Frequencies of PARP1 Gene Polymorphisms in OSCC and Controls (Smokers)

Genotype	OSCC smokers	Controls	OR	(95% CI)	c2 Value	P*- Value
rs1136410						
TT	54 (0.75)	62 (0.86)	Ref			
TC	13 (0.18)	10 (0.14)	1.493	0.606-3.677	0.76	0.38203
CC	5 (0.07)	0 (0)	12.615	0.682-233.37	5.48	0.01923
T	121 (0.84)	134 (0.93)	Ref			
C	23 (0.16)	10 (0.07)	2.547	1.165-5.568	5.78	0.01617
rs3219090						
TT	12 (0.17)	15 (0.21)	Ref			
TC	29 (0.40)	27 (0.37)	1.343	0.534-3.377	0.39	0.53085
CC	31 (0.43)	30 (0.42)	1.292	0.520-3.209	0.3	0.58112
T	53 (0.63)	57 (0.40)	Ref			
C	91 (0.37)	87 (0.60)	1.125	0.699-1.810	0.24	0.62759

Table 4. Genotype Frequencies of PARP1 Gene Polymorphisms in OSCC and Controls (Non-Smokers)

Genotype	OSCC non-smokers	Controls	OR	(95% CI)	c2 Value	P*- Value
rs1136410						
TT	18 (0.64)	20 (0.72)	Ref			
TC	8 (0.29)	6 (0.21)	1.481	0.431-5.095	0.39	0.53179
CC	2 (0.07)	2 (0.07)	1.111	0.142-8.725	0.01	0.92015
T	44 (0.79)	46 (0.82)	Ref			
C	12 (0.21)	10 (0.08)	1.255	0.492-3.197	0.23	0.63431
rs3219090						
TT	6 (0.22)	5 (0.18)	Ref			
TC	9 (0.32)	12 (0.43)	0.625	0.144-2.713	0.4	0.52915
CC	13 (0.46)	11 (0.39)	0.985	0.235-4.127	0.001	0.98334
T	21 (0.38)	22 (0.61)	Ref			
C	35 (0.62)	34 (0.39)	1.078	0.503-2.310	0.04	0.84595

were 82 (0.82), 16 (0.16), and 2 (0.02), respectively.

PARP1 SNP rs3219090 did not show any association with OSCC patients in South Indian patients (Table 2). As shown in (Table 2), the frequency of the rs3219090, (T>C) T/T, T/C, and C/C genotypes were 18(0.18), 38(0.38), and 44 (0.44), respectively, in OSCC patients and 20 (0.20), 39 (0.39), and 41 (0.41), respectively, in controls. OSCC patients did not show any risk when compared with healthy individuals (Table 2).

The frequencies of the rs1136410, (T>C) T/T, T/C, and C/C genotypes were 1872(0.72), 21(0.21), and 7 (0.07), respectively, in OSCC patients and 82 (0.20), 16 (0.16), and 2 (0.02), respectively, in controls. A strong association was observed with PARP1 SNP rs1136410 minor allele "C" polymorphism. PARP1 SNP rs1136410 homozygous minor allele C showed significant risk in OSCC patients when compared to controls (p=0.02942; OR=1.909; CI, 1.060-3.439). OSCC patients showed nearly 2 fold higher risk compared to healthy individuals. Minor allele frequency was significantly higher in OSCC patient's (0.175) when compared to healthy individuals (0.10) (Table 2).

Interaction of PARP1 genetic polymorphisms with smoking status

We have categorized the patients as smoker (Table 3) and non-smoker (Table 4). Individuals who smoked for more than five years were defined as smokers. The individuals who had never smoked in their lifetime were regarded as non-smokers. PARP1 SNP rs1136410 variant genotype CC and homozygous minor allele C showed significant risk in OSCC patients when compared to controls (Table 3). A strong association was observed with PARP1 SNP rs1136410 homozygous genotype "C/C" polymorphism in OSCC patients with smoking habit. OSCC patients with smoking habit showed nearly 12 fold higher risk compared to healthy individuals with "CC" genotype of SNP rs1136410. (p=0.01923; OR=12.615;

CI, 0.682-233.37). Similarly, variant allele C imposed 2.547 folds increased risk of OSCC patients with smoking habit (p=0.01617; OR=2.547; CI, 1.165-5.568) (Table 3). PARP1 SNP rs3219090 which didn't show any association with OSCC cancer in overall study, showed no association with OSCC patients with smoking habit.

In the present study no significant association was observed in case of interaction of smoking status with any genotypes in PARP1 SNP rs3219090 and rs1136410 for OSCC risk in non-smokers (Table 4).

Discussion

In this case-control study we have analyzed two SNPs selected from previous genome-wide association study (GWAS) and literature related with cancer and other disease with the intention of evaluating their role and association with OSCC in South Indian population. This is the first study in Indian population to report about PARP1 SNP's role in progression of OSCC.

In the present case-control study out of the two SNP's studied, we observed a significant association between the PARP1 rs1136410 (T>C) polymorphism and the risk of oral squamous cell carcinoma in South Indian population. PARP1 rs1136410 (T>C) showed significant risk at "C" allele in overall study (Table 2). In stratified analysis of OSCC patients based on smoking status rs1136410 showed significantly higher risk in smoking patients at homozygous variant allele CC and also at minor allele C (Table 3). This association was not observed in non-smoker patients.

In this study, we found for the first time that the PARP-1 rs1136410 (T>C) genotype significantly contributes to OSCC susceptibility in Indian population, which further extend the important role of PARP-1 in carcinogenesis. The increased risk of OSCC in subjects with the PARP-1 rs1136410 genotype is likely attributable to the reduction of PARP-1 activity (M. Alanazi et al., 2014). PARP1 SNP rs3219090 didn't show any association with OSCC in overall analysis and even in smokers and non-smokers.

It has been reported that PARP-1 rs1136410 (T>C) which is leading to change of amino acid Valine at 762 position to Alanine, significantly contributes to lowering the PARP-1 catalytic activity by 30-40%, in a dosage-dependent manner, which might diminish the BER ability to repair damaged DNA and thus increasing cancer risk (Wang et al., 2007). Previous studies have found a positive association between the Val762Ala SNP and increased risk of many types of cancer, including thyroid (Chiang et al., 2008), breast (M. Alanazi et al., 2013), esophagus (Hao et al., 2004), lung (X. Zhang et al., 2005), stomach (Q. Zhang et al., 2009), urinary bladder (Figueroa et al., 2007), cervical (Ye et al., 2012), and prostate (Lockett et al., 2004). While others found the Ala762 variant to be a protective allele from cancer, it was significantly associated with decreased risk of non-hodgkin lymphoma (NHL) in Korean males and Connecticut females (Jin et al., 2010), squamous cell carcinoma of the head and neck (Li et al., 2007), and breast cancer (Smith et al., 2008). Interestingly, the frequencies of the PARP-1 Ala762 allele are known to be different among ethnic populations.

In conclusion, our findings suggest that SNP variants in PARP-1 may play an important role in the development of OSCC. Despite our data supports for a clear association between PARP-1 and OSCC in South Indian population, PARP-1 gene plays a major role in the susceptibility of the disease. As the sample size of this study is not sufficiently large and is restricted to South Indian population, the present data should be validated in larger samples and in other ethnic groups.

References

Alanazi M, Pathan AAK, Abduljaleel Z,et al (2013). Association between PARP-1 V762A polymorphism and breast cancer susceptibility in saudi population. *PLoS One*, **9**, 85541.

Alanazi M, Pathan AAK, Ajaj S A, et al (2013). DNA repair genes XRCC1, XRCC3, XPD, and OGG1 polymorphisms among the central region population of Saudi Arabia. *Biological Res*, **46**, 161-7.

Alanazi M, Pathan AAK, Shaik JP, et al (2013). The C Allele of a synonymous SNP (rs1805414, Ala284Ala) in PARP1 is a risk factor for susceptibility to breast cancer in Saudi patients. *Asian Pac J Cancer Prev*, **14**, 3051-6.

Aly HA (2012). Cancer therapy and vaccination. *J Immunol Methods*, **382**, 1-23.

Brennan JA, Boyle JO, Koch WM, et al (1995). Association between cigarette smoking and mutation of the p53 gene in squamous-cell carcinoma of the head and neck. *New England J Med*, **332**, 712-7.

Chiang FY, Wu CW, Hsiao PJ, et al (2008). Association between polymorphisms in DNA base excision repair genes XRCC1, APE1, and ADPRT and differentiated thyroid carcinoma. *Clin Cancer Res*, **14**, 5919-24.

Da Silva SD, Ferlito A, Takes RP, et al (2011). Advances and applications of oral cancer basic research. *Oral Oncol*, **47**, 783-91.

De Camargo Cancela M, Voti L, Guerra-Yi M, et al (2010). Oral cavity cancer in developed and in developing countries: population-based incidence. *Head Neck*, **32**, 357-67.

Feinberg AP, Tycko B (2004). The history of cancer epigenetics. *Nat Rev Cancer*, **4**, 143-153.

Figueroa JD, Malats N, Real FX, et al (2007). Genetic variation in the base excision repair pathway and bladder cancer risk. *Hum Genet*, **121**, 233-242.

Gupta GP, Massague J (2006). Cancer metastasis: building a framework. *Cell*, **127**, 679-695.

Hainaut P, Plymoth A (2013). Targeting the hallmarks of cancer: towards a rational approach to next-generation cancer therapy. *Curr Opin Oncol*, **25**, 50-1.

Hanahan D, Weinberg RA (2000). The hallmarks of cancer. *Cell*, **100**, 57-70.

Hanahan D, Weinberg RA (2011). Hallmarks of cancer: the next generation. *Cell*, **144**, 646-674.

Hao B, Wang H, Zhou K, et al (2004). Identification of genetic variants in base excision repair pathway and their associations with risk of esophageal squamous cell carcinoma. *Cancer Res*, **64**, 4378-84.

Jagtap P, Szabo C (2005). Poly (ADP-ribose) polymerase and the therapeutic effects of its inhibitors. *Nat Rev Drug Discov*, **4**, 421-40.

Jin XM, Kim HN, Lee IK, et al (2010). PARP-1 Val762Ala polymorphism is associated with reduced risk of non-Hodgkin lymphoma in Korean males. *BMC Med Genetics*, **11**, 38.

Lacko M, Braakhuis BJ, Sturgis EM, et al (2014). Genetic susceptibility to head and neck squamous cell carcinoma.

Int J Radiat Oncol Biol Phys, **89**, 38-48.
Li C, Hu Z, Lu J, et al (2007). Genetic polymorphisms in DNA base-excision repair genes ADPRT, XRCC1, and APE1 and the risk of squamous cell carcinoma of the head and neck. *Cancer*, **110**, 867-75.
Lockett KL, Hall MC, Xu J, et al (2004). The ADPRT V762A genetic variant contributes to prostate cancer susceptibility and deficient enzyme function. *Cancer Res*, **64**, 6344-8.
Napier SS, Speight PM (2008). Natural history of potentially malignant oral lesions and conditions: an overview of the literature. *J Oral Pathol Med*, **37**, 1-10.
Petti S (2009). Lifestyle risk factors for oral cancer. *Oral Oncol*, **45**, 340-50.
Rai NP, Anekar J, Shivaraja Shankara Y M, et al (2015). Comparison of serum fucose levels in leukoplakia and oral cancer patients. *Asian Pac J Cancer Prev*, **16**, 7497-500.
Rai NP, Divakar DD, Al Kheraif AA, et al (2015). Outcome of palliative and radical radiotherapy in patients with oral squamous cell carcinoma - a retrospective study. *Asian Pac J Cancer Prev*, **16**, 6919-22.
Shishodia NP, Divakar DD, Al Kheraif AA, et al (2015). End stage palliative care of head and neck cancer: a case study. *Asian Pac J Cancer Prev*, **16**, 1255-1258.
Smith TR, Levine EA, Freimanis RI, et al (2008). Polygenic model of DNA repair genetic polymorphisms in human breast cancer risk. *Carcinogenesis*, **29**, 2132-8.
Wang XG, Wang ZQ, Tong WM, et al (2007). PARP1 Val762Ala polymorphism reduces enzymatic activity. *Biochem Biophys Res Commun*, **354**, 122-126.
Wu X, Zhao H, Suk R, et al (2004). Genetic susceptibility to tobacco-related cancer. *Oncogene*, **23**, 6500-23.
Ye F, Cheng Q, Hu Y, et al (2012). PARP-1 Val762Ala polymorphism is associated with risk of cervical carcinoma. *PLoS One*, **7**, 37446.
Zhang Q, Li Y, Li X, et al (2009). PARP-1 Val762Ala polymorphism, CagA+ H. pylori infection and risk for gastric cancer in Han Chinese population. *Mol Biol Rep*, **36**, 1461-7.
Zhang X, Miao X, Liang G, et al (2005). Polymorphisms in DNA base excision repair genes ADPRT and XRCC1 and risk of lung cancer. *Cancer Res*, **65**, 722-6.