Original Research

Association of X-ray Repair Cross-Complementing Group 1 Arg399gln Polymorphisms with the Susceptibility to Develop Oral Squamous Cell Carcinoma in Tamol Chewer's Population in Assam, India

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Abstract

Background: Various environmental factors have been reported to play key role in the development of oral squamous cell carcinoma (OSCC). A lesser known risk factor of oral cancer in India is the uncontrolled use of areca nut chewing. In North-East India, Areca nut, locally called as "Tamol" in Assam, is raw betel nut, lime and betel leaf without tobacco, which are more effective as compared to dried which can be the important contributing factor for OSCC. **Objectives:** The aim of the study was to detect the association between XRCC1 polymorphisms and increased risk of OSCC in tamol chewers population in Assam, India. **Methods:** 50 OSCC patients, 50 tamol chewers and 50 controls were enrolled in the study. XRCC1 Arg399Gln polymorphisms were determined by using polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP). **Results:** There was a significant association for XRCC1 codon 399 (Arg/Gln+Gln/Gln) ($p<0.05$; OR=1.909, CI= 0.8622- 4.227) with the wild type in cancer sample as compared with control sample. Similarly, the positive association for 399G/G ($p<0.05$; OR =2.842, CI = 0.919-8.79) genotypes with oral carcinoma and control sample. In case of tamol chewers, the AA genotype was found to be associated with 2-fold (OR- 2.25, CI= 0.709-7.14) increase risk of developing oral cancer while GA+AA genotype was associated with one and half fold (OR-1.62, CI=0.7354- 3.568) risk of developing oral cancer. **Conclusions:** Based on these results, the XRCC1399G>A genotype could be used as a useful molecular biomarker to predict genetic susceptibility in tamol chewers population and its susceptibility to develop OSCC.

Keywords: Oral squamous cell carcinoma, polymerase chain reaction–restriction fragment length polymorphism, Tamol, X-ray repair cross-complementing group

Submitted: 30-Apr-2020; **Accepted:** 23-Oct-2020; **Published:** 22-Mar-2021

Introduction

Oral cancer is currently the 6th most common malignancy in the world.[1] The worldwide oral cancer incidence is around 500,000 new cases every year, accounting for approximately 3% of all malignancy, creating a world health problem significantly.[2] In India, it is the most common malignancy among men and one of the five most common malignancies among women.[3] Etiology of oral squamous cell carcinoma (OSCCs) comprises risk factors such as exposure to tobacco product, alcohol, infection, dietary factors, and chemical irritants. A lesser known risk factor of oral cancer in India can also be attributed to uncontrolled chewing of areca nut.[4] In Northeast India, locally called as "Tamol" in Assam, "Kwai" in Meghalaya, and "Kuba" in Mizoram, is a raw betel nut chewed in combination with betel leaf and lime and often without tobacco. It has a high content of chemicals such as alkaloids, polyphenol, and tannins

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Access this article online

Quick Response Code:

Website: www.ijds.in

DOI: 10.4103/IJDS.IJDS_60_20

How to cite this article: Lepcha L, Sarma MP, Kataki AC, Unni BG. Association of X-ray repair cross-complementing group 1 Arg399gln polymorphisms with the susceptibility to develop oral squamous cell carcinoma in tamol chewer's population in Assam, India. Indian J Dent Sci 2021;13:73-9.

as compared to the dried ones, which can be the major risk factor for the development of OSCC.[5] The northeast regions, especially Assam and Meghalaya, are the major regions for areca nut consumption.[6] Regular consumption can lead to scratches and eventually form ulcers in the oral cavity. The slaked lime also contains a strong chemical compound that will further form scars or ulcers in the soft tissue of the oral cavity.[7] Such factors can induce chromosomal instability or abnormal DNA damage response, further leading to cell death or unregulated proliferation. In such cases, the DNA repair system works to maintain genomic stability and repair further DNA damage. Therefore, any disruption or transcription of DNA repair genes accounts for the lethal effects of DNA damage and increases the risk of carcinoma.[8]

The important DNA repair pathways involved are nucleotide excision repair, base excision repair (BER), and double-strand break. The X-ray repair cross-complementing group 1 (XRCC1) involved in the BER pathway plays a key role in protecting the genome from a variety of risk factors. XRCC1 acts in BER encoding scaffolding protein that assembles together of the DNA repair complex.[9]

The three commonly studied single-nucleotide polymorphisms in the XRCC1 gene in different population include Arg194Trp (C to T substitution at exon 6 resulting in an arginine (Arg) to Trp amino acid change), Arg280His (G to A substitution at exon 9 resulting in an Arg to His amino acid change), and Arg399Gln (G to A substitution at exon 10 resulting in an Arg to glutamine [Gln] amino acid change).[8,9,10]

Several studies have also reported the association of head-and-neck cancer risk with DNA repair genes XRCC1 Arg194Trp, XRCC1 Arg280His, and XRCC1 Arg399Gln polymorphism.[10] In this present study, we investigate the interaction of XRCC1 (Arg399Gln) polymorphisms in OSCC patients and their role in modulating the relationship between habitual Tamol chewers and OSCC risk.

Methodology

Study subjects

The study consisted of 50 histologically confirmed, untreated OSCC cases (diagnosed between 2015 and 2019), 50 samples from Tamol chewers, and 50 controls samples without a family history of cancer prior to cytological confirmation. All subjects included are living in the northeastern states of India. The oral swab and saliva sample of the participating subjects were collected upon written consent. Controls were individually matched to cases in sex, age, ethnicity, and neighborhood. The study was approved by the Institutional Ethics Committee, BBCI, Guwahati, Assam. Precautions were taken to avoid contaminations while handling the samples.

Data collection

A standard predesigned questionnaire was used to collect general and exposure information of the subjects. Each subject was requested to report information on sociodemographic characteristics such as tobacco smoking, alcohol consumption, and betel quid chewing status. In this study, Tamol chewers, smokers, tobacco chewers, and alcohol consumers were excluded from control samples, and in case of Tamol chewers, the populations with smokers, tobacco chewers, and alcohol consumers were excluded.

Cytomorphological studies for Tamol chewers

In Tamol chewers group, cytomorphological studies had been conducted to screen the sample before proceeding to DNA analysis. The sample collection criteria were of individuals who are nonsmoker, nontobacco chewer, and habitual Tamol chewers. Cytologically confirmed OSCC case samples were done only on Tamol chewers and further assessments were carried. The subject was instructed to rinse the oral cavity with water and the smear was taken from the buccal mucosa using a wooden spatula. The materials collected were smeared on two slides and immediately fixed on 95% ethyl alcohol for 15 min. One slide was stained according to the hematoxylin and eosin staining method and Papanicolaou (PAP) staining method. The smear was the observed under ×40 and ×100 magnification. The cytological features of the smear have been observed and compared with the control sample.

DNA extraction and genotyping

The genomic DNA was isolated from the collected saliva and oral swaps using the Trizol reagent, T9424 (Sigma), according to the protocol provided by the company and were stored at −20ºC before genotyping. The XRCC1 (Arg399Gln) gene was amplified using forward and reverse primers: 5'-TTGTGCTTTCTCTGTGTCCA-3' and 5'-TCCTCCAGCCTTTTCTGATA-3', the primer has been taken from the previous published paper with slight modification.[9] An amplicon of 615 bp was obtained. The polymerase chain reaction (PCR) product was digested with MspI restriction enzyme (New England Biolabs, USA); two fragments of 240 and 375 bp represent the wild-type allele GG (Arg/Arg), three fragments of 615, 375, and 240 bp indicate for heterozygous GA (Arg/Gln) and a single 615 bp fragment for the variant allele AA (Gln/Gln). The restriction fragment length polymorphism (RFLP) results were confirmed by sequencing 10% of the randomly selected samples from both cases and controls by Sanger sequencing using Genetic Analyzer 3500, Applied BioSystems (AgriGenome, Kerala, India).

Statistical analysis

Statistically significant differences of demographic characteristics in the study populations were assessed by Chi-square test. Analysis of risk of association between tamol chewing and cancer status of XRCC1 Arg399Gln genotypes is expressed in terms of odds ratios (ORs), 95% confidence intervals (95% CIs), and their corresponding P values. $P = 0.05$ was taken as statistically significant.

Results

A total of 150 samples, 50 each from OSCC patients, Tamol chewers, and control subjects, were included in the studies with

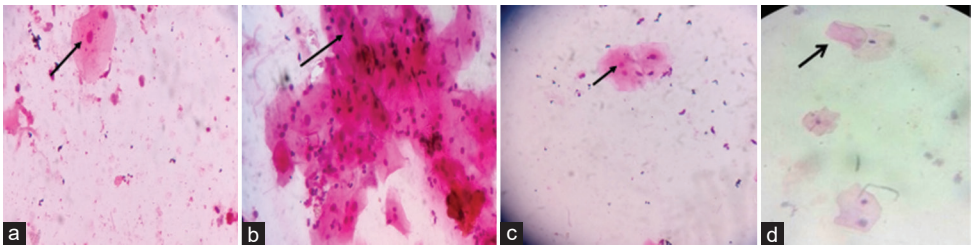

Figure 1: Papanicolaou staining method in habitual Tamol chewers. (a) Micronucleate cell (b) disturbed nuclei-cytoplasmic ratio and inflammatory cells (c) binucleated cells (d) anucleate cells

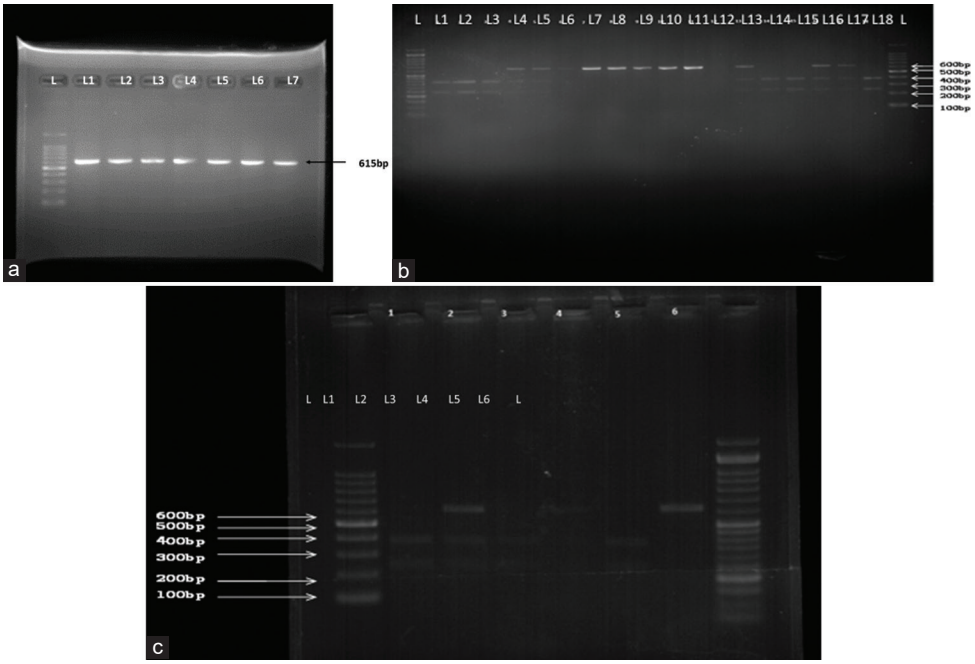

Figure 2: (a) X-ray repair cross-complementing group 1 gene amplicon of 615bp. L represents the DNA ladder and Lanes 1, 2, 3, 4, 5, 6, and 7 represent polymerase chain reaction product. (b) Digested product after restriction fragment length polymorphism with MspI in case lanes 1, 2, 3, 14, 15, and 18 represent wild Arg/Arg (G/G) genotype, lanes 5, 6, 13, 16, and 17 represent heterozygous-type Arg/Gln (G/A) genotypes, and lanes 7, 8, 9, 10, and 11 represent mutant Gln/Gln (A/A) genotypes. (c) Digested product after RFLP with MspI in Tamol chewers L: DNA ladder, lanes 1, 3, and 5 represent wild Arg/Arg (G/G) genotype, lanes 2 and 4 represent heterozygous-type Arg/Gln (G/A) genotypes, and lane 6 represents mutant Gln/Gln (A/A) genotypes. Arg: Arginine, Gln: Glutamine

Table 1: Characteristics of included subjects

Characteristics	Cancer (OSCC)	Tamol pan chewers	Control
Age (mean±SD)	55±12	46±11	50±11
Sex			
Female	14	41	31
Male	36	09	19
Tamol pan (years)			
5-10	5	24	Never
>10	45	26	Never
Tamol pan/day			
10-20	37	28	Never
>20	13	22	Never

OSCC: Oral squamous cell carcinoma, SD: Standard deviation

prior consent. The mean age of the OSCC patients, Tamol pan chewers, and control were 55 ± 12, 46 ± 11, and 50 ± 11 years, respectively. Of 50 samples of cancer patients, 36 were male and 14 were female. In Tamol pan chewers group, the maximum number was female and it must be noted that male population are often involved in alcohol and tobacco consumptions which are already an established carcinogen, thereby ruling male patient out. Not much of a family history with oral cancer or head-and-neck cancer cases as such was observed [Table 1]. In Tamol chewers' group, cytomorphological studies had been conducted to screen the sample before proceeding to DNA analysis. The buccal smear stained with PAP stain showed oral epithelial dysplasia commonly observed in habitual Tamol chewers [Figure 1]. The samples which showed positive for oral epithelial dysplasia were included in XRCC1 399 codon polymorphism studies.

The DNA samples were amplified by the PCR method and the samples were separated on 2% agarose gel where 615 bp DNA has been observed [Figure 2a]. An association between XRCC1

Arg399Gln polymorphisms was determined by detecting PCR-RFLP band pattern on 2% agarose gel and XRCC1 Arg399Gln polymorphism was investigated by PCR/RFLP. After digestion with MspI enzymes, two fragments of 241 bp and 374 bp are obtained for wild-type Arg/Arg genotypes. The homozygous variant genotypes display only one fragment of 615 bp, Gln/Gln genotypes, and the heterozygous display all three fragments of Arg/Gln genotypes. Lanes 1, 2, 3, 14, 15, and 18 represent wild Arg/Arg (G/G) genotype, lanes 5, 6, 13, 16, and 17 represent heterozygous-type Arg/Gln (G/A) genotypes, and lanes 7, 8, 9, 10, and 11 represent mutant Gln/Gln (A/A) genotypes [Figure 2b]. In Tamol pan chewers, lanes 1, 3 and 5 represent wild Arg/Arg (G/G) genotype, lanes 2 and 4 represent heterozygous-type Arg/Gln (G/A) genotypes, and lane 6 represents mutant Gln/Gln (A/A) genotypes [Figure 2c]. Genotyping results [Tables 2 and 3] show the distribution of Arg/Arg, Arg/Gln, and Gln/Gln for XRCC1 at codon 399. The allele frequency of XRCC1 codon 399 among case was Arg/Arg (40%), Arg/Gln (36%), and Gln/Gln (24%). Similarly, in Tamol chewers and control case, the allele frequency of Arg/Arg, Arg/Gln and Gln/Gln of 44% and 36%, 20% and 56%, 34% and 10% respectively was found.

According to the results in Table 2, there was a significant association for 399 (Arg/Gln + Gln/Gln) ($P < 0.05$; OR = 1.909, CI = 0.8622–4.227) with the wild type in cancer sample as compared with the control sample. Similarly, there was a positive association for 399G/G (OR = 2.842, CI = 0.919–8.79) genotypes with oral carcinoma and control sample. It is

Table 2: Genotyping results shows the distribution of Arg/Arg, Arg/Gln and Gln/Gln for X-ray repair cross-complementing group 1 at codon 399 of control and cases

Genotypes	Controls (*n*=50), *n* (%)	Cases (*n*=50), *n* (%)	OR (95% CI)	*P*
GG (Arg/Arg)	28 (56)	20 (40)	1.00 (reference)	-
GA (Arg/Gln)	17 (34)	18 (36)	1.093 (0.4799-2.484)	0.41
AA(Gln/Gln)	5 (10)	12 (24)	2.842 (0.919-8.79)	0.03
GA (Arg/Gln) + AA (Gln/Gln)	22 (44)	30 (60)	1.909 (0.8622-4.227)	0.05

n: Number of samples, OR: Odds ratio, CI: Confidence interval, Arg: Arginine, Gln: Glutamine

Table 3: Genotyping results show the distribution of Arg/Arg, Arg/Gln, and Gln/Gln for X-ray repair cross-complementing group 1 at codon 399 of control and Tamol chewers

Genotypes	Controls (*n*=50), *n* (%)	Tamol chewers (*n*=50), *n* (%)	OR (95% CI)	*P*
GG (Arg/Arg)	28 (56)	22 (44)	1.00 (reference)	-
GA (Arg/Gln)	17 (34)	18 (36)	1.093 (0.4799-2.484)	0.41
AA (Gln/Gln)	5 (10)	10 (20)	2.25 (0.709-7.14)	0.08
GA (Arg/Gln) + AA (Gln/Gln)	22 (44)	28 (56)	1.62 (0.7354-3.568)	0.1196

n: Number of samples, OR: Odds ratio, CI: Confidence interval, Arg: Arginine, Gln: Glutamine

Table 4: Allele frequencies for X-ray repair cross-complementing group 1 at codon 399 in cases and control

Variables	Controls (*n*=50) (%)	Cases (*n*=50) (%)	OR (95% CI)	*P*
G (Arg) allele frequency	73 (73)	58 (58)	1.958 (1.081-3.545)	0.01348
A (Gln) allele frequency	27 (27)	42 (42)		

n: Number of samples, OR: Odds ratio, CI: Confidence interval, Arg: Arginine, Gln: Glutamine

Table 5: Allele frequencies for X-ray repair cross-complementing group 1 at codon 399 in Tamol chewer's and control

Variables	Controls (*n*=50) (%)	Tamol chewers (*n*=50) (%)	OR (95% CI)	*P*
G (Arg) allele frequency	73 (73)	62 (62)	1.657 (0.911-3.014)	0.05011
A (Gln) allele frequency	27 (27)	38 (38)		

n: Number of samples, OR: Odds ratio, CI: Confidence interval, Arg: Arginine, Gln: Glutamine

Table 6: Allele frequencies for X-ray repair cross-complementing group 1 at codon 399 in Tamol chewer's and cases

Variables	Cases (*n*=50) (%)	Tamol chewers (*n*=50) (%)	OR (95% CI)	*P*
G (Arg) allele frequency	58	62 (62)	0.8464 (0.4804-1.491)	0.2839
A (Gln) allele frequency	42	38 (38)		

n: Number of samples, OR: Odds ratio, CI: Confidence interval, Arg: Arginine, Gln: Glutamine

statistically significant, as tested indicated by $P < 0.05$. Based on these results, the AA and GA genotype can be observed as risk factors that increase the risk by approximately three- and twofold, respectively. When comparisons were drawn between control and Tamol chewers, AA genotype was found to be associated with twofold (OR – 2.25, CI = 0.709–7.14) increase risk of developing oral cancer, while GA + AA genotype was associated with one and half fold (OR – 1.62, CI = 0.7354–3.568) risk of developing oral cancer. However, no significant association was observed with respect to either of the genotype [Table 3].

Tables 4-6 represents allele frequencies of XRCC1 399 codon. For XRCC1 Arg399Gln gene, the frequency of Arg allele in cases was 42.0%, whereas in controls and Tamol chewers, it was 27% and 38%, respectively, the frequency of Gln allele in cases was 58% and 73% in controls, while the frequency of Gln allele was 62% in Tamol chewers. Allele G was associated with twofold increase risk when control category was compared with cases (OR – 1.958, CI = 1.081–3.545; $P < 0.05$). When control was compared against Tamol chewers, G allele was to be a risk factor that increases the risk of developing the cancer by one and half fold (OR – 1.657, CI = 0.911–3.014; $P < 0.05$). However, no significance value was observed when cases are compared against Tamol chewers.

Discussion

This study investigates the association between DNA repair genes, i.e., XRCC1 Arg399Gln polymorphism and its susceptibility toward the development of OSCCs in Tamol chewers population in Assam, India. [14-16] Our finding suggests that Tamol pan chewers showed epithelial dysplasia which is often found in more than 90% in Tamol chewers population for more than 10 years. A significant positive correlation was found between the grading of epithelial dysplasia with duration of Tamol consumption in a year ($r = 0.267$, $P = 0.036$). These variables were positively related with grading of epithelial

Table 7: Literature review in X-ray repair cross-complementing group 1 polymorphism and cancer cases in Indian population

First author (year)	Country/state	Tumor site	SNPs studied	Sample size	Conclusive remarks
Raktim Borkokoty *et al*, 2020[13]	India (Assam)	OSCC	XRCC1 Arg399Gln XRCC-1 Arg194Trp XRCC1 Arg280His	152/190	Polymorphisms in XRCC1 gene is associated with OSCC pathogenesis in Kamrup Urban District, Assam, India, and is of prognostic significance. Also suggestive of the importance of base excision repair pathway alterations in OSCC pathogenesis
Vijay Parshuram Raturia *et al*., 2019[21]	India (Lucknow and Tamil nadu)	Laryngeal squamous cell carcinoma	XRCC-1 Arg194Trp	150/150	Habit of alcohol intake, tobacco smoking and chewing habits and XRCC-1 variant have statistically significant and thus loco-regionally increased the case of laryngeal squamous cell carcinomas
Sambit Swarup Nanda *et al*., 2018[8]	India (Lucknow)	HNC	XRCC1 Arg194Trp	101/00	Relation XRCC1 Arg194Trp polymorphism with head and neck squamous cell carcinoma patients who are treated with CCRT and found that genotypic variant of XRCC1 in HNSCC patients treated with CCRT
Serum anil singh *et al*., 2018[9]	Northeast India (Manipuri, Nagaland and Mizoram)	Nasopharyngeal carcinoma	XRCC1 Arg399Gln XRCC2 Arg188His	100/120	Studies showed that XRCC1 Arg399Gln polymorphic variant is a strong predisposing risk factor for NPC in the northeast Indian population
Goutham Hassan Venkatesh *et al*., 2013[20]	India (Karnataka)	HNC	XRCC1 Arg399Gln XRCC-1 Arg194Trp XRCC1 Arg280His	183/00	They report of gene variants of DNA repair gene and its associated with the risk of developing oral mucositis in HNC patients undergoing therapy (chemoradiotherapy or radiotherapy)
Kumar A *et al*., 2012[3]	North India	HNC	XRCC-1 Arg194Trp	278/278	In XRCC1 polymorphisms, Arg194Trp variants showed a reduced risk, with XRCC1-Arg280His variants, there was no association with HNC risk
Sobti RC *et al*., 2007[12]	Northern India	Esophageal cancer	XRCC1 Arg399Gln	120/160	In smokers, the XRCC1 Arg/Gln genotype was marginally and statistically non significantly associated with increased risk of esophageal cancer
Ramachandran S *et al*., 2006[10]	South Indian population (Travancore)	OSCC	XRCC1 Arg399Gln XRCC-1 Arg194Trp XRCC1 Arg280His	110/110	SNPs were investigated in XRCC1 Arg194Trp and found no associated with increased risk of developing oral cancer
Mousumi Majumder *et al*., 2005[16]	India (Calcutta)	OSCC	XRCC1 Arg399Gln	31/348	Genetic variant of XRCC1 (codon 280), increased the risk of both leukoplakia and cancer among smokers Therefore, XRCC1 can be a marker to identify which leucoplakias can progress to cancer

XRCC: X-ray repair cross-complementing gene, HNC: Head and neck cancer, NPC: Nasopharyngeal cancer, OSCC: Oral Squamous cell carcinoma, CCRT: Concurrent chemoradiation therapy, SNPs: single nucleotide polymorphisms, Arg: Arginine, Gln: Glutamine

dysplasia and this study concludes that epithelial dysplasia was common among chronic pan chewers. Waris and Nagi *et al*. also highlighted the presence of epithelial dysplasia in 57.7% of the habitual pan chewers. This study can also serve as a primary screening process before proceeding to any further expensive and complex test.[11]

Tamol pan, a mixture of raw betel nut, betel leaf, and lime, contains a large number of alkaloids and phenolic compounds. These compounds are considered risk factors of cancer which can form DNA adducts causing DNA damage, further lead mutation and genomic instability.[6] The XRCC1 is involved in BER pathway that plays a key role in protecting the genome from various risk factors. XRCC1s act in BER encoding scaffolding protein that assembles together the DNA repair complex.[11,20,21] The three commonly studied single-nucleotide polymorphisms in the XRCC1 gene in various population include Arg194Trp, Arg280His, and Arg399Gln [Table 7]. Our investigation demonstrates a significant association between XRCC Arg399Gln polymorphism in cases with control ($P = 0.05$). In case of Tamol chewers group, XRCC1 (Gln/Gln) was at twofold risk to develop cancer as compared with the control group (OR = 2.25, CI = 0.709–7.14), which are in accordance with a previous data report by Borkokoty *et al.* (2020) where they reported that the presence of XRCC1 399 variant genotype increased the risk of oral cavity cancer (OR = 1.566, $P = 0.049$) in smokers and alcoholics in the India population.[12] Adampourezare *et al*. (2017) also reported a positive association for 399G/G ($P < 0.001$, OR = 3.304, CI = 1.624–6.780) and 399 A/A ($P < 0.001$, OR = 14.143, CI = 1.861–296.277) genotypes with differentiated thyroid carcinoma and concluded that the XRCC1 399G > A genotype could be used as a useful molecular biomarker to predict genetic susceptibility for differentiated thyroid carcinoma in Iranian-Azeri populations.[14]

Various studies had done in association with XRCC1 vatients in Indian population in relation with tobacco related cancer cases and found a significant association.[15,16] Avci *et al*. investigated the association of XRCC1 Arg399Gln polymorphisms with the susceptibility of developing OSCC in the Turkish population. One hundred and eleven case and 148 controls were studied with PCR/RFLP approach. It was found that the XRCC1 Arg399Gln Gln/Gln genotype and Gln allele were risk factors for OSCC.[2] Another report from the Indian population by Singh and Ghosh (2016) found that XRCC1Arg399Gln polymorphic variant is a strong predisposing risk factor for nasopharyngeal cancer in the northeast Indian population.[9] In our studies, we have also found the positive relation between XRCC1Arg399Gln polymorphic variant and OSCCs, which can serve as a strong predisposing risk factor for OSCC in the northeast population.

In XRCC1 Arg399Gln polymorphism, the wild-type allele and the variant allele are arginine (Arg) and glutamine (Gln) at codon 399. Gln allele was found to be statistically associated with cancer cases compared to the Arg allele in both the groups by twofold and one and half fold. In cancer case, the risk was twofold with $P < 0.05$, similar result was also found in Tamol chewers group where the risk was one and half fold with $P < 0.05$ which may indicate the Tamol consumption the responsible risk factor. Allele frequencies for Arg and Gln observed in this study are found similar to other cancer studies. Cho *et al*. observed XRCC1 Arg399Gln polymorphism and risk of NPC among Taiwanese in China. Arg allele frequency in cases was 0.71, whereas in controls, it was 0.73. Gln allele frequency in cases was 0.29, and in controls, it was 0.27. In the Cantonese population, Arg allele frequency in nasopharyngeal carcinoma patients was 75.0%, whereas in controls, it was 74.0%. Gln allele frequency in cases was 25.0% and 26.0% in controls.[17] A similar study conducted in North Africa reported that Arg allele frequency in cases was 72.0% and 75.6% in controls, while the Gln allele frequency in cases was 28.0% and 24.4% in controls.[18] However, in the study of Li *et al*., a statistically significant difference was found between cases and controls.[19] Habit of other addictive sunbstances and patinets under therapy also have positive impact on XRCC-1 varients on Head and Neck cancer.[20,21] Our investigation suggested a significant association between cancer case or Tamol chewers and the allelic frequencies for XRCC1 399 codon. ($P = 0.01348$, $P = 0.05011$). These are encouraging results and suggest that this genetic variation can be the contributing factor for the progression of cancer and also suggest that the association of Tamol chewing habit may induce various type of DNA damage.

Conclusion

This study showed that the XRCC1 Arg399Gln polymorphic variants can serve as a predisposing risk factor for OSCC in Tamol chewers' population in Assam. Saliva can serve as an appropriate sample since it can be collected easily because it is a noninvasive procedure making sample collection easy. Our studies might have certain limitations with regard to sample size, so further studies with a larger sample size and also in other population are required before the clinical implication can be conducted.

Acknowledgment

The author acknowledges the financial support received from Assam Downtown University in the form of a seed grant and Dr. Bhubaneswar Borooah Cancer Institute and North East Cancer Hospital and Research Institute for providing research samples and technical help for undertaking the research work.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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