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# Short Communication
# Polymorphism of Two Genes and Oral Lesion Risk in North Indian Population

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## Abstract

**Background and Objective:** Inherited polymorphisms in carcinogen metabolizing and DNA repair genes may contribute to variations in carcinogen metabolism and DNA repair capacity and thus genetic susceptibility to cancer. This study was performed to evaluate the association between polymorphism of two genes namely XPC and GSTT1 and risk for development of oral lesion. **Materials and Methods:** In a hospital-based case-control study a total of 46 histopathologically proven leukoplakia, erythroplakia, lichen planus and oral submucous fibrosis patients and equal number of age, sex, ethinicity and habit matched healthy control subjects were taken. Subjects were genotyped for XPC Intron 9 (Ins/Del) polymorphisms with allele specific PCR method: Whereas, XPC Exon 16 (A>C) polymorphism were genotyped by PCR-RFLP. For genotyping of GSTT1, multiplex PCR was used. The association between DNA damage response gene polymorphisms and oral lesion occurring risk was assessed by calculating odds ratios (OR) with 95% confidence intervals (CI). The combined ORs were calculated under the dominant genetic model for each polymorphism. All the statistical analyses were two sided and conducted using the EPIinfo software. **Results:** Overall, a significant association of XPC poly AT Del/Del (D/D) genotype with increase risk of oral lesion was observed. Similarly AA genotypes for XPC exon 16 variant presented statistically significant $p<0.05$ increased risk of oral lesions. In contrary, AC and CC genotypes from the same polymorphism found to be protective for the development of oral lesion with OR-0.387 and 0.174, respectively for AC and CC genotype. The study demonstrates that absence of GSTT1 gene increases the risk of developing oral lesions. **Conclusion:** The present study supports that polymorphism in XPC gene may reduce the risk of developing oral lesions in North Indian population, whereas GSTT1 null genotype increases the risk of oral lesions.

Key words: Single nucleotide polymorphism, DNA repair gene, oral lesions, genotyping, oral submucous fibrosis

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Data Availability: All relevant data are within the paper and its supporting information files.

## INTRODUCTION

A precancerous lesion is an altered tissue associated with a significantly increased risk of cancer, thus if left untreated, these conditions may lead to cancer. These lesions often present as either white or red patches, known as leukoplakia and erythroplakia, lichen like patches known as lichen planus or loss of elasticity of intra-oral muscle fibers which lead to restricted opening of the mouth known as submucous fibrosis. Several molecular studies have evaluated the association of head and neck cancer with polymorphisms in DNA repair genes[1-4]. There are three major pathways involved in DNA damage repair, depending on the type of the damage, nucleotide excision repair (NER), base excision repair (BER) and double strand break (DSB).

Nucleotide excision repair (NER) is one of the most important pathways and eliminates a wide variety of DNA damage[5]. Mutations and single nucleotide polymorphisms (SNPs) in NER genes may contribute to deficient NER capacity and human cancer risk[6,7]. Among the many genes XPC play a major role in NER pathway, in damage recognition, open complex formation and repair protein complex formation. Previous studies have identified that polymorphism at XPC gene on chromosome 3, were associated with various types of cancer[8].

Many polymorphic variants in the XPC gene have been identified and two most common polymorphisms are Lys939Gln (XPC A33512C, rs2228001) an A>C transversion in exon 16 and a poly (AT) ins/del polymorphism (XPC PAT I/D) at intron 9 which have been associated with the risk of many human malignancies including cancers of bladder, breast, head and neck, lung, oesophagus, oral cavity and skin[9].

The protein encoded by GSTT1, glutathione S-transferase (GST) theta 1, is a member of a superfamily of proteins that catalyze the conjugation of reduced glutathione to a variety of electrophilic and hydrophobic compounds. The GSTs in human can be divided into five classes: alpha, mu, pi, theta and zeta. The theta class includes GSTT1, GSTT2 and GSTT2B. GSTT1 and GSTT2/GSTT2B may play a role in human carcinogenesis. The present study was aimed to identify any association of XPC and GSTT1 polymorphisms with the risk of development of oral lesion.

## MATERIALS AND METHODS

**Study group:** Forty six patients admitted with oral lesion problem (leukoplakia, erythroplakia, lichen planus and oral submucous fibrosis) in Saraswati Dental College and Hospitals, Lucknow from both rural and urban areas were enrolled for the study. The patients were of both sexes and within the age group of 21-50 years. The study was carried out between May 2016-March, 2017 after the approval of ethical committee of Saraswati Dental College, Lucknow

**Data collection management:** A pro forma directed history was taken and examination performed on admission. A detailed case history of the patients with emphasis on their habits (chewing Gutka and/or Tobacco and/or betel nut, smoking Cigarettes and/or Bidi, taking gulmanjan or alcohol) was taken and recorded on a standard pro forma along with through clinical examinations. Histopathologically OSMF patients were divided into grade 1 2 and 3 on the basis of staging system.

**Collection of blood sample:** About 5 mL of peripheral blood sample were collected from both the study and control group by venous arm puncture under aseptic precautions and transferred into a presterilized EDTA vials. The collected blood was then subjected to centrifugation (Remi cooling centrifuge (Model no.: NT2178GK), Remi elektrotechnik limited, instrument division, Vasai, India) at 3000 rpm for 10 min to segregate plasma and erythrocytes. Genomic DNA was extracted from the stored peripheral blood by salting out method. Consent was taken and care was carried out according to the guidelines laid down by the Ethical Committee.

**PCR based genotyping:** Subjects were genotyped for XPC Intron 9 (Ins/Del) polymorphisms with allele specific PCR method, whereas, XPC Exon 16 (A>C) polymorphism were genotyped by PCR-RFLP. For genotyping of GSTT1, multiplex PCR (Surecycler 8800, Agilent technologies, USA) was used.
All the gene polymorphisms were successfully genotyped in both the patients and control group. Precise quality control procedures were applied during the genotyping process. The PCR mix without DNA sample was used as a negative control to ensure contamination-free PCR product. Samples that failed to genotype were scored as missing and subjected to repetition. Ten percent of samples from both, patients and control groups were repeated to evaluate the quality of genotyping which showed 100% concordance. The PCR primer and anealing temperature used for genotyping are described elsewhere[10,11].

**Statistical analysis:** The $p<0.05$ was considered statistically significant. Chi-square ($\chi^2$) analysis was used to assess

deviation from Hardy-Weinberg's equilibrium and to compare the genotype frequency between patients and controls. All the statistical analyses were two sided and conducted using the EPIinfo software, version 7 (Centers for Disease Control and Prevention (CDC), United States).

## RESULTS

A total of forty-six patients having one or more indicators of oral lesions were included in the study. The breakup of this study group according to the four lesions was as follows: Leukoplakia-10, erythroplakia-05, lichen planus-09, oral submucous fibrosis-22. Control group consisted of the same numbers of normal healthy individuals. Both groups were genotyped for XPC polymorphisms with allele specific PCR and PCR RFLP method. For genotyping of GSTT1 multiplex PCR was used in a group of oral pre-cancerous patient samples and healthy controls.

Among the patients, thirty seven (80%) were male and 09 (20%) were female, with a mean age of 33.96±8.43. In the control group 32 (64%) were male and 18 (36%) were female, with a mean age of 32.23±8.98. All ninety-two samples were recruited for the study.

The results in Table 1 represent the genotype distribution and polymorphism in study and control groups both. In comparison to the D/D genotype of XPC poly AT polymorphism D/I and I/I genotypes were higher in control than in cases. It was also observed that the frequency of D/I genotype was significantly more in control than in cases, which indicates a protective association of I allele with the development of oral lesions. A similar protection was also observed with the C allele of XPC ex16 A>C polymorphism. The CC genotype was significantly $p<0.05$ higher in the control group as compared to the cases. The study further demonstrates that absence of GSTT1 gene increases the risk of oral lesions by 4 times because the frequency of GSTT1 null genotype was found to be significantly $p<0.05$ higher in cases than in control.

## DISCUSSION

The present study done on four oral lesion patients demonstrates the association of two genes, XPC and GSTT1 with prevalence of oral lesions. The results also suggest that the D/D genotype of XPC poly AT and AA genotype of XPC ex 16 are significantly responsible for increased risk of oral lesions. This preliminary case-control study provides a strong platform that absence of GSTT1 gene increases the risk of developing oral lesions.

The two most common polymorphisms, Lys939Gln (rs2228001) in exon 15 and a poly (AT) insertion/deletion polymorphism in intron 9, have been associated with an increased risk of many human malignancies[12]. In a study by Mittal and Mandal, variant genotype of XPC gene demonstrated significant association with prostate cancer as well as in bladder cancer[13]. In one more similar study by Mandal *et al.*[14], a positive correlation with NER gene, XPC PAT I/I genotype and prostate cancer risk was observed as well as in XPC exon 16 variant CC genotypes presented statistically significant risk of prostate cancer. Marin *et al.*[15] found that frequency of the PAT+/+ genotype was higher in the cases (20.6%) than in the controls and that the PAT+/+ subjects were at significantly increased risk for lung cancer.

Wang and co-workers have demonstrated significant association between oral premalignant lesions and polymorphism in nucleotide excession repair (NER) genes. Major NER genes included XPC, XPA, XPD etc. and polymorphisms in these may lead to changes in DNA repair capacity, leading to increased susceptibility to premalignant lesions as well as malignant lesions[16]. In another study, it was

Table 1: Genotype distributions of XPC Poly AT, Exon 16 (A>C) and GSTT1 +/- polymorphisms among the oral lesion cases and control

| | | Case | | Control | | | |
|---|---|---|---|---|---|---|---|
| Polymorphism | Genotype | N | (%) | N | (%) | OR(95%CI) | p-value |
| Poly AT (Deletion/Insertion) N(case/control): 39/39 | D/D | 25 | 64.0 | 14 | 35.80 | Ref | Ref |
| | D/I | 11 | 28.0 | 21 | 53.80 | 0.2933 (0.110-0.781) | 0.024 |
| | I/I | 3 | 7.70 | 4 | 10.25 | 0.420 (0.082-2.151) | 0.522 |
| XPC ex16 (A/C) N(case/control): 46/46 | AA | 31 | 67.3 | 18 | 39.15 | Ref | Ref |
| | AC | 12 | 26.0 | 18 | 39.15 | 0.387 (0.152-0.984) | 0.074 |
| | CC | 3 | 6.52 | 10 | 21.70 | 0.174 (0.042-0.717) | 0.022 |
| GSTT1 (Null/ Non null) N(case/control): 44/44 | + | 28 | 63.6 | 39 | 88.60 | Ref | Ref |
| | - | 16 | 16.0 | 5 | 11.30 | 4.457 (1.461-13.597) | 0.012 |

All forty-six samples were included for polymorphism study, however there was PCR failure seen with seven and two samples in poly AT and GSTT1, respectively. Same number of control was used to maintain equilibrium. Statistically significant association is represented in bold. N: Number of sample, OR: Odds ratio, CI: Confidence interval, Ref: Reference value taken for respective polymorphism

shown that the XPC PAT and Ala499Val polymorphisms may be associated with an increased risk of head and neck cancer[17]. Glutathione S-transferase theta 1 (GSTT1) has been shown to be a strong indicator of human carcinogenesis. The present study also correlated absence of GSTT1 with higher oral lesion risk. GSTT1 deletion polymorphism is known to abolish enzyme activities and modulate lung cancer risk[18]. In a similar study designed to investigate the GSTT1 null polymorphism and the risk of oral leukoplakia in individuals with tobacco smoking habits in Brazilian population, it was concluded that GSTT1 null genotype may increase the risk of developing oral leukoplakia because the frequency of the GSTT1 null genotype in the group with oral leukoplakia (48.6%) was statistically different from the controls (27.8%)[19].

In a study by D'mello *et al.*, fifty-two cases were evaluated using buccal mucosal scrapes of tobacco habituates for 8 or more years, without clinically evident lesion (Group I) and from mucosa of tobacco habituates with clinically evident and histopathologically confirmed OSCC (Group II). 90.66% of subjects had GSTT1 null genotype in Group I subjects. In Group II, subjects with both clinically and histopathologically diagnosed oral cancer, about 76.96% had GSTT1 null genotype[20]. Zhang *et al.*[21], have also demonstrated similar results where an association between head and neck squamous cell carcinoma and GSTT1 null genotype was found in South American population. On the other hand, contrary to this study, the GSTT1 null genotype was not associated with increased cervical cancer risk in the work done by Economopoulos *et al.*[22]. In an extensive meta-analysis in Chinese population to check the association of GSTT1 polymorphism with lung cancer risk, found an increased lung cancer risk among subjects carrying GSTT1 null genotype compared with those carrying present genotype[23].

## CONCLUSION

The results based on the study of three polymorphisms in two genes involved in DNA repair, show that the variant allele in exon 16 of the XPC gene is associated with a decreased risk of oral lesion, although the functional explanation for such an association remains undetermined. In addition, we report an association of increased oral lesion risk with the GSTT1 null genotype. Therefore, it becomes significant to study inherited polymorphisms of DNA repair genes in a big population.

## SIGNIFICANCE STATEMENTS

This study discovers the role of XPC and GSTT1 genes in the development of oral lesions that can be beneficial for the population. This study will help the researcher to uncover the critical areas of genetic susceptibility of oral lesions that many researchers were not able to explore. Thus a new theory on genetic susceptibility for oral lesions may be arrived at.

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