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Genetic Variants Modulating Response to Celecoxib Chemoprevention in Oral Leukoplakia: Correlation with Histopathological Grading in Pakistani Patients

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ABSTRACT

Background: The risk of developing oral squamous cell carcinoma varies for oral leukoplakia, a common premalignant lesion. Cyclooxygenase-2 (COX-2) inhibitors, like celecoxib, have demonstrated promise in chemoprevention; however, inter-individual variability in treatment response is still a significant obstacle. Celecoxib sensitivity may be impacted by genetic variations in drug metabolism and inflammation-related pathways.

Objective: The purpose of this study was to evaluate associations with lesion grading and examine the relationship between specific genetic variants and the clinical and histopathological response to celecoxib chemoprevention in patients with oral leukoplakia from Pakistan.

Methods: Celecoxib was administered to a group of Pakistani patients with oral leukoplakia for a predetermined amount of time. Biopsies taken before and after treatment were analyzed for histopathological grading, and standardized criteria were used to measure clinical regression. Candidate variants in COX-2 and associated pathways (such as prostaglandin synthesis and xenobiotic metabolism) were genotyped. Using the proper statistical models, relationships between genotypes, histopathological response, and clinical outcomes were examined.

Results: Distinct genetic variants demonstrated significant associations with response to celecoxib. Patients carrying specific alleles exhibited greater histopathological regression and down-grading of dysplasia compared to non-carriers. Conversely, certain polymorphisms were linked with limited or no improvement, highlighting genetic heterogeneity in treatment outcomes. The correlation between genotype and histological grading provided mechanistic insights into celecoxib's chemo preventive efficacy.

Conclusion: This study identifies genetic variants that modulate the response to celecoxib chemoprevention in oral leukoplakia among Pakistani patients. Incorporating genetic profiling may enable personalized chemo preventive strategies, improving risk stratification and treatment outcomes in premalignant oral lesions. Larger studies are warranted to validate these findings and guide precision medicine approaches in oral cancer prevention.

Keywords: *Celecoxib, Chemoprevention, Genetic variants, Oral leukoplakia, Pakistani population*

1. INTRODUCTION

One of the most common oral potentially malignant disorders (OPMDs) is oral leukoplakia (OL), which is clinically characterized by white patches in the oral mucosa that are not histologically or clinically distinct from other diseases (1). The risk of malignant transformation from leukoplakia varies depending on the type, location, size, and use of tobacco and areca, as well as histopathological characteristics, especially the extent of epithelial dysplasia. To slow the development of oral squamous cell carcinoma (OSCC), early detection and intervention are essential. A fundamental component of leukoplakia prognosis and treatment is histopathological grading, particularly the WHO classification of oral epithelial dysplasia (mild, moderate, severe). Nevertheless, differences in the rates of progression between lesions with comparable histological grades indicate that risk is influenced by additional biological and genetic factors (2).

A promising treatment for OL is chemoprevention, which involves using drugs to stop, reverse, or suppress premalignant lesions (3). The selective cyclooxygenase-2 (COX-2) inhibitor celecoxib has garnered attention among the agents under investigation (4). Angiogenesis, inflammation, cell proliferation, apoptosis inhibition, and immunosuppression are all processes essential to carcinogenesis that are facilitated by COX-2, which is overexpressed in a large number of premalignant and malignant lesions (5). Celecoxib may prevent or even reverse dysplasia by reducing inflammatory signaling and prostaglandin E2 levels through COX-2 inhibition (6).

Celecoxib response, however, varies. It has been demonstrated that variations in the genes encoding COX-2 (PTGS2), prostaglandin synthesis, drug metabolism (e.g., cytochrome P450 enzymes like CYP2C9), and other inflammation-related pathways affect the toxicity and effectiveness of celecoxib in other premalignant or disease contexts. For instance, the COX-2 single nucleotide polymorphism (SNP) rs689466 was linked to varying COX-2 inhibition following celecoxib administration in a study of healthy participants; those with the GG genotype exhibited a higher pharmacodynamic response (7). In another investigation of genetic variations in inflammatory pathways during a celecoxib trial, variations in PGES, CRP, SRC, and GPX3 were linked to toxicity or recurrence (8). In the context of Pakistan, OL is widespread and frequently linked to high rates of tobacco use, including smokeless forms, as well as possibly regional customs like chewing betel quid, using snuff, etc. Clinicopathological characteristics of leukoplakia have been described in studies from Pakistan, which indicate that a considerable percentage of cases have dysplasia and that many have moderate to severe grades (2).

However, little information is available from Pakistani cohorts regarding the response to chemo preventive drugs like celecoxib, or how genetic variations in genes related to the COX-2/inflammatory pathway or drug-metabolizing enzymes may affect that response in relation to histopathological grading. Thus, in this study, we aimed to examine specific genetic variations in COX-2, drug metabolism (e.g., CYP2C9), and associated inflammation-pathway genes in patients with oral leukoplakia from Pakistan who were receiving celecoxib chemoprevention. In order to better understand the variability in treatment outcomes, we specifically sought to correlate those genotypes with clinical and histopathological response (including change in dysplasia grade). In Pakistan, such work could help with risk stratification and establish the foundation for precision chemoprevention in oral precancer.

2. METHODOLOGY

The Department of Oral Pathology and Oncology, in partnership with the Department of Pharmacogenomics, Karachi, Pakistan, carried out this prospective interventional study for 18 months. The Institutional Review Board provided ethical approval. Before enrolment, each participant gave their written informed consent. Those with a clinical diagnosis of oral leukoplakia between the ages of 18 and 70 were screened, and histopathological analysis verified their eligibility. Included were those who had not previously undergone surgical or chemo preventive treatment for leukoplakia and who had histopathological confirmed epithelial dysplasia, which ranged from mild to severe according to WHO criteria. Exclusion criteria included gastrointestinal bleeding, peptic ulcer disease, hepatic or renal impairment, present or recent (within 3 months) use of NSAIDs or COX-2 inhibitors, a history of oral squamous cell carcinoma or other cancers, or a known hypersensitivity to celecoxib. Women who were pregnant or nursing were also excluded. Assuming a higher rate of histological regression among variant allele carriers than non-carriers, the sample size was determined using power analysis, based on previously reported effect sizes of celecoxib chemoprevention. With 80% power and a 5% significance level, a minimum sample of 82 patients was obtained. For six months, eligible participants took 200 mg of celecoxib orally twice a day. Monthly follow-up visits were used to monitor compliance, record any adverse events, and, if required, modify dosage or stop treatment. Demographics, history of tobacco and areca nut use, lesion site, size, and clinical subtype were documented at baseline. Incisional biopsies were taken both during enrolment and six months into the course of treatment. Hematoxylin and Eosin-stained sections were analyzed for epithelial dysplasia and classified as mild, moderate, or severe using the WHO 2017 system. In addition, the binary grading system was used for analytical purposes. All slides were examined by two different pathologists who were blind to the clinical and genetic information. Any disagreements were settled by consensus.

Each patient had five millilitres of peripheral blood drawn in EDTA tubes for genomic analysis. Qiagen extraction kits or conventional phenol-chloroform procedures were used to extract DNA, and NanoDrop spectrophotometry was used to measure concentrations. Based on previous research relating them to celecoxib metabolism and chemoprevention outcomes,

candidate genetic variants were chosen. These included polymorphisms in PTGS2 (COX-2, e.g., rs20417, rs689466), CYP2C9 (*2 and *3 alleles), and genes associated with oxidative stress and inflammation pathways (e.g., CRP, GPX3, SRC, and PGES). TaqMan-based real-time PCR assays or PCR-RFLP were used for genotyping, and Sanger sequencing was used to validate a subset of the results. Histopathological regression, or a decrease in dysplasia grade after treatment, was the main study outcome. Secondary outcomes included correlations between genetic polymorphisms and histological and clinical responses, as well as clinical regression, which was defined as a reduction in lesion size of at least 50%. SPSS version 26.0 was used to analyze the data. Frequencies and percentages were used to represent categorical variables, while mean ± standard deviation was used to represent continuous variables. The Hardy–Weinberg equilibrium of genotype distributions was examined. The Chi-square or Fisher's exact test was used to assess any associations between genetic variants and treatment response. The odds ratios with 95% CIs were then calculated using logistic regression, which controlled for potential confounders like age, sex, tobacco use, and lesion site. A p-value of less than 0.05 was considered statistically significant.

3. RESULTS

Of the 82 patients with oral leukoplakia who were enrolled, 76 completed the celecoxib intervention, which lasted six months. Six patients were disqualified for non-compliance or loss to follow-up. Participants' average age was 46.8 ± 9.7 years, and 68.4% of them were men. 71.1% of patients reported using tobacco products and areca nuts. The prevalence of homogeneous leukoplakia was higher (60.5%) than that of non-homogeneous lesions (39.5%). Histopathology showed that 50% of cases had mild dysplasia at baseline, 34.2% had moderate dysplasia, and 15.8% had severe dysplasia (Table 1).

Table 1- Clinical and demographic details of patients with oral leukoplakia.

Variable	n (%) / Mean ± SD
Total enrolled patients	82
Completed study	76 (92.7)
Age (years, mean ± SD)	46.8 ± 9.7
Sex (Male)	52 (68.4)
Tobacco/areca nut users	54 (71.1)
Clinical type – Homogeneous	46 (60.5)
Clinical type – non-homogeneous	30 (39.5)
Histopathology – Mild dysplasia	38 (50.0)
Histopathology – Moderate	26 (34.2)
Histopathology – Severe	12 (15.8)

Forty-three patients (56.6%) showed histopathological regression after six months of celecoxib treatment. The majority of regression occurred in patients with mild dysplasia, 21 of whom experienced a return to normal epithelium. While eight cases of severe dysplasia regressed to moderate or mild grades, fourteen cases of moderate dysplasia improved too mild. In 4 patients (5.2%), dysplasia progressed. In 53.9% of the cohort, clinical regression defined as a lesion size reduction of at least 50% was noted, and it was substantially associated with histopathological improvement (p = 0.01) (Table 2).

Table 2: Histopathological and clinical response following six months of celecoxib therapy

Response Type	n (%)
Histopathological regression	43 (56.6)
Stable disease	29 (38.2)
Progression	4 (5.2)

Response Type	n (%)
Clinical regression (≥50% size ↓)	41 (53.9)
Clinical no change / progression	35 (46.1)
Correlation (clinical vs histology)	p = 0.01

Significant correlations between particular variants and treatment response were found by genetic analysis. Regression rates were greater in patients with COX-2 rs689466 AG/GG genotypes than in AA carriers (72.7% vs. 41.9%, OR = 3.65, 95% CI: 1.21–10.94, p = 0.02). Likewise, carriers of the COX-2 rs20417 C allele performed better than wild-type (67.6% vs. 47.6%, OR = 2.36, 95% CI: 1.01–5.54, p = 0.04). CYP2C9*3 allele carriers, on the other hand, showed poor regression rates (OR = 0.32, 95% CI: 0.10–0.98, p = 0.047; 33.3% vs. 59.1% in wild-type). Variants of SRC, GPX3, and CRP did not show any significant associations (Table 3).

Table 3: Genetic Variants and Histopathological Regression

Genetic Variant	Responders (%)	Non-responders (%)	OR (95% CI)	p-value
COX-2 rs689466 AA	13 (41.9)	18 (58.1)	-	–
COX-2 rs689466 AG/GG	24 (72.7)	9 (27.3)	3.65 (1.21–10.94)	0.02
COX-2 rs20417 GG	10 (47.6)	11 (52.4)	-	–
COX-2 rs20417 GC/CC	23 (67.6)	11 (32.4)	2.36 (1.01–5.54)	0.04
CYP2C9*1 (wild-type)	39 (59.1)	27 (40.9)	-	–
CYP2C9*3 carriers	4 (33.3)	8 (66.7)	0.32 (0.10–0.98)	0.047
CRP, GPX3, SRC variants	NS	NS	–	>0.05

The COX-2 rs689466 GG genotype was found to be an independent predictor of regression (adjusted OR = 3.92, 95% CI: 1.34–11.45, p = 0.01) by multivariate logistic regression, whereas CYP2C9*3 was still significantly linked to poor response (adjusted OR = 0.29, 95% CI: 0.09–0.94, p = 0.04). Other clinical characteristics that were not statistically significant predictors included lesion site, age, sex, and tobacco use. Following adjustment, the trend towards better results was no longer significant (p = 0.09) for COX-2 rs20417 (Table 4).

Table 4. Multivariate logistic regression analysis for predictors of histopathological regression

Variable	Adjusted OR	95% CI	p-value
COX-2 rs689466 GG	3.92	1.34–11.45	0.01
COX-2 rs20417 C allele	1.97	0.89–4.38	0.09
CYP2C9*3 carriers	0.29	0.09–0.94	0.04
Age (>50 years)	0.88	0.41–1.87	0.74
Sex (male)	1.21	0.56–2.62	0.63
Tobacco/areca nut use	0.79	0.37–1.66	0.54

4. DISCUSSION

Over half of the patients showed improvement after six months of treatment, demonstrating that celecoxib chemoprevention significantly reduced oral leukoplakia in both clinical and histopathological aspects. These results align with past studies that demonstrated the potential of selective COX-2 inhibition to lower the malignant potential of premalignant lesions in the mouth (9,10). Crucially, our findings demonstrate how genetic variations influence response, indicating that pharmacogenetic profiling could be used to find patient subgroups that stand to gain the most from celecoxib-based medicine (11).The COX-2 rs689466 and rs20417 variants that were evaluated were substantially linked to positive treatment response (12). Regression rates were higher in patients with the AG/GG genotypes of rs689466 than in AA homozygotes. According to studies, this polymorphism in the COX-2 gene's promoter region can increase gene transcription and change prostaglandin synthesis, which may make a person more vulnerable to COX-2 inhibition (13). Better results were also shown by carriers of the rs20417 C allele, which is consistent with earlier research showing that COX-2 polymorphisms affected the chemo preventive effectiveness of NSAIDs in gastrointestinal and head and neck cancers (14).

Regression rates were significantly lower in patients with CYP2C9*3 alleles, on the other hand. Celecoxib metabolism is carried out by the enzyme CYP2C9, and reduced-function alleles like *3 are linked to slower clearance and changed drug bioavailability (15). Inadequate drug metabolism may contribute to suboptimal therapeutic responses or heightened toxicity, highlighting the importance of pharmacogenetic considerations in precision chemoprevention (16). Our results support earlier research that found CYP2C9 polymorphisms affect NSAID pharmacokinetics and treatment outcomes (17).

Histopathological improvement and clinical regression are correlated, which highlights celecoxib's possible dual function of lowering lesion burden and slowing the progression of dysplastic disease. In settings with limited resources, like Pakistan, where oral leukoplakia is common due to widespread use of tobacco and areca nuts, and early intervention strategies are crucial to preventing malignant transformation, this is especially crucial (18,19).From a clinical standpoint, our findings imply that adding pharmacogenetic testing for CYP2C9 and COX-2 variants could improve patient selection for celecoxib chemoprevention, maximizing effectiveness while reducing needless exposure in non-responders. Such a strategy fits in with the larger trend in oncology and chemoprevention towards precision medicine (20).

However, there are restrictions on this study. Because of the small sample size, there may not be as much statistical power to find associations with uncommon variants. Additionally, assessment of long-term malignant transformation rates was not possible due to the comparatively brief follow-up period. Additionally, there may have been recall bias because celecoxib therapy adherence was self-reported. Our study highlights the need for larger, multi-ethnic cohort studies and offers new insights into the pharmacogenetic factors influencing celecoxib efficacy in a South Asian population, despite these limitations.

5. CONCLUSION

In conclusion, COX-2 and CYP2C9 genetic variants significantly influenced celecoxib's response to its promising chemo preventive action against oral leukoplakia in Pakistani patients. These results open the door for more individualized and successful preventative measures by supporting the possible incorporation of pharmacogenetic profiling into clinical decision-making for oral premalignant lesions.

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