Journal of Oral Biology and Craniofacial Research 14 (2024) 169–174

Contents lists available at ScienceDirect

Journal of Oral Biology and Craniofacial Research

journal homepage: www.elsevier.com/locate/jobcr

Evaluation of CYP1B1, oxidative stress and phase II detoxification enzyme status in oral cancer progression model

Anirudha R. Bartake [a,b], Sachin Sarode [b,c,*], Sangeeta Palaskar [a], Amit Girme [d], Gargi Sarode [a], Samruddhi Kamble [a], Bindiya Narang [a], Pradnya Bhale [a]

[a] Department of Oral Pathology and Microbiology, Sinhgad Dental College and Hospital, Pune, Maharashtra, India
[b] Department of Oral Pathology and Microbiology, Dr. D.Y. Patil Dental College & Hospital, Dr. D.Y. Patil Vidyapeeth, Pune, Maharashtra, India
[c] Dr. D. Y. Patil Institute of Pharmaceutical Sciences and Research, Dr. D.Y. Patil Unitech Society, Pimpri Pune, 411018, India
[d] Department of Surgery, Dr. DY Patil Medical College & Research Centre, Dr. D.Y. Patil Vidyapeeth, Pune, Maharashtra, India

ARTICLE INFO

Keywords:
CYP1B1
Malonaldehyde
Oral squamous cell carcinoma
Oral epithelial dysplasia
Tobacco

ABSTRACT

Background: Tobacco is one of the main etiological factors for oral squamous cell carcinoma (OSCC) and oral potentially malignant disorders (OPMD). CYP1B1 is an enzyme which plays a major role in the phase I detoxification of tobacco, the byproducts of which are subsequently detoxified by phase II enzymes Glutathione S Transferase (GST). We attempted to evaluate the L432V polymorphism and tissue expression of CYP1B1, along with the oxidant-antioxidant status in OSCC progression model.

Method: ology: Tissue biopsies and blood samples were collected from the subjects; L432V polymorphism was evaluated by TaqMan RT-PCR, immunohistochemistry was performed on the tissue sample using CYP1B1 polyclonal primary antibody and Allred quick scoring system was used to evaluate the stained slides. Malonaldehyde (MDA) and GST activity were measured spectrophotometrically to assess oxidative-antioxidative status.

Results: When the L432V polymorphism was analyzed, it was observed that in oral epithelial dysplasia (OED) and OSCC, CG was more common than GG genotype. Highest mean Allred score was observed in tobacco users (6.27), highest GST activity was seen in oral epithelial dysplasia (5.006 U/ml) and highest MDA activity was observed in OSCC (1553.94 nm/ml).

Conclusion: Tobacco users with CG and GG genotypes are at equal risk of developing oral epithelial dysplasia or OSCC and L432V polymorphism does not appear to increase the risk of malignant transformation in oral epithelial dysplasia. Moreover, tobacco users with GG genotype and tissue expression of CYP1B1 may be at a greater risk of oxidative damage.

Funding source

The author received no specific funding for this work.

1. Introduction

Oral Squamous Cell Carcinoma (OSCC) is a crippling disease with a worldwide prevalence, more so in India and the subcontinent region. Globally, it affects approximately 300,000 subjects annually and accounts for 2%–4% of all the cancer cases.[1,2] The five-year survival rate for OSCC has been reported ranging from 30 to 90% depending on whether or not it is associated with metastasis and recurrences.[3] Many of the OSCC cases develop from oral potentially malignant disorders (OPMDs), the world-wide prevalence of these PMDs ranging from 1 to 5%.[4,5] Histopathologically OPMD's generally present with epithelial dysplasia.[5,6] Oral epithelial dysplasia (OED) is characterized by cytological and architectural alterations reflecting the loss of normal maturation and stratification pattern of epithelium, the malignant transformation rate for OED ranges from 8% to 24%.[7] Tobacco is one of the main etiological factors responsible for OSCC and OPMDs, it is used in various forms such as betel quid, tobacco with lime, bidi, hookah, smoking etc. Tobacco is known to promote carcinogenesis by multiple

* Corresponding author.Department of Oral Pathology and Microbiology, Dr. D.Y. Patil Dental College and Hospital, Dr. D.Y. Patil Vidyapeeth Sant-Tukaram Nagar, Pimpri, Pune: 18, Maharashtra, India.
E-mail address: drsachinsarode@gmail.com (S. Sarode).

https://doi.org/10.1016/j.jobcr.2024.02.001
Received 6 November 2023; Received in revised form 22 December 2023; Accepted 5 February 2024

pathways including epigenetic alterations in oral epithelial cells (abnormal expression of p53, p16, GLUT-1, DAPK), inhibiting multiple systemic immune function (IL-2, IL-4, Fas, FasL) and causing alterations in oxidative stress which is ultimately responsible for DNA damage.[8] Tobacco contains more than 500 different polycyclic aromatic hydrocarbons (PAHs) most of them are carcinogenic and the most potent among them being benzo [*a*]pyrene (BaP).[9]

The PAHs in tobacco including BaPs are metabolized by phase I enzymes such CYP1A1 and CYP1B1, which are also involved in drug metabolism and synthesis of lipids, cholesterol, and steroids. CYP1B1 is induced by the aromatic hydrocarbon receptor (Ahr) and it plays a pivotal role in the phase I metabolism of tobacco and its byproducts, giving rise to reactive metabolites. These metabolites are then further conjugated by phase II detoxification enzymes like glycine *N*-acyltransferase (GLYAT), glutathione *S*-transferases (GST) and methyltransferases.[10,11]

The phase II enzyme glutathione-*S*-transferase (GST) is a member of a multigene family that protects the cells from chemical stress and carcinogens by combining them with cellular glutathione (GSH) and removing them via GSH conjugate-recognizing transport.[12] Increasing evidence shows a connection between impaired detoxification and cancer development. Additionally, smoking exposes the oral epithelium to high levels of toxic reactive oxygen species like hydrogen peroxide (H_2O_2) and hydroxyl radicals. These free radicals' target the polyunsaturated fatty acids in membrane lipids causing lipid peroxidation, resulting in the formation of aldehydes which play a vital role in the development of various diseases including cancer by altering the tonicity, permeability, rigidity and integrity of cell membrane proteins. Malonaldehyde (MDA) is one of the reactive aldehydes produced as a result of lipid peroxidation.[13] Thus, the oxidative stress and the anti-oxidant status can be effectively evaluated by using MDA and GST markers respectively.

Overexpression of CYP1B1 has been reported in a wide array of tumours and it has also been implicated in tumor progression.[14] The human CYP1B1 gene consists of three exons and it has been mapped to chromosomal region 2p21–22. Single nucleotide polymorphisms (SNPs) have been reported in CYP1B1 the most common amongst them being Ala119Ser (A119S), Leu432Val (L432V), Arg48 Gl y (R48G), and Asn453Ser (N453S).[15] Various carcinomas including breast, lung and prostrate have frequently reported Leu432Val (rs1056836) polymorphism, it has also been reported to be strongly associated with head-and-neck cancer subjects' survival.[16]

This study attempted to evaluate the genetic polymorphism and tissue expression of CYP1B1 along with the oxidant-antioxidant status in oral cancer progression model. The L4322V polymorphism and immunohistochemical expression of CYP1B1 were correlated with the GST and MDA activity in OPMD's and OSCC.

2. Material and methods

2.1. Study design

The present study was a case-control study designed to analyze the genotypic and immunohistochemical expression of CYP1B1, along with oxidant-antioxidant status in oral cancer progression model. The study was approved by the institutional ethics committee of xxxxxxxxxxxxx (Ref No. DYPV/EC/98/18).

2.2. Setting and participants

A total of 88 subjects divided in 4 groups were included in the study. Group 1 (22) included apparently healthy controls without history of tobacco usage (CWOT), group II (22) were apparently healthy controls with history of tobacco usage (CWT), group III (22) were histopathologically diagnosed patients of oral epithelial dysplasia (OED) with history of tobacco usage and group IV (22) were histopathologically diagnosed patients of oral squamous cell carcinoma (OSCC) with history of tobacco usage. Subjects with any disease which can potentially affect the oxidant-antioxidant status i.e. cardiovascular diseases, diabetics on medication, hypertensives on medication, liver diseases, renal failures along with subjects with history of other sarcomas or carcinomas were excluded from the study.

2.3. Sample collection

After obtaining prior informed consent, blood samples of all the four study group subjects were collected in plain vial (2 ml for serum separation) and EDTA vial (4 ml for DNA isolation). Tissue biopsies were obtained from patients with oral potentially malignant disorders and squamous cell carcinoma. Tissue samples from apparently healthy subjects were obtained during routine minor surgical procedures like disimpaction, flap surgery etc. The collected venous blood was used for DNA extraction and serum separation which was subsequently utilized for glutathione s transferase (GST) and malonaldehyde (MDA) analysis.

2.4. Genotyping

Genomic DNA extraction from EDTA blood samples was done using Macherey Nagel NucleoSpin® tissue Kit. For performing CYP1B1 genotyping, rs1056836 was procured from ThermoFisher Scientific with assay ID C__3099976_30 and the Premix Taq™ DNA polymerase was procured from TaKara®. PCR setup was done using the reagents and aliquoted in a 96 well plate, gDNA was then added to the plate. The amplification was done using Real Time PCR and the genotype was recorded based on the amplification graph (Fig. 1).

2.5. Immunohistochemistry methodology

The staining was carried out using Poly HRP Path-InSitu kit. The primary antibody used was CYP1B1 rabbit polyclonal (50 µl, Sino Biological) and the procedure followed was as per the instructions of the manufacturer. Sections from all the four groups were dewaxed, rehydrated and washed in cold water and heat-based antigen retrieval in 0.01 M citrate buffer, pH 6 for 20 min was done, this was followed by immuno-staining with the *anti*-CYP1B1 antibody. Sections of breast cancer were used as the positive control and normal esophageal tissue was used as negative control.

2.6. Immunohistochemistry evaluation

The stained sections were then observed under high magnification (40x), with computer assisted image analyzer (LAS Version-4.1) for cytoplasmic staining and Allred (Quick) scoring system was used to analyze immunohistochemical expression.[17–19] In the Allred scoring system score A depicts the proportion of positively stained cells out of hundred it ranges from 0 to 5 (0 = 0%, 1 = 1 %, 2 = 2–10%, 3 = 11–33%, 4 = 34–66%, 5 = 67–100%) and score B depicts the intensity of the stain which ranges from 0 to 3 (0 = Negative, 1 = Weak, 2 = Intermediate and 3 = Strong). The combination of the percentage of positively stained cells and the staining intensity (A + B) ranges from 0 to 8, wherein 0-1 score is considered to be negative, 2–3 is weak, 4–6 is intermediate and 7–8 is considered to be strong positivity and as such a particular score can be assigned to the slide.

2.7. GST analysis

GST analysis was carried out by using the Elabscience Biotechnology kit. The instructions followed were as per the manufacturer. The estimation was based on the reaction between reduced glutathione and the 1-chloro-2,4-dinitrobenzene (CDNB). The absorbance was measured at 340 nm using a spectrophotometer and the GST activity was calculated.[20]

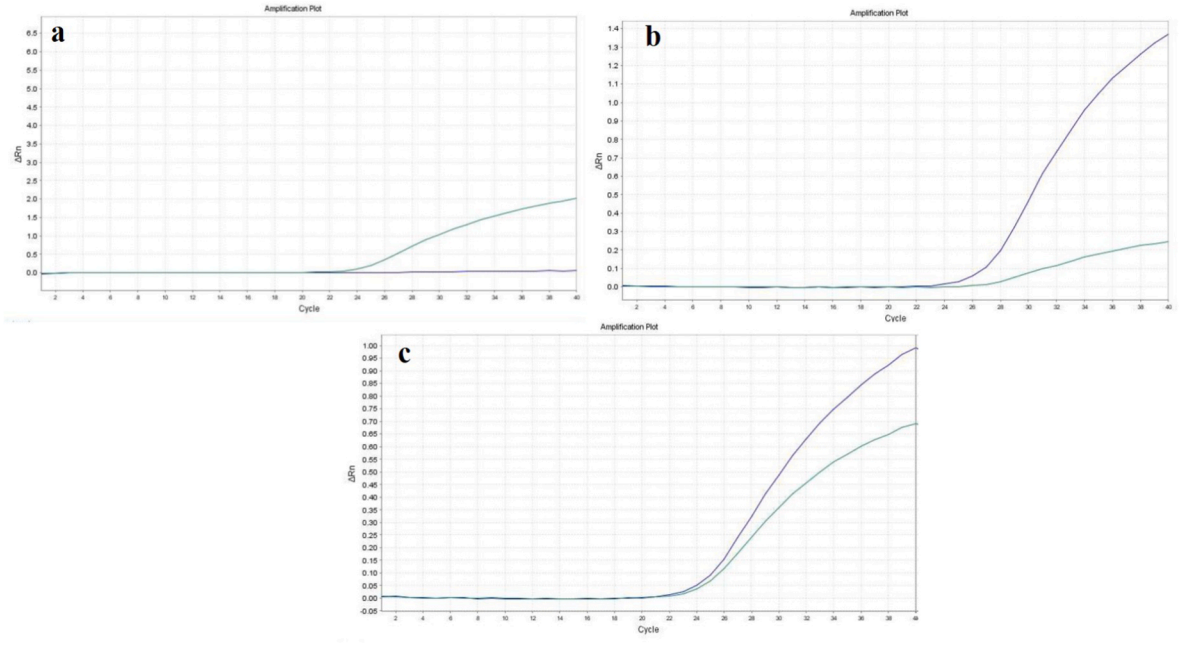

Fig. 1. (a) Amplification graph with CC (b) Amplification graph with GG (c) Amplification graph with CG.

2.8. MDA analysis

The thiobarbituric acid-reacting substances (TBARS) assay was used to determine the MDA levels. In this method, MDA and Thio Barbituric Acid (TBA) react thermally to produce a trimethine-colored substance which can be measured spectrophotometrically at 532 nm. Then, using the MDA-TBA complex's molar extinction coefficient, the MDA concentration was determined.[21]

2.9. Statistical analysis

Microsoft excel Version 13 was used to enter the data and IBM statistical packages for social sciences (SPSS) or windows, version 21.0 (IBM Corp., Armonk, NY) was used to perform statistical analysis. After obtaining the mean and standard deviation for continuous data, the Chi square test of proportion was used to evaluate differences in proportion, and Post Hoc Tukey's was used to compare the data between groups. Odds ratio calculations were made in order to determine the relationship between the genotype and study groups. $P < 0.05$ was regarded as statistically significant, and all statistical tests were run with the confidence interval set at 95%.

3. Results

3.1. Demographic details

The mean age distribution of study participants in group I (CWOT), group II (CWT), group III (OED) and group IV (OSCC) was 37.63 ± 10.63, 43.27 ± 9.71, 49.68 ± 11.90 and 54.68 ± 12.15 respectively. The gender distribution (n = 22) in group I was 8 (36.4%) male and 14 (63.6%) female, in group II it was 14 (63.6%) male and 8 (36.4%) female. In group III there were 19 (86.4%) male and 3 (13.6%) female and in group IV there were 16 (72.7%) male and 6 (27.3%) female.

3.2. CYP1B1 L432V polymorphism

When the L432V polymorphism was compared between the groups it was observed that CG was most common in CWOT, OED and OSCC, while GG was most common in CWT though the difference in proportion was not statistically significant ($p > 0.05$). The genotype distribution has been represented in Table 1. When the odds ratio was calculated between CWT & OED and CWT & OSCC it was observed that the CG genotype was associated 4.08 times in OED and 5.95 times in OSCC ($P < 0.05$). Similarly, the odds ratio of GG genotype between CWT & OED and CWT & OSCC was 0.25 times in OED and 0.21 times in OSCC respectively. ($P < 0.05$). No such statistically significant results were observed between other groups.

3.3. Association of CYP1B1 polymorphism and IHC expression

When comparison of the IHC Allred staining score and CYP1B1 polymorphism done between the study groups it was observed that the GG genotype had the highest mean Allred score in CWT, OED and OSCC as shown in Table 2. There was no statistically significant difference in the mean Allred staining score and genotypes between the four groups ($p > 0.05$).

3.4. IHC analysis

The evaluation of Allred score for IHC staining depicted that the mean score was highest for CWT and least for CWOT (**Table 3**). This difference in mean was statistically significant ($p < 0.05$). Furthermore, it was also observed that the difference in mean between CWOT and OED as well CWOT and OSCC was also statistically significant ($p < 0.05$) (**Table 4**) (**Fig. 2**).

Table 1
Distribution of L432V SNP among groups.

Study Group	CC	CG	GG	Total	p Value
CWOT	1	11	10	22	0.180
	4.5%	50.0%	45.5%	100%	
CWT	1	5	16	22	
	4.5%	22.8%	72.7%	100%	
OED	1	12	9	22	
	4.5%	54.6%	40.9%	100%	
OSCC	0	14	8	22	
	0%	63.3%	36.7%	100%	

CWOT-Control without tobacco, CWT-Control with tobacco, OED-Oral epithelial dysplasia, OSCC-Oral squamous cell carcinoma, p value < 0.05 = significant.

Table 2
Comparison of the IHC Allred score and Genotype.

Study Groups	Genotype	N	Mean	p Value
Group 1 (CWOT)	CC	1	7.0000	.291
	CG	11	3.2727	
	GG	10	3.7000	
Group 2 (CWT)	CC	1	5.0000	.508
	CG	5	6.2000	
	GG	16	6.3750	
Group 3 (OED)	CC	1	5.0000	.152
	CG	12	5.7500	
	GG	9	6.5556	
Group 4 (OSCC)	CG	14	5.5714	.300
	GG	8	5.8750	

CWOT-Control without tobacco, CWT-Control with tobacco, OED-Oral epithelial dysplasia, OSCC-Oral squamous cell carcinoma, p value < 0.05 = significant.

Table 3
L432V SNP distribution and mean Allred score, GST and MDA in study groups.

Study Group	Genotype	IHC (Allred Score)	GST (U/ml)	MDA (nm/100 ml)
CWOT	CG-50% GG-45.5% CC- 4.5%	3.63 ± 2.23	2.83 ± 3.14	750.65 ± 382.94.
CWT	CG-22.8% GG-72.7% CC- 4.5%	6.27 ± 1.12	3.54 ± 1.842	1106.67 ± 372.51
OED	CG-54.6% GG-40.9% CC- 4.5%	6.04 ± 1.09	5.00 ± 2.56	808.17 ± 520.10
OSCC	CG-63.3% GG-36.7% CC- 0%	5.68 ± 0.64	3.80 ± 1.51	1553.94 ± 287.20

CWOT-Control without tobacco, CWT-Control with tobacco, OED-Oral epithelial dysplasia, OSCC-Oral squamous cell carcinoma.

Table 4
Pairwise Comparison of IHC Allred Score, GST and MDA among groups.

	Control Group	Study Group	Mean Difference	p Value
IHC (Allred Score)	CWOT	CWT	−2.63636	.000*
		OED	−2.40909	.000*
		OSCC	−2.04545	.000*
	CWT	OED	.22727	.950
		OSCC	.59091	.504
	OED	OSCC	.36364	.825
GST (U/mL)	CWOT	CWT	−.71364	.746
		OED	−2.17273	.015*
		OSCC	−.97227	.521
	CWT	OED	−1.45909	.176
		OSCC	−.25864	.983
	OED	OSCC	1.20045	.334
MDA (nm/100 ml)	CWOT	CWT	−356.01818	.021*
		OED	−57.52091	.964
		OSCC	−803.29273	.000*
	CWT	OED	298.49727	.071
		OSCC	−447.27455	.002*
	OED	OSCC	−745.77182	.000*

CWOT-Control without tobacco, CWT-Control with tobacco, OED-Oral epithelial dysplasia, OSCC-Oral squamous cell carcinoma, p value < 0.05 = significant, * statistically significant results.

3.5. GST analysis

When the GST levels among the groups were compared it was observed that the mean GST was highest in OED and least in CWOT (Table 3). This difference in mean was found to be statistically significant ($p < 0.05$) but no such significant difference was observed between OED and OSCC (Table 4).

3.6. MDA analysis

On comparison of MDA (nm/100 ml) among study groups it was observed that the mean MDA was highest for OSCC and least for CWOT (Table 3). This difference in mean was statistically significant ($p < 0.05$). Statistically significant difference was also observed between CWOT and CWT tobacco as well as between CWT and OSCC (Table 4).

3.7. Association of CYP1B1 polymorphism and oxidant-antioxidant status

On evaluation of the genotype and GST activity it was observed that highest level of GST activity (5.92 ± 3.07 U/mL) was seen in CG genotype in OED, followed by GG genotype in OSCC (4.2588 U/ml). The highest level of MDA (1731 nm/100 ml) activity was observed in CC genotype in CWOT followed by GG genotype in OSCC (1626.3 nm/100 ml). It was noted that there was no significant difference in GST or MDA activity and genotype in any of the study groups. ($p > 0.05$), except for the MDA activity in CWT. ($p < 0.05$).

4. Discussion

One of the main causes of cancer-related deaths globally is oral cancer. And despite advances in the diagnostics and treatment protocols the mortality and morbidity associated with OSCC has not improved.[3] Various risk factors have been attributed to OSCC which include age, sex, race, gender, diet, nutrition, tobacco, alcohol and betel nut the chief among them being tobacco.[20] The phase I detoxification group of enzymes known as cytochrome P450 family form the primary line of defense against substances such as alcohol and tobacco. They convert these substances in to less toxic byproducts by oxidation or hydrolysis.[22] These byproducts are highly reactive free radicals which still possess a threat to the body and can damage the proteins or DNA. The phase II enzymes such as GST neutralize these harmful byproducts of the phase I metabolism by conjugation and subsequent excretion.[23] A crucial member of the cytochrome p450 family is CYP1B1, which is essential for phase I metabolism. It detoxifies procarcinogens such as polycyclic aromatic hydrocarbons (PAH), which have long been implicated in the etiopathogenesis of oral cancer. These PAH's act by forming DNA adducts which alter the expression of certain genes involved in tumor-associated signaling pathways, thereby promoting the proliferative and tumorigenic activity of OSCC.[24,25]

Numerous studies have been conducted to ascertain the association of CYP1B1 polymorphism and susceptibility to cancers such as endometrial and urinary cancer.[26,27] But studies regarding its association with oral cancer susceptibility are woefully lacking. To the best of our knowledge, till date no study has been conducted which analyses the CYP1B1 polymorphism and its association with the oxidant -antioxidant status in oral premalignancy and malignancy.

Of the various CYP1B1 polymorphisms studied the L432V polymorphism has been frequently associated with increased risk of cancers including lung.[28] In our study there were no meaningful differences in the distribution of CYP1B1 polymorphisms among the controls and OED or OSCC patients. It was observed that tobacco users with both the CG (Leu-Val alleles) and GG (Val-Val alleles) genotypes were at equal risk of developing OED or OSCC. Polymorphisms like the N453S may play a more significant role in OSCC given that it has been shown to be associated with increased adduct formations and subsequent altered cancer risks.[29]

Significant difference was found in the immunohistochemical expression of CYP1B1 between the study groups with least expression seen in CWOT and the highest in CWT. Interestingly, there was not much

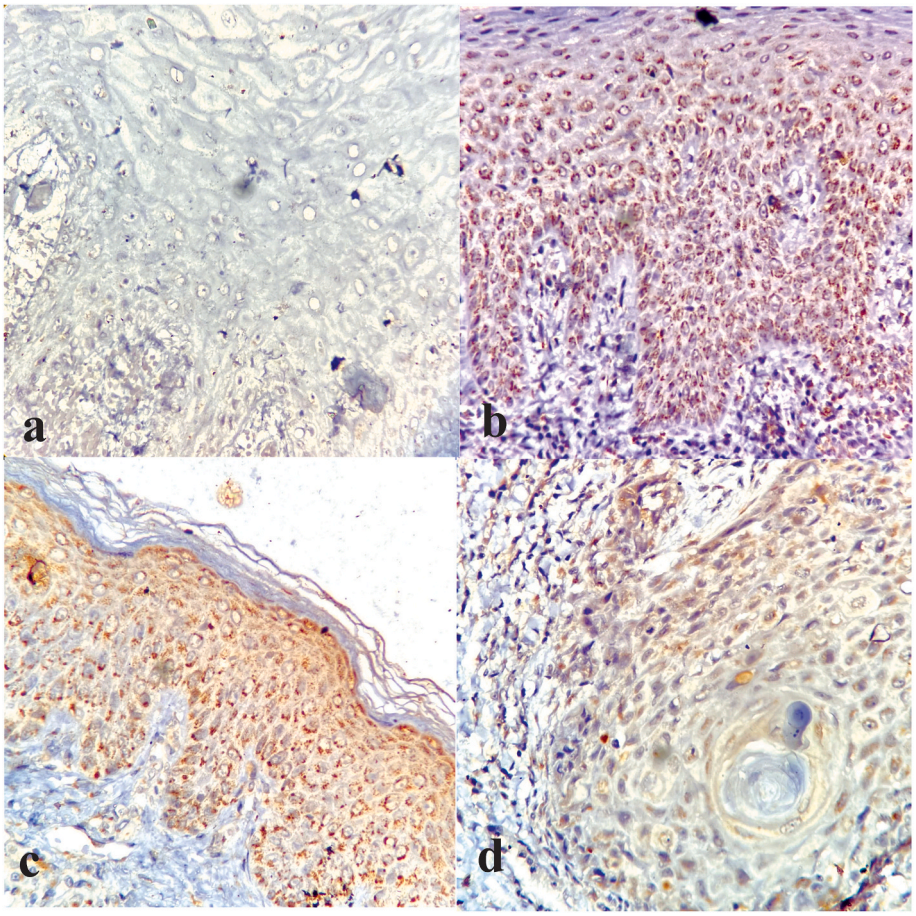

Fig. 2. (a) Negative CYP1B1 expression in apparently normal mucosa (CWOT) (40x) (b) CYP1B1 expression in apparently healthy mucosa with tobacco exposure (CWT) showing intense cytoplasmic positivity (40x) (c) CYP1B1 expression in OED showing moderate cytoplasmic positivity (40x) (d) CYP1B1 expression in OSCC showing moderate cytoplasmic intensity (40x).

difference in the expression of CYP1B1 between OED and OSCC, with decreased expression in OSCC as compared to OED. To summarize the expression of CYP1B1 was found to be highest in CWT > OED > OSCC > CWOT. Our findings were consistent with previous studies wherein overexpression of CYP1B1 has been demonstrated in breast, and ovarian cancer.[30–32] It is well known that tobacco exposure upregulates CYP1B1 in target tissues of local epithelium, particularly in the squamous cells of the pharynx and larynx.[29] On the contrary, some studies have demonstrated that there is downregulation of CYP1B1 in OSCC as compared to healthy oral tissue.[33] We found that the expression of CYP1B1 was slightly higher in OSCC as compared to heathy tissue (CWOT). But when the expression of CYP1B1in OSCC was compared with tissue exposed to tobacco (CWT) it was noted that the CYP1B1 expression was downregulated in OSCC. Thus, we can infer that when the healthy tissue is exposed to tobacco it shows significant upregulation of CYP1B1, but there is no further upregulation in either OED or OSCC, rather a gradual downregulation may be seen. Our findings are similar to a study on endometrial cancer, where lower level of CYP1B1 in cancerous endometrial tissue was found as compared to precancerous and normal endometrial tissue.[34] This initial upregulation of CYP1B1 expression in tissues could be construed as the body's immediate response to tobacco exposure in an attempt for detoxification. We found no significant association between the mean Allred score and CYP1B1 polymorphisms. Interestingly though the highest Allred score was observed for the GG genotype in CWT, OED and OSCC, suggesting that the tissue expression of CYP1B1 may be more intense in subjects with GG genotype when they are exposed to tobacco.

GST is a well-studied phase II metabolism enzyme which eliminates harmful byproducts of phase I metabolism by conjugation with GSH.[35] We found that there was a gradual increase in the GST activity from CWOT to CWT and finally OED with significantly increased levels seen OED as compared to CWOT. But subsequently, decrease in the GST levels was noted in OSCC as compared to OED. This suggests that there is an increase in the levels of GST initially in an attempt to eliminate the harmful byproducts of PAH metabolism like reactive oxygen species (ROS). This subsequent depletion in GST activity in OSCC may depict the overwhelming of the oxidant-antioxidant mechanism causing accumulation of carcinogens leading to malignant changes.[36,37] When the MDA activity which is a known marker for lipid peroxidation and oxidative stress was studied, a gradual increase in the activity was observed from CWOT, CWT, OED and finally OSCC with significantly increased levels seen in OSCC as compared to healthy individuals without tobacco habits. This may depict a gradual accumulation of harmful byproducts which can cause subsequent oxidative damage to the cells.[37,38] On comparison of GST and MDA activity in the oral cancer progression model, it could be summarized that decreasing GST activity in patients with oral premalignant disorders may signal progression to malignancy. When CYP1B1 polymorphism was compared with GST & MDA activity, the only significant finding observed, was that tobacco users with GG genotype had significantly higher MDA activity, indicating that tobacco users with GG genotype may be at greater risk of oxidative damage as compared to individuals with CG genotype.

The role of CYP1B1 in OSCC pathogenesis may be limited to the activation of tobacco related carcinogens, nevertheless studies have demonstrated it is overexpressed in OSCC cells.[39] Since CYP1B1 plays an important role in the activation of various carcinogens it can be targeted

during chemotherapy by agents like *trans*-stilbenes and carbazoles. Moreover, it is also known to activate prodrugs like chalcone DMU-135 and an oxime DMAKO-20, this property can be useful to selectively target cancer cells which overexpress CYP1B1.[40,41]

4.1. Limitations

Even though our study has provided an insight into the L432V polymorphism and its role in oral pre-cancer and cancer, there are several limitations that need to be taken into consideration. First of all, the study had a limited sample size due to which generalizability of the results would be difficult. Additionally, we have studied only one polymorphism in CYP1B1, which limits the scope of the study. Furthermore, our cross-sectional approach only provides a static picture of the CYP1B1 polymorphism and its association with the oxidant-antioxidant status in OED and OSCC, whereas a longitudinal investigation can offer a more dynamic viewpoint.

5. Conclusion

Tobacco users with CG and GG genotypes are at equal risk of developing oral epithelial dysplasia or OSCC and L432V polymorphism does not appear to increase the risk of malignant transformation in oral epithelial dysplasia. Moreover, CYP1B1 was upregulated in healthy oral tissues when they were exposed to tobacco and this upregulation was more commonly associated with the GG genotype, also tobacco users with GG genotype may be at a greater risk of oxidative damage as compared to CG genotype. Hence tissue expression of CYP1B1 and L432V polymorphism analysis may prove to be a useful tool to assess the oxidative damage as well as risk of disease in tobacco users. Additionally, decreasing GST and increasing MDA activity in patients with oral premalignant disorders may signal progression to malignancy.

Declaration of competing interest

All the authors associated with the present manuscript declared no potential conflict of interest concerning the research, authorship, and/or publication of this article.

References

1. Singh MP, Kumar V, Agarwal A, Kumar R, Bhatt ML, Misra S. Clinico-epidemiological study of oral squamous cell carcinoma: a tertiary care centre study in North India. *J Oral Biol Craniofac Res*. 2016 Jan-Apr;6(1):31–34.
2. Markopoulos AK. Current aspects on oral squamous cell carcinoma. *Open Dent J*. 2012;6:126–130.
3. Camisasca DR, Silami MA, Honorato J, Dias FL, de Faria PA, Lourenço Sde Q. Oral squamous cell carcinoma: clinicopathological features in Subjects with and without recurrence. *ORL J Otorhinolaryngol Relat Spec*. 2011;73(3):170–176.
4. Mortazavi H, Baharvand M, Mehdipour M. Oral potentially malignant disorders: an overview of more than 20 entities. *J Dent Res Dent Clin Dent Prospects*. 2014 Winter;8(1):6–14.
5. Ranganathan K, Kavitha L. Oral epithelial dysplasia: classifications and clinical relevance in risk assessment of oral potentially malignant disorders. *J Oral Maxillofac Pathol*. 2019 Jan-Apr;23(1):19–27.
6. Abidullah M, Kiran G, Gaddikeri K, Raghoji S, Ravishankar TS. Leuloplakia - review of a potentially malignant disorder. *J Clin Diagn Res*. 2014 Aug;8(8). ZE01-4.
7. Chiu SF, Ho CH, Chen YC, et al. Malignant transformation of oral potentially malignant disorders in Taiwan: an observational nationwide population database study. *Medicine (Baltim)*. 2021 Mar 5;100(9), e24934.
8. Jiang X, Wu J, Wang J, Huang R. Tobacco and oral squamous cell carcinoma: a review of carcinogenic pathways. *Tob Induc Dis*. 2019 Apr 12;17:29.
9. Vu AT, Taylor KM, Holman MR, Ding YS, Hearn B, Watson CH. Polycyclic aromatic hydrocarbons in the mainstream smoke of popular U.S. Cigarettes. *Chem Res Toxicol*. 2015 Aug 17;28(8):1616–1626.
10. Moorthy B, Chu C, Carlin DJ. Polycyclic aromatic hydrocarbons: from metabolism to lung cancer. *Toxicol Sci*. 2015 May;145(1):5–15.
11. Esteves F, Rueff J, Kranendonk M. The central role of cytochrome P450 in xenobiotic metabolism-A brief review on a fascinating enzyme family. *J Xenobiot*. 2021 Jun 22;11(3):94–114.
12. Singh RR, Reindl KM. Glutathione S-transferases in cancer. *Antioxidants*. 2021 Apr 29;10(5):701.
13. Ayala A, Muñoz MF, Argüelles S. Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. *Oxid Med Cell Longev*. 2014;2014, 360438.
14. Kwon YJ, Baek HS, Ye DJ, Shin S, Kim D, Chun YJ. CYP1B1 enhances cell proliferation and metastasis through induction of EMT and activation of wnt/β-catenin signaling via Sp1 upregulation. *PLoS One*. 2016 Mar 16;11(3), e0151598.
15. Zhang H, Li L, Xu Y. CYP1B1 polymorphisms and susceptibility to prostate cancer: a meta-analysis. *PLoS One*. 2013 Jul 4;8(7), e68634.
16. Morvan VL, Richard É, Cadars M, et al. Cytochrome P450 1B1 polymorphism drives cancer cell stemness and patient outcome in head-and-neck carcinoma. *Br J Cancer*. 2020;123:772–784.
17. Ahmad Fauzi MF, Wan Ahmad WSHM, Jamaluddin MF, et al. Allred scoring of ER-IHC stained whole-slide images for hormone receptor status in breast carcinoma. *Diagnostics*. 2022 Dec 8;12(12):3093.
18. Tarle M, Raguž M, Muller D, Lukšić I. Nuclear epidermal growth factor receptor overexpression as a survival predictor in oral squamous cell carcinoma. *Int J Mol Sci*. 2023;24(6):5816.
19. Al-Saraireh YM, Alshammari FOFO, Satari AO, et al. Cytochrome 4Z1 expression connotes unfavorable prognosis in ovarian cancers. *Medicina*. 2022 Sep 13;58(9):1263.
20. Remmerie B, Vandenbroucke K, De Smet L, et al. Expression, purification, crystallization and structure determination of two glutathione S-transferase-like proteins from Shewanella oneidensis. *Acta Crystallogr, Sect F: Struct Biol Cryst Commun*. 2008 Jun 1;64(Pt 6):548–553.
21. Khadir F, Pouramir M, Joorsaraee SG, Feizi F, Sorkhi H, Yousefi F. The effect of arbutin on lipid peroxidation and antioxidant capacity in the serum of cyclosporine-treated rats. *Caspian J Intern Med*. 2015 Fall;6(4):196–200.
22. Jiang X, Wu J, Wang J, Huang R. Tobacco and oral squamous cell carcinoma: a review of carcinogenic pathways. *Tob Induc Dis*. 2019 Apr 12;17:29.
23. Aronica L, Ordovas JM, Volkov A, et al. Genetic biomarkers of metabolic detoxification for personalized lifestyle medicine. *Nutrients*. 2022 Feb 11;14(4):768.
24. Alsubait A, Aldossary W, Rashid M, Algamdi A, Alrfaei BM. CYP1B1 gene: implications in glaucoma and cancer. *J Cancer*. 2020 May 19;11(16):4652–4661.
25. He H, Huang Y, Lu Y, et al. Effect of benzo[a]pyrene on proliferation and metastasis of oral squamous cell carcinoma cells: a transcriptome analysis based on RNA-seq. *Environ Toxicol*. 2022 Nov;37(11):2589–2604.
26. Zhu ZY, Mu YQ, Fu XM, Li SM, Zhao FX. Association of CYP1B1 gene polymorphisms and the positive expression of estrogen alpha and estrogen beta with endometrial cancer risk. *Eur J Gynaecol Oncol*. 2011;32(2):188–191.
27. Jiang W, Sun G, Xiong J, Xi X, Shi Z. Association of CYP1B1 L432V polymorphism with urinary cancer susceptibility: a meta-analysis. *Diagn Pathol*. 2014 Jun 9;9:113.
28. Motovali-Bashi M, Biglari M, Rezaei H, Dehghanian F. CYP1B1 L432V polymorphism and lung cancer risk in the Iranian population. *Iran J Biotechnol*. 2013;11(3):199–204.
29. Schults Marten A, Chiu Roland K, Nagle Peter W, et al. Genetic polymorphisms in catalase and CYP1B1 determine DNA adduct formation by benzo(a)pyrene ex vivo. *Mutagenesis*. March 2013;28(2):181–185.
30. McFadyen MC, Breeman S, Payne S, et al. Immunohistochemical localization of cytochrome P450 CYP1B1 in breast cancer with monoclonal antibodies specific for CYP1B1. *J Histochem Cytochem*. 1999 Nov;47(11):1457–1464.
31. McFadyen MC, Cruickshank ME, Miller ID, et al. Cytochrome P450 CYP1B1 over-expression in primary and metastatic ovarian cancer. *Br J Cancer*. 2001 Jul 20;85(2):242–246.
32. Roos PH, Bolt HM. Cytochrome P450 interactions in human cancers: new aspects considering CYP1B1. *Expet Opin Drug Metabol Toxicol*. 2005 Aug;1(2):187–202.
33. Pradhan S, Nagashri MN, Gopinath KS, Kumar A. Expression profiling of CYP1B1 in oral squamous cell carcinoma: counterintuitive downregulation in tumors. *PLoS One*. 2011;6(11), e27914.
34. Hevir N, Sinkovec J, Rižner TL. Disturbed expression of phase I and phase II estrogen-metabolizing enzymes in endometrial cancer: lower levels of CYP1B1 and increased expression of S-COMT. *Mol Cell Endocrinol*. 2011 Jan 1;331(1):158–167.
35. Selvendiran K, Banu SM, Sakthisekaran D. Oral supplementation of piperine leads to altered phase II enzymes and reduced DNA damage and DNA protein cross links in benzo(a)pyrene induced experimental lung carcinogenesis. *Mol Cell Biochem*. 2005;268:141–147.
36. Ma HL, Yu C, Liu Y, et al. Decreased expression of glutathione S. transferase correlates with poorly differentiated grade in patients with oral squamous cell carcinoma. *J Oral Pathol Med*. 2015;44(3):193–200.
37. Verma S, Saxena R, Siddiqui MH, Santha K, Sethupathy S. Evaluation of CYP1B1 expression, oxidative stress and phase 2 detoxification enzyme status in oral squamous cell carcinoma patients. *J Clin Diagn Res*. 2017 Mar;11(3):BC01–BC05.
38. Mohideen K, Sudhakar U, Balakrishnan T, et al. Malondialdehyde, an oxidative stress marker in oral squamous cell carcinoma-A systematic review and meta-analysis. *Curr Issues Mol Biol*. 2021 Aug 28;43(2):1019–1035.
39. Chi AC, Appleton K, Henriod JB, et al. Differential induction of CYP1A1 and CYP1B1 by benzo [a] pyrene in oral squamous cell carcinoma cell lines and by tobacco smoking in oral mucosa. *Oral Oncol*. 2009 Nov 1;45(11):980–985.
40. Fabris M, Luiza Silva M, de Santiago-Silva KM, de Lima Ferreira Bispo M, Goes Camargo P. CYP1B1: a promising target in cancer drug discovery. *Anti Cancer Agents Med Chem*. 2023;23(9):981–988.
41. McFadyen MC, Murray GI. Cytochrome P450 1B1: a novel anticancer therapeutic target. *Future Oncol*. 2005 Apr;1(2):259–263.