https://doi.org/10.48047/AFJBS.6.8.2024.270-292

African Journal of Biological Sciences

Research Paper Open Access

Evaluation of genomic changes in saliva in patients with Oral Potentially Malignant Disorders

1.Dr. Mimansha Patel, 2. Dr Pankaj Gupta, 3. .Dr. Ila Tiwari, 4. Dr.Manish A.T, 5. Dr. Amit Kumar, 6. Dr. Kaustubh Suresh Thakare

[1]Professor & HOD, Department of Oral and Maxillofacial Pathology and Microbiology, Triveni Dental College, Bodri, Bilaspur, Chhattisgarh.

[2] Assistant Professor, Amaltas Institute of Medical Sciences, Dewas M.P.

[3]Senior Periodontist and Implantologist , Clove Dental, Bangalore , Karnataka

[4]Senior Lecturer, Department of Oral and Maxillofacial Pathology and Microbiology, Triveni Institute of Dental Sciences, Hospital & Research Centre, Bilaspur Chhattisgarh

[5]PG Student, Department of Oral and Maxillofacial Pathology and Microbiology, Bhabha College of Dental Sciences, Bhopal, M.P.

[6]Professor, Department of Periodontology and Implantology, VYWS dental college and Hospital, Amravati – 444602

Corresponding author

Dr. Mimansha Patel, Professor & HOD, Department of Oral and Maxillofacial Pathology and Microbiology, Triveni Dental College, Bodri, Bilaspur, Chhattisgarh

mimanshapatel_24@yahoo.com

Article History

Volume 6,Issue 8, 2024

Received:05 Mar 2024

Accepted : 06 Apr 2024

doi: 10.33472/AFJBS.6.8.2024.270-292

Abstract

Background: Attempts should be made to identify the molecule based biomarkers in saliva which give indication about the progession of precancer and malignancy of the oral area. It can act as valuable aid in diagnosis , proper analysis of transformation of precancerous lesions into malignancy and proper management of the malignancy of oral cavity.

Aim: Evaluation of genomic changes in saliva in patients with Oral Potentially Malignant Disorders

Methods and Materials:. 48 cases of "OPMDS" diagnosed histopathologically would be included. Statistically significant tests would be used to compare and link the "DNA methylation" patterns in saliva of tobacco (smoking) users without disease with those of tobacco (smoking) users with disease (OPMDS). Approximately 1 mL of saliva was collected from each individual. They were instructed to rinse their mouth five times with water (to remove food debris) and to scrap their buccal mucosa with a new set of toothbrush so, as to get a quality saliva sample containing buccal epithelial cells. The saliva samples were collected in sterile containers. The samples collected outside the laboratory were placed in a sealed plastic bag and transported in dry ice to the laboratory. Tissue samples would also be obtained from healthy individuals & individuals with smoking habit but without disease. The "HiPurA Mammalian Genomic DNA Purification Kit (HiMedia Labs.)" would then be used to isolate DNA from saliva.

Results: Methylation of DNA at p16 in saliva samples was observed 25.6% of the oral premalignant lesion, methylation of DAP-K in saliva samples was observed in 32.3% lesions while methylation of MGMT in saliva samples was observed in 30.3% oral premalignant lesions. Methylation of DNA at p16 in saliva specimens among tobacco users with no diseases was observed in 18.3% cases, methylation of DAP-K in saliva specimens was observed in 23.3% cases while methylation of MGMT in saliva specimens was observed in 34.4 cases. Methylation of DNA at p16 in saliva specimens among normal healthy individuals was observed in 5.6% cases, methylation of DAP-K in saliva specimens was observed in 2.3% cases while methylation of MGMT in saliva specimens was observed in 7.3% cases. The finding was statistically non-significant. ($p \leq 0.01$)

Conclusion: The specificity and sensitivity of p16, DAP-K and MGMT in saliva specimens in diagnosis of oral potential malignant disorders is quite significant in tobacco users as well as non tobacco users.

Keywords: Genomic changes, saliva, OPMDs

Introduction

Malignancy in the oral region is one of the most commonly observed malignancy of human beings especially males. It is one of the commonest malignancies in Asian countries especially the Indian subcontinent.[1-5] But globally there is also increased cases of malignancy of oral area in the European countries as well the western countries like USA and UK. It has been found that more than ten lakhs new cases of cancer of oral and pharyngeal region are found in these western developed countries. Earlier it was reported mainly in the old age males but nowadays young adults have also became vulnerable to this disease.[6-8]Malignancy observed in oral area is a gradual pathology which usually start from the stage of precancer and then undergoes several sequential histological changes in the tissues to get transformed into a dangerous condition. Lesions of the oral cavity having premalignant nature are detected quite easily through oral clinical examination.[9-12] Besides the procedure of cytology and biopsy can be carried easily in the oral cavity for confirmation of the precancerous lesions and conditions. If the cancer of oral cavity is diagnosed at an early stage then there is descreased chances of death of patient due to malignancy reported in oral area. But malignancy of oral area is normally detected at late stage which increase possibility of death of the patient. This finding is more common in the countries of Indian subcontinent.[13-16]

Conditions and lesions of the oral cavity having premalignant nature are collectively termed as oral potentially malignant diseases. Lesions of oral area having premalignant nature include a variety of lesions such as erythroplakia, leukoplakia and smokers palate. On the other hand the precancerous conditions include DLE (discoid lupus erythematosus), lichen planus, oral submucous fibrosis, actinic keratosis.[17-19] It has been documented that the chances of conversion of pre malignant lesions with dysplasia into carcinoma is around 13 to 20 percent over a duration of 6 months to sixteen years. Although biopsy and cytology are believed to be standard methods of diagnosis of precancer and cancer of oral cavity but they are not much useful in analysing the progression of these conditions. This is because there cannot be biopsy at regular intervals as it will cause increased morbidity to patients. Besides there are variations in the findings of histopathological and clinical investigations.[20-22] It has been also reported that changes in genes can also indicate the progress of precancer and cancer of oral cavity. [17,18] These changes in genes results in formation of certain molecules which can act as biomarkers for the progress of the disease.[19,20] Therefore attempts should be made to identify the molecule based biomarkers which give indication about the progession of precancer and malignancy of the oral area. It can act as valuable aid in diagnosis , proper analysis of transformation of precancerous lesions into malignancy and proper management of the malignancy of oral cavity.

Alongwith tissue sample there can be use of saliva and whole blood for the analysis of methylation. But there is some disadvantage with these samples. In case of whole blood there are different cells which have different patterns for methylation process. As a result whole blood become non specific sample for analysis. In case of saliva there are chances of contamination of saliva due to debris of food, microorganisms and residual cells. However there are few studies which have demonstrated

better results by using saliva and whole blood samples for detecting the biomarkers specifically found in saliva. Therefore this study was carried out to evaluate genomic changes in saliva of patients suffering from OPMDs.

Methods and Materials

Participants

Criteria for inclusion

• Individuals who use tobacco in any form, with or without sickness.
• Cases of oral possibly malignant conditions that have been histopathologically diagnosed.

Criteria for exclusion

• Presence of other condition inside mouth producing inflammatio , like tooth having sharp cusp, inflammation of gingiva, or inflammation of periodontium in non-smokers.
• Those who refused to provide their consent.

Plan of investigation

• After informed consent, "tobacco" (smoking) users with/without disease would be included, and a complete history of habit would be gathered.
• Cases of "OPMDS" diagnosed histopathologically would be included.
• Statistically significant tests would be used to compare and link the "DNA methylation" patterns of tobacco (smoking) users without disease with those of tobacco (smoking) users with disease (OPMDS).

Outcomes/Results Expected:

This research will aid in determining the utility of saliva as a tool for detecting high and low risk "Oral Potentially Malignant Disorders."
Individuals would be enrolled in each group at random using random table procedures. As a result, the trial would cover a total of 48 patients. As a result, each group would have 16 people in it.
Individuals in Group A are in good health.
Group B: Study participants using tobacco and suffering from disease
Group C: Study participants who are not suffering from disease.
Tobacco use – full case history – quantitative variables
"DNA methylation" — methylation-specific quantitative real-time PCR (qMSP)

Variables are the things that can change.

• **Result –** "possibly malignant oral diseases"
• **Confounding factors** – These include history of habit other than tobacco, hereditary history of disease, presence of condition in mouth that can cause inflammatory response.
• **Dependent variable** – "DNA methylation"
• **Independent variable** – cigarette usage

METHODOLOGY

Approximately 1 mL of saliva was collected from each individual. They were instructed to rinse their mouth five times with water (to remove food debris) and to scrap their buccal mucosa with a new set of toothbrush so, as to get a quality saliva sample containing buccal epithelial cells. The saliva samples were collected in sterile containers. The samples collected outside the laboratory were placed in a sealed plastic bag and transported in dry ice to the laboratory. Tissue samples would also be obtained from healthy individuals & individuals with smoking habit but without disease.

The "HiPurA Mammalian Genomic DNA Purification Kit (HiMedia Labs.)" would then be used to isolate DNA from saliva. Nanodrop 2000 would test the purity and aggregation of DNA (ThermoScietific). The EZ DNA methylation Gold Kit (Zymo Research, USA) would select around 500 ng of genomic DNA from each individualspecimen for sodium bisulfitetranslation as per the manufacturer's instructions. The Illumina Infinium HumanMethylation450 BeadChip (Illumina Inc, USA) would be used to assess genome-wide DNA methylation according to the manufacturer's instructions.

Bisulfite sequencing PCR (BSP) was done with bisulfite conversion-specific primers produced by MethPrimer. The BSP compounds will be quantified on a one percent agarose gel. A fraction of the products obtained in BSP were then analysed with the help of qMSP. This qMSP related reactions would be carried out utilizing an ABI 7900HT Fast Real-Time PCR System Instrument (USA) with the help of primers specific for methylation and Rox Fast Start Universal SYBR Green Master Mix (Roche, Switzerland).[6]

The products of BSP were then choosen for the purpose of sequencing and cloning. Another portion of the products of BSP products would be purified with the help of device namely MiniElute Gel Extraction Kit (USA). Nearly one hundred sixty five ng of real product of BSP was attached to the TA basedvector namely PTZ57R/T with the help of T4 Deoxyribonucleic acid Ligase (Japan). To confirm the likely positive clones, colony PCR will be conducted under normal conditions with universal M13 forward and reverse primers. The positive clones will next be sequenced using the 3100 Genetic Analyzer (ABI, USA). With the help of chromatogram collected and the percentage of methylation calculated, the methylation status of each clone was established.[6]

Gender bias and age bias are two examples of bias (would be removed by regression analysis)

Statistical Analysis

- The "Predictive Analytics Software (SPSS 16.0 version.)" would be used for the analysis.
- To establish correlation among the continuous variables in different groups, a recurring steps "ANOVA" (with post hoc as Bonferroni correction) would be utilised.
- Categorical variables would be assessed with the help of "chi-square test" or "Fisher's exact test."

The level of statistically significance was fixed at $p \leq 0.05$.

Results

In this research the percentage of study subjects belonging to age group of 30 to 40 years was 22.22, the percentage of study subjects belonging to the age group of forty to fifty years was 33.33 and the percentage of study subjects belonging to age group of 50 to 60 years was 44.44.(table 1, graph 1). In this study the percentage of male study subjects was 66.66 while the percentage of female study subjects was 33.34%. (table 2, graph 2)

The percentage of daily wager was 22.22%, percentage of rickshaw puller was 11.11%, percentage of housewife was 22.22%, percentage of agriculture population was 11.11%, percentage of businessman was 11.11% and percentage of study participants working in service sector was 22.22%. (table 3, graph 3). The education level of the study participants were analysed and it was observed that 33.33% of total study participants had education level below matriculation, 22.22% had education level of matriculation, 11.11% study participants were educated upto intermediate level and 33.33% study participants were educated upto graduation level. (table 4, graph 4)

33.33% of the study participants were of poor socioeconomic status, 33.33% of study participants were of average socioeconomic status and 33.33% of the study participants were of good socioeconomic status (table 5, graph 5).

33.33% of the study participants were found to have leukoplakia , 11.11% study participants were found to have OSMF, while 44.44% study participants were found to have no oral potential malignant diseases (table 6, graph 6). 50% of the study participants having OPMD were having OPMD in their family members while 50% don't had genetic susceptibility (table 7, graph 7)

In this study methylation of DNA at p16 in saliva samples was observed 25.6% of the oral premalignant lesion, methylation of DAP-K in saliva samples was observed in 32.3% lesions while methylation of MGMT in saliva samples was observed in 30.3% oral premalignant lesions. The finding was statisrically significant. ($p \leq 0.01$). (table 8, graph 8).

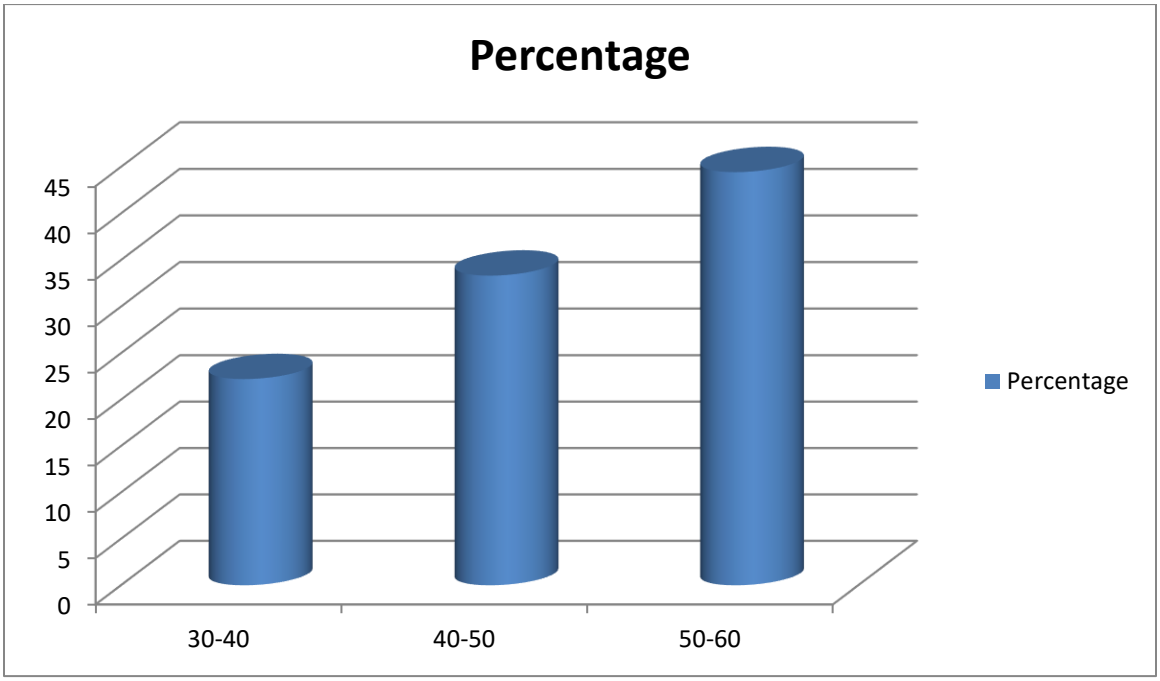
In this study, methylation of DNA at p16 in saliva specimens among tobacco users with no diseases was observed in 18.3% cases, methylation of DAP-K in saliva specimens was observed in 23.3% cases while methylation of MGMT in saliva specimens was observed in 34.4 cases. The finding was statistically significant. ($p \leq 0.01$). (table 9, graph 9)

In this study methylation of DNA at p16 in saliva specimens among normal healthy individuals was observed in 5.6% cases , methylation of DAP-K in saliva specimens was observed in 2.3% cases while methylation of MGMT in saliva specimens was observed in 7.3% cases. The finding was statistically non-significant. ($p \leq 0.01$) (table 10, graph 10).

The specificity and sensitivity of p16, DAP-K and MGMT in saliva specimens in diagnosis of oral potential malignant disorders is quite significant in tobacco users having diseases.(table 11). The specificity and sensitivity of p16, DAP-K and MGMT in saliva specimens diagnosis of oral potential malignant disorders is also significant in tobacco users having no diseases.(table 12). The methylation of combination of gene in saliva in tobaaco consumers having disease was quite significant (table 13). The methylation of combination of gene in saliva specimens tobaaco consumers having no disease was also significant.(table 14).

Table 1: Details of age of the Study participants

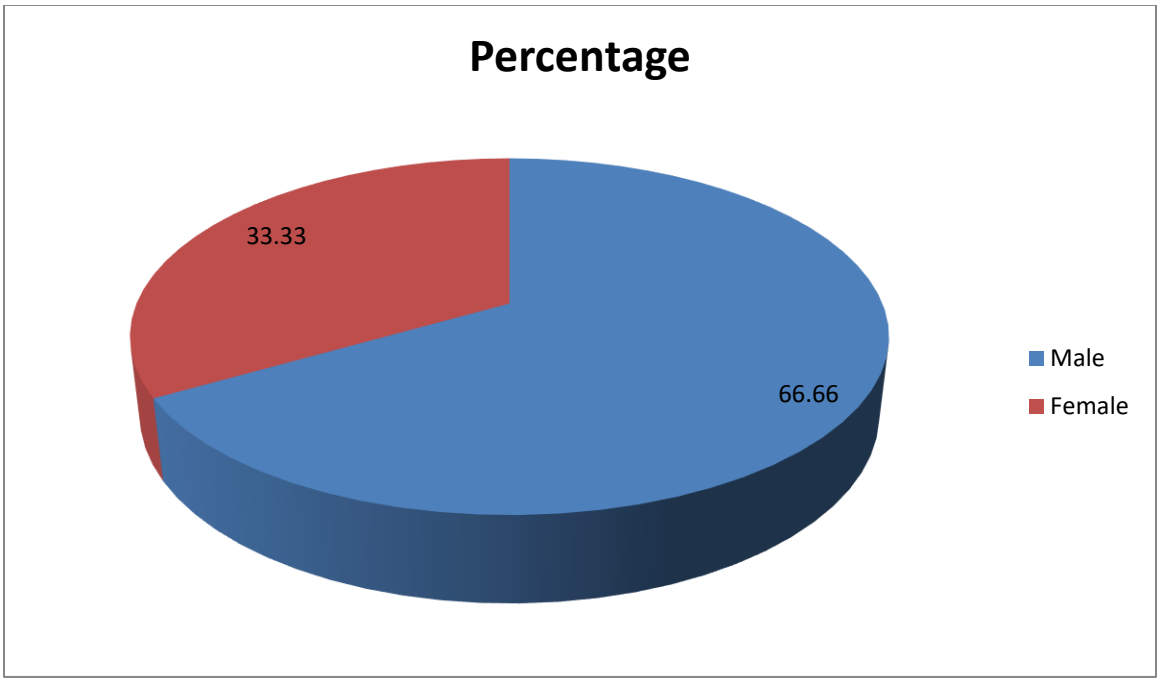
Different Age Groups	Percentage
30-40	22.22
40-50	33.33
50-60	44.44



Graph 1: Details of age of the Study participants

Table 2: Gender distribution

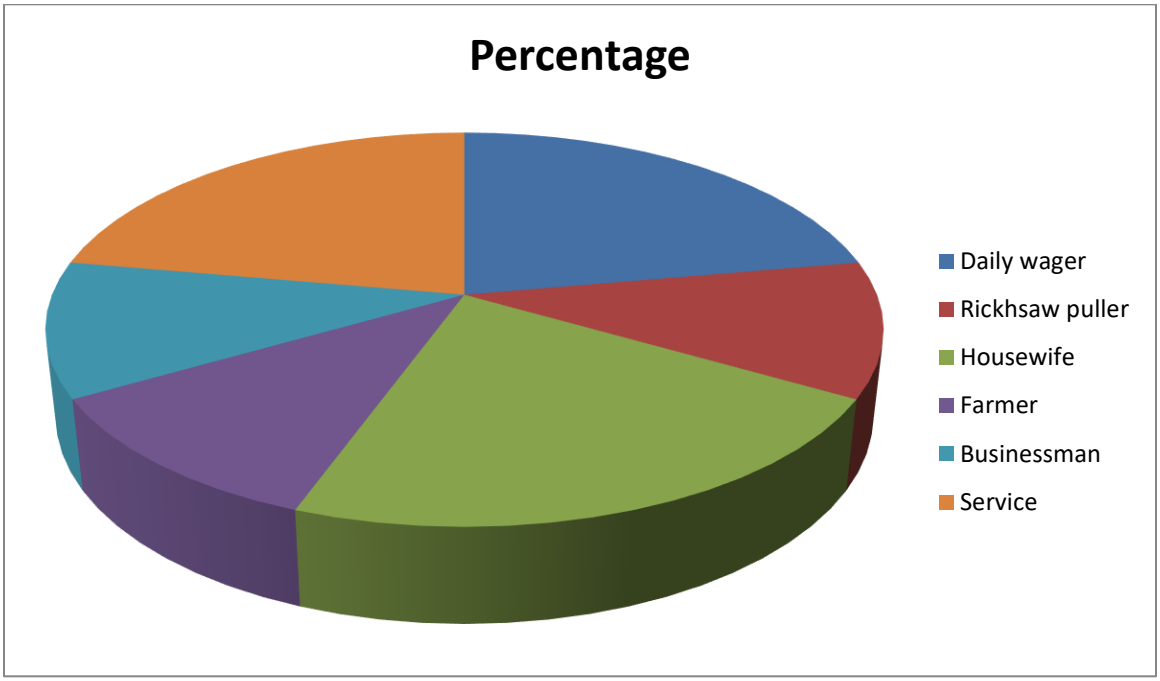
Gender	Percentage
Male	66.66
Female	33.34



Graph 2: Gender distribution

Table 3: Details about occupation of the study subject

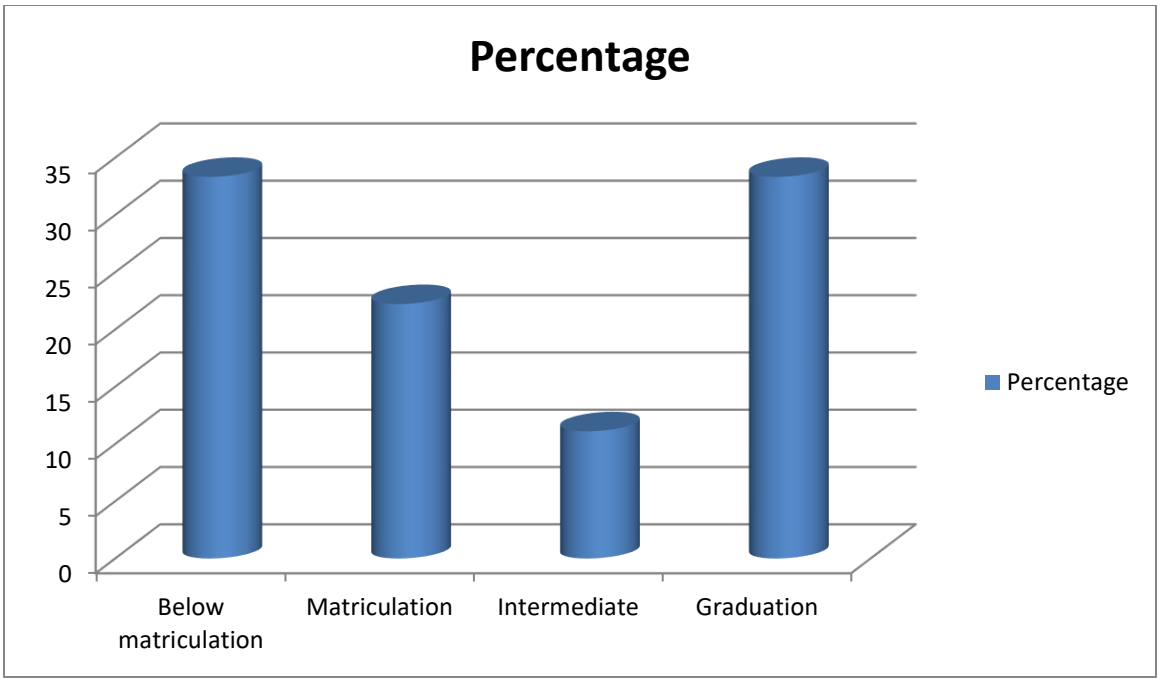
Occupation	**Percentage**
Daily wager	22.22
Rickhsaw puller	11.11
Housewife	22.22
Farmer	11.11
Businessman	11.11
Service	22.22



Graph 3: Details about occupation of the study subject

Table 4 : Level of education

Level of education	**Percentage**
Below matriculation	33.33
Matriculation	22.22
Intermediate	11.11
Graduation	33.33

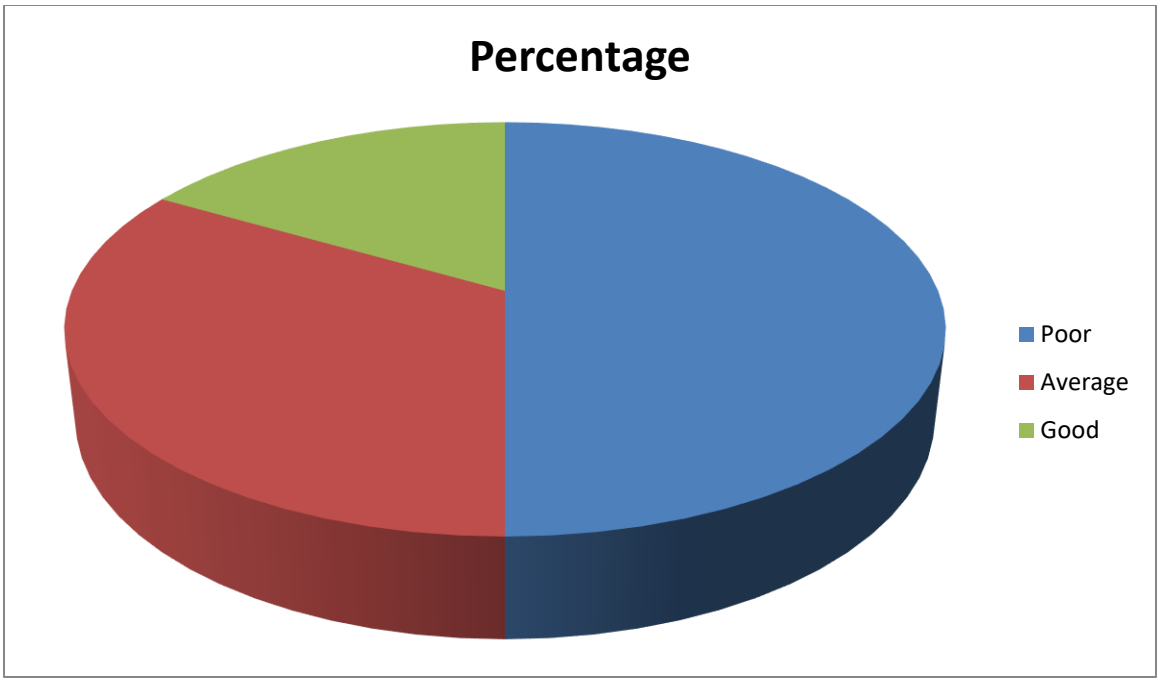


Graph 4 : Level of education

Table 5 : Socioeconomic status of the study participants

Socioeconomic status	**Percentage**
Poor	33.33
Average	33.33
Good	33.33

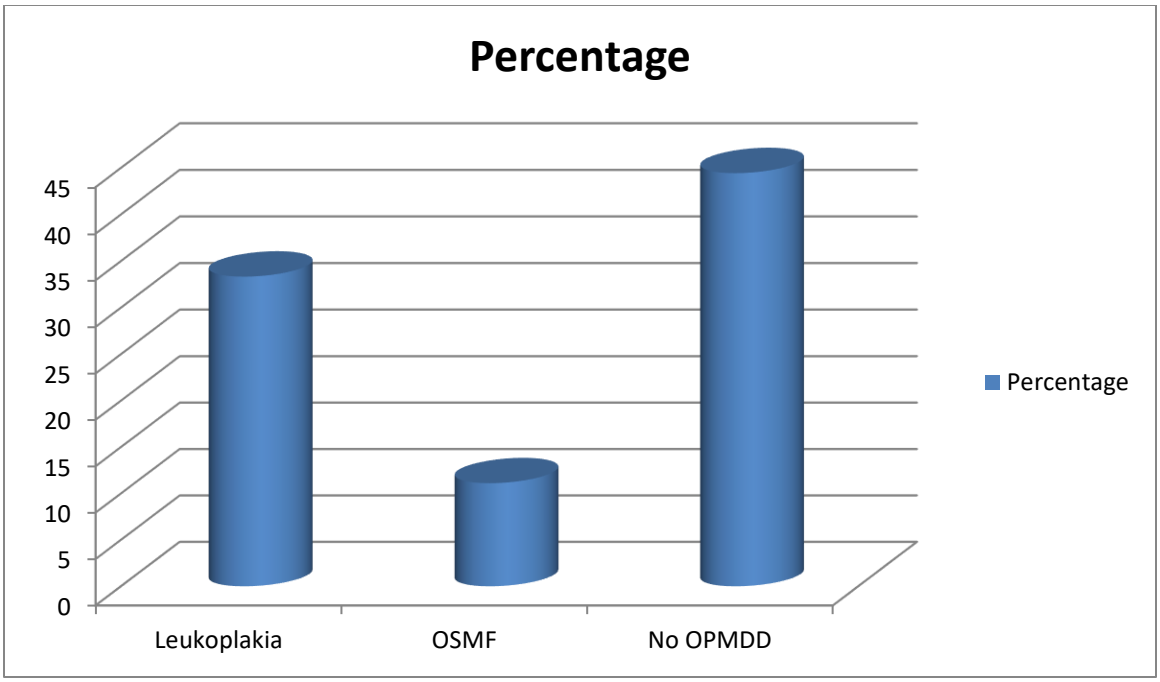
.



Graph 5 : Socioeconomic status of the study participants

Table 6: Type of oral potential malignant disease

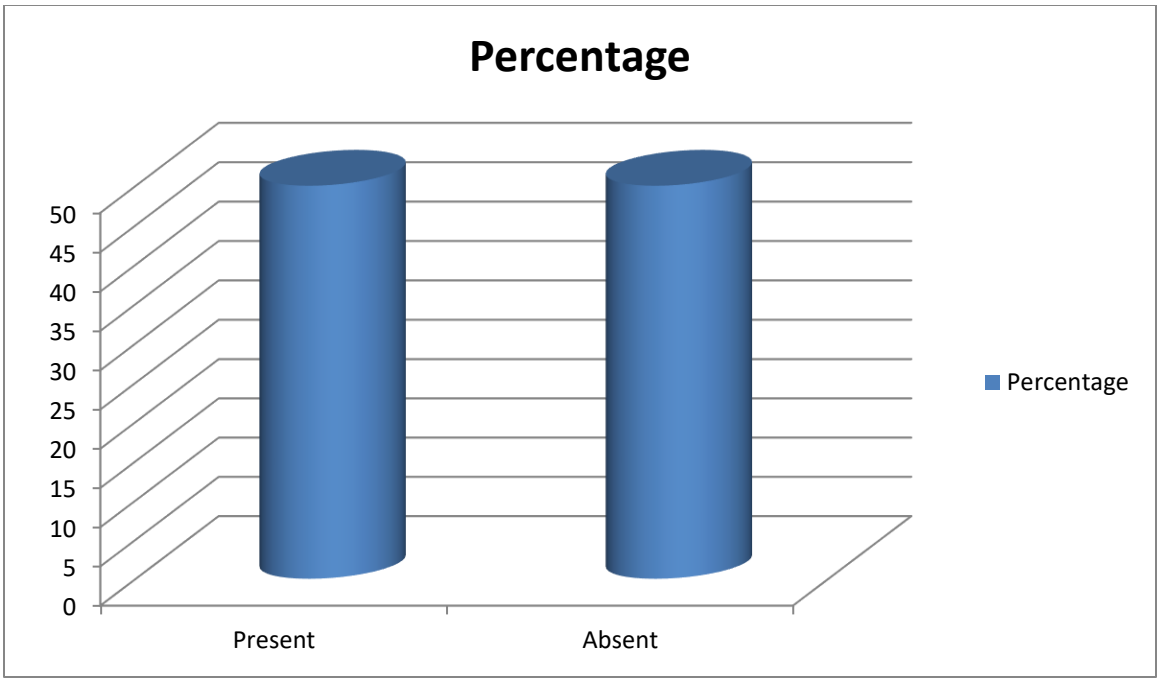
OPMD	**Percentage**
Leukoplakia	33.33
OSMF	11.11
No OPMD	55.55



Graph 6: Type of oral potential malignant disease

Table 7: Genetic Susceptibility in study participants regarding OPMDS

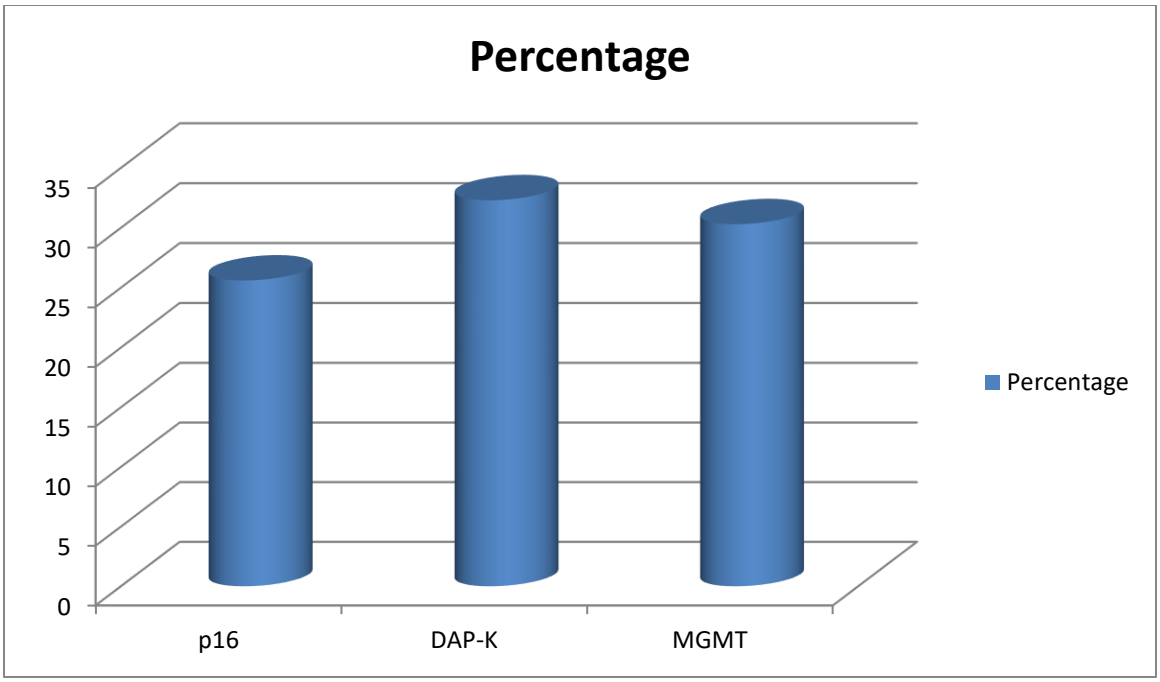
Genetic susceptibility	**Percentage**
Present	50
Absent	50



Graph 7: Genetic Susceptibility in study participants regarding OPMDS

Table 8: DNA methylation of gene p16, gene DAP-K and gene MGMT in saliva of tobacco users with oral potential malignant disorders.

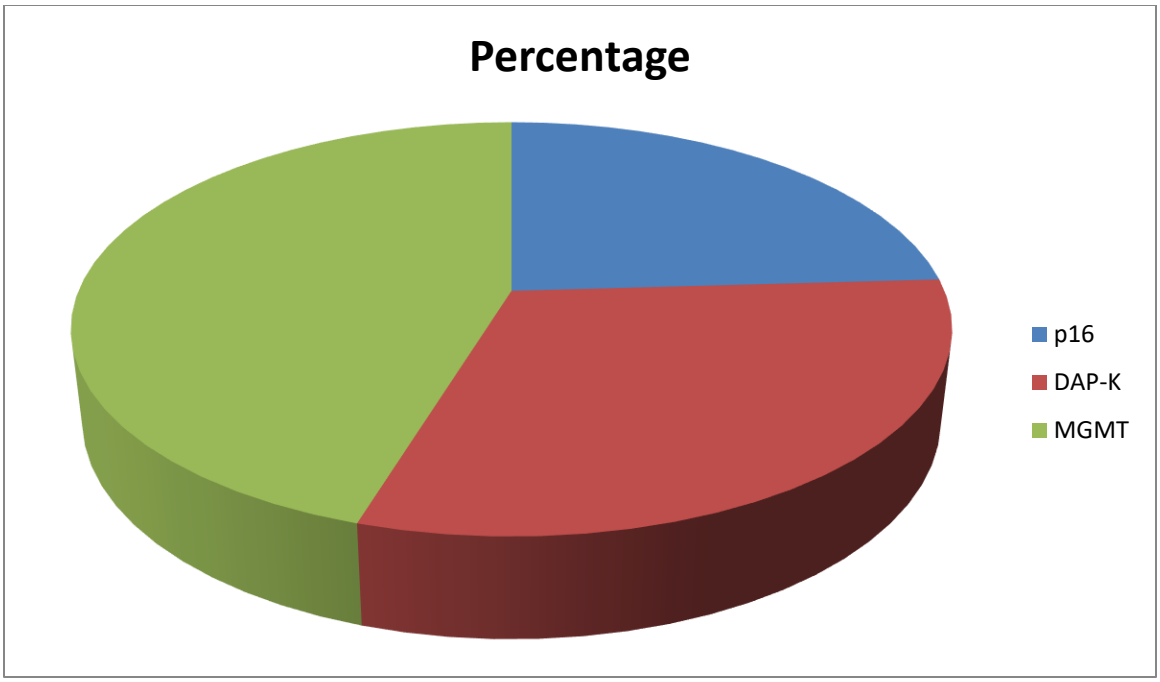
Gene loci	DNA methylation %	P value
p16	25.6	
DAP-K	32.3	0.001
MGMT	30.3	



Graph 8: DNA methylation of gene p16, gene DAP-K and gene MGMT in saliva of tobacco users with oral potential malignant disorders

Table 9: DNA methylation of gene p16, gene DAP-K and gene MGMT in saliva samples of tobacco users with no oral potential malignant disorders.

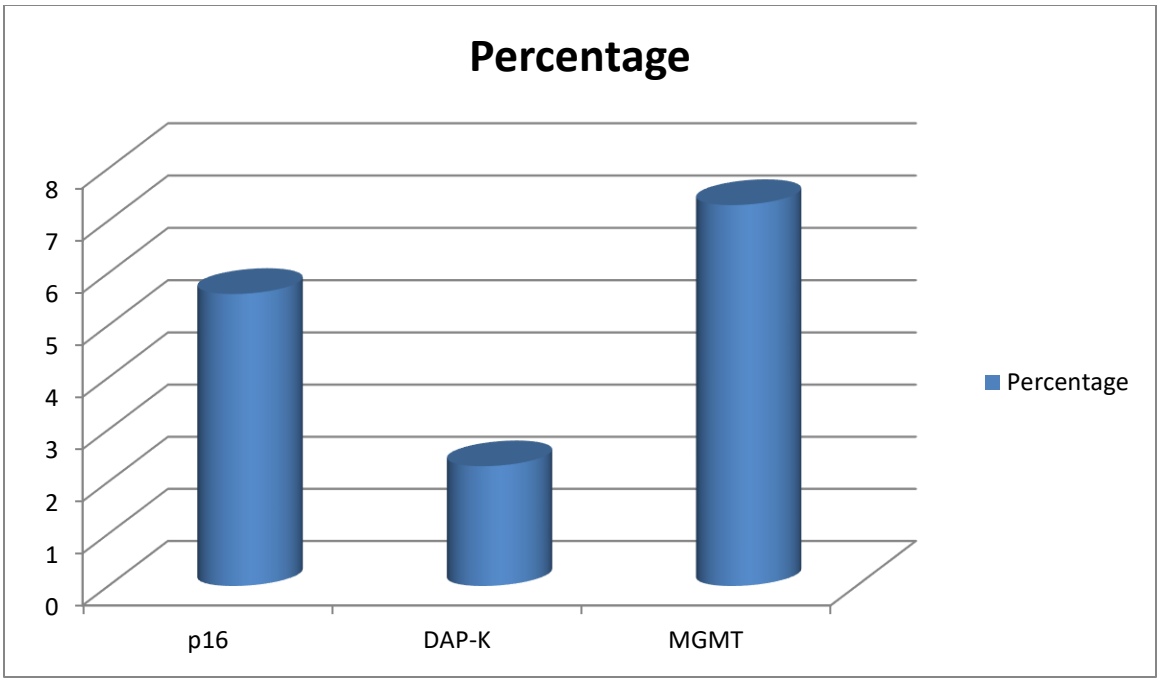
Gene loci	**DNA methylation %**	**P value**
p16	18.3	
DAP-K	23.3	0.001
MGMT	34.4	



Graph 9: DNA methylation of gene p16, gene DAP-K and gene MGMT in saliva samples of tobacco users with no oral potential malignant disorder

Table 10: DNA methylation of gene p16, gene DAP-K and gene MGMT in saliva specimens in normal healthy individuals.

Gene loci	**DNA methylation %**	**P value**
p16	05.6	
DAP-K	02.3	0.07
MGMT	07.3	



Graph 10: DNA methylation of gene p16, gene DAP-K and gene MGMT in saliva specimens in normal healthy individuals.

Table 11 : Identification of oral potential malignant disorders based on Single-Gene Methylation in saliva using cutoff values in tobacco consumers with disorders in oral cavity

Gene/ Sample	**Values of P, Fisher Exact Test**	**Values of (95% CI), % Sensitivity**	**Values of (95% CI), % Specificity**	**Values of (95% CI), % PPV**	**Values of (95% CI), % NPV**
P16 OPMD Control	0.002	91.2 (83.1-98.87)	77.3 (62.0-97.5)	82.1 (72.4-94.3)	91.4 (79.1-99.5)
DAP- K OPMD Control	0.003	91.4 (86.1-98.76)	75.4 (61.0-98.6)	82.6 (72.9-94.3)	91.1 (78.1-99.5)
MGMT OPMD Control	0.001	91.8 (81.1-98.67)	72.9 (62.4-96.5)	82.7 (72.1-93.3)	91.9 (77.1-98.5)

Table 12: Identification of oral potential malignant disorders based on single-Gene Methylation Using Cutoff Values in tobacco consumers without disorders in oral cavity.

Gene/ Sample	**Values of P, Fisher Exact Test**	**Values of (95% CI), % Sensitivity**	**Values of (95% CI), % Specificity**	**Values of (95% CI), % PPV**	**Values of (95% CI), % NPV**
P16 OPMD Control	0.001	91.7 (82.1-93.87)	76.4 (62.1-97.6)	81.9 (70.3-94.7)	91.1 (79.0-99.7)
DAP- K OPMD Control	0.002	91.4 (82.1-91.76)	75.7 (61.1-93.7)	82.1 (75.8-91.4)	91.2 (78.8-98.4)
MGMT OPMD Control	0.007	90.7 (80.1-91.67)	71.2 (61.4-91.5)	82.1 (72.0-93.8)	91.2 (77.9-98.6)

Table 13: Identification of oral potential malignant disorders based on Multiple-Gene Methylation in saliva using cutoff values in tobacco consumers with disorders in oral cavity

Gene/ Sample	**Values of P, Fisher Exact Test**	**Values of (95% CI), % Sensitivity**	**Values of (95% CI), % Specificity**	**Values of (95% CI), % PPV**	**Values of (95% CI), % NPV**
P16+MGMT+DAP-K OPMD Control	0.002	91.3 (84.1-91.87)	77.9 (62.0-97.5)	80.1 (72.4-94.3)	90.4 (79.1-99.5)
DAP- K+P16 OPMD Control	0.003	91.4 (86.1-98.76)	75.1 (61.0-98.6)	82.6 (72.9-94.3)	91.1 (78.1-99.5)
MGMT+P16 OPMD	0.001	91.8 (81.1-98.67)	72.9 (62.4-96.5)	82.7 (72.1-93.3)	91.9 (77.1-98.5)

Control					
MGMT+DAP-K OPMD Control	0.001	90.2 (80.1-91.87)	77.4 (62.0-97.5)	82.1 (72.4-94.3)	91.4 (79.1-99.5)

Table 14: Identification of oral potential malignant disorders based on Multiple-Gene Methylation in saliva specimens using cutoff values in tobacco consumers with disorders in oral cavity

Gene/ Sample	**Values of P, Fisher Exact Test**	**Values of (95% CI), % Sensitivity**	**Values of (95% CI), % Specificity**	**Values of (95% CI), % PPV**	**Values of (95% CI), % NPV**
P16+MGMT+DAP-K OPMD Control	0.007	87.3 (84.1-91.87)	73.9 (62.0-97.5)	76.1 (72.4-94.3)	84.4 (79.1-99.5)
DAP- K+P16 OPMD Control	0.003	91.4 (86.1-98.76)	75.1 (61.0-98.6)	82.6 (72.9-94.3)	91.1 (78.1-99.5)
MGMT+P16 OPMD Control	0.001	91.8 (81.1-98.67)	72.9 (62.4-96.5)	82.7 (72.1-93.3)	91.9 (77.1-98.5)
MGMT+DAP-K OPMD Control	0.001	90.2 (80.1-91.87)	77.4 (62.0-97.5)	82.1 (72.4-94.3)	91.4 (79.1-99.5)

Discussion

This study was conducted to evaluate genomic changes in saliva of individuals with OMPDs. It was found that 33.33% of the study participants were found to have leukoplakia , 11.11% study participants were found to have OSMF, while 44.44% study participants were found to have no oral potential malignant diseases (table 6, graph 6). 50% of the study participants having OPMD were having OPMD in their family members while 50% don't had genetic susceptibility (table 7, graph 7)

In this study methylation of DNA at p16 in saliva samples was observed 25.6% of the oral premalignant lesion, methylation of DAP-K in saliva samples was observed in 32.3% lesions while methylation of MGMT in saliva samples was observed in 30.3% oral premalignant lesions. The finding was statisrically significant. ($p \leq 0.01$).

Despite the fact that oral malignancies are usually accompanied by a well-defined pre-cancerous phase, there are few symptomatic and anatomical criteria for detecting and signalling development between pre-cancer towards cancerous tumors. Shridhar K et al [17] did a critical assessment to summarise the data for abnormal DNA epigenetic modifications as a prospective diagnostic marker for progression prediction. p16 gene, p14 gene, MGMT gene, and DAPK gene were among the highly modified loci identified in multiple studies. 2 longitudinal investigations found that pre-malignant lesions that escalated to carcinoma had more p16 hyper-methylation than lesions that reverted.[20-23] 3 new hyper-methylated regions were discovered in one study that looked at epigenetic modification methylation status likely TRHDE, ZNF454 and KCNAB3). The number of papers that were assessed were short, cross-sectional research with ill-defined control groups.[24-25]

While size of the sample and research designing limitations prevent clear findings, existing data proves that DNA epigenetic modifications sequences could be useful as a predictive marker in mouth pre-cancer advancement.[12-16] To substantiate the recently described signals and find added risk and molecular pathways of progression of the disease, massive epigenetic modification methylation analyses of mouth premalignant lesions with longitudinal tracking are required. The most common oral premalignant lesion, leukoplakia, has a varied incidence of malignant development.[17-19]

In this study, methylation of DNA at p16 in saliva specimens among tobacco users with no diseases was observed in 18.3% cases, methylation of DAP-K in saliva specimens was observed in 23.3% cases while methylation of MGMT in saliva specimens was observed in 34.4 cases. The finding was statistically significant. ($p \leq 0.01$).

In this study methylation of DNA at p16 in saliva specimens among normal healthy individuals was observed in 5.6% cases , methylation of DAP-K in saliva specimens was observed in 2.3% cases while methylation of MGMT in saliva specimens was observed in 7.3% cases. The finding was statistically non-significant. ($p \leq 0.01$). López M et al [18] postulated that detecting molecular abnormalities in oral cytological specimens from individuals at danger of experiencing primary or recurring tumours, such as promoter epigenetic modifications of DNA, could be a useful predictive and therapeutic step in the process of advancement of such lesions to malignancies.

Two categories of subjects were studied, each with a different danger of developing mouth carcinoma . Every patient's mouth lavage was used to isolate DNA. A MSP was used to evaluate the methylation condition of the p16gene promoters , p14 gene promoters , and MGMT gene promoters. P16 and MGMT methylation was found in forty four and fifty six percent of the oral specimens, respectively. P14 methylation was found in just twelve percent of the samples. Patients with a history of OSCC had higher rates of DNA enhanced methylation status. Hypermethylation

of DNA promoters is common in the early stages of oral carcinogenesis, and considerably more so in the advanced stages. MSP with oral washes is a non traumatic , extremely sensitive method for monitoring individuals with malignant and malignant lesions. The potential tumour suppressor genes like kinesin family member 1A and endothelin receptor type B are down regulated in malignancies.[17-24]

In our study the specificity and sensitivity of p16, DAP-K and MGMT in saliva specimens in diagnosis of oral potential malignant disorders is quite significant in tobacco users having diseases. The specificity and sensitivity of p16, DAP-K and MGMT in saliva specimens diagnosis of oral potential malignant disorders is also significant in tobacco users having no diseases. The methylation of combination of gene in saliva in tobaaco consumers having disease was quite significant. The methylation of combination of gene in saliva specimens tobaaco consumers having no disease was also significant.

In prospective obtained salivary washes from participants with precancerous and malignant lesion of oral cavity, Pattani KM et al [19] investigated enhanced methylation promoter region of EDNRB gene and KIF1A gene and their potential utility for risk categorization. The methylation condition of EDNRB gene and KIF1A gene in salivary washes from one hundred ninety one patients was investigated using quantification methylation-specific PCR technique. The next step was to evaluate the relationship between methylation condition and histologic assessment, as well as to evaluate accuracy of classification. On univariate assessments, dysplasia or malignancy diagnosis was linked to ageing and modification of KIF1A gene or EDNRB gene . EDNRB methylation was substantially linked with KIF1A methylation. Histopathologic assessment was significantly linked with EDNRB gene or KIF1A gene methylation in multivariable modelling. Without past biopsy-proven cancer, a subgroup of subjects (n = one hundred sixty one) were given a medical risk approach that relies on examination. EDNRB gene and risk categorization were linked with dysplasia or malignancy diagnosis on univariate modelling and stayed noteworthy on multivariate assessment.

With such a sensitivity of seventy one percentage and specificity of fifty eight percentage, medical risk categorization detected dysplasia or malignancy.[14-18] Clinical risk categorization sensitivity increased to seventy five percent when paired with EDNRB gene modification. EDNRB gene methylation in salivary washes was linked to histopathological diagnoses of precancer and cancer, indicating that it could be used to categorise people in danger for oral pre cancer and cancer lesions in circumstances where a trained dental practitioner is unavailable. This may also potentially identify patients with premalignant and malignant lesions that do not meet the criteria for high clinical risk based on skilled dental examination.[19-24] The tumour suppressor gene death-associated protein kinase (DAPK) has been proposed. In face and neck malignancies as well as other neoplasms, a higher incidence of DAPK gene promoter epigenetic modification has been observed, and this has been employed as a diagnostic biomarker in molecular typing methodologies.[20-25]

Conclusion

The specificity and sensitivity of p16, DAP-K and MGMT in saliva specimens in diagnosis of oral potential malignant disorders is quite significant in tobacco users as well as non tobacco users. The specificity and sensitivity of p16, DAP-K and MGMT in saliva specimens in diagnosis of oral potential malignant disorders is quite significant in tobacco users having diseases. The specificity and sensitivity of p16, DAP-K and MGMT in saliva specimens diagnosis of oral potential malignant disorders is also significant in tobacco users having no diseases. The methylation of combination of gene in saliva in tobaaco consumers having disease was quite significant. The methylation of combination of gene in saliva specimens tobaaco consumers having no disease was also significant

References

1. Breuer RH, Snijders PJ, Sutedja GT, et al. Expression of the p16(INK4a) gene product, methylation of the p16(INK4a) promoter region and expression of the polycomb-group gene BMI-1 in squamous cell lung carcinoma and premalignant endobronchial lesions. Lung Cancer. 2005; 48(3):299–306.
2. Mao L, Lee JS, Fan YH, et al. Frequent microsatellite alterations at chromosomes 9p21 and 3p14 in oral premalignant lesions and their value in cancer risk assessment. Nat Med. 1996;2(6):682–685.
3. Rosin MP, Cheng X, Poh C, et al. Use of allelic loss to predict malignant risk for low-grade oral epithelial dysplasia. Clin Cancer Res. 2000;6(2): 357–362.
4. Cohen O, Inbal B, Kissil JL, et al. DAP-kinase participates in TNF-alpha- and Fas-induced apoptosis and its function requires the death domain. J Cell Biol. 1999;146(1):141–148.
5. Levy-Strumpf N, Kimchi A. Death associated proteins (DAPs): From gene identification to the analysis of their apoptotic and tumor suppressive functions. Oncogene. 1998;17(25):3331–3340.
6. Merlo A, Herman JG, Mao L, et al. 5’ CpG island methylation is associated with transcriptional silencing of the tumour suppressor p16/CDKN2/MTS1 in human cancers. Nat Med. 1995;1(7): 686–692.
7. Nuovo GJ, Plaia TW, Belinsky SA, Baylin SB, Herman JG. In situ detection of the hypermethylation-induced inactivation of the p16 gene as an early event in oncogenesis. Proc Natl AcadSci U S A. 1999; 96(22):12754–12759.
8. Pegg AE, Dolan ME, Moschel RC. Structure, function, and inhibition of O6-alkylguanine-DNA alkyltransferase. Prog Nucleic Acid Res Mol Biol. 1995;51:167–223.
9. Esteller M, Risques RA, Toyota M, et al. Promoter hypermethylation of the DNA repair gene O(6)-methylguanine-DNA methyltransferase is associated with the presence of G:C to A:T transition mutations in p53 in human colorectal tumorigenesis. Cancer Res. 2001;61(12): 4689–4692.

10. Esteller M, Toyota M, Sanchez-Cespedes M, et al. Inactivation of the DNA repair gene O6-methylguanine-DNA methyltransferase by promoter hypermethylation is associated with G to A mutations in K-ras in colorectal tumorigenesis. Cancer Res. 2000;60(9):2368–2371.

11. Dawes K, Andersen A, Reimer R, Mills JA, Hoffman E, Long JD, Miller S, Philibert R. The relationship of smoking to cg05575921 methylation in blood and saliva DNA samples from several studies. Sci Rep. 2021 Nov 3;11(1):21627. doi: 10.1038/s41598-021-01088-7. PMID: 34732805; PMCID: PMC8566492.

12. Philibert R, Dogan M, Beach SRH, Mills JA, Long JD. AHRR methylation predicts smoking status and smoking intensity in both saliva and blood DNA. Am J Med Genet B Neuropsychiatr Genet. 2020 Jan;183(1):51-60. doi: 10.1002/ajmg.b.32760. Epub 2019 Aug 27. PMID: 31456352.

13. Dawes K, Andersen A, Vercande K, et al. Saliva DNA Methylation Detects Nascent Smoking in Adolescents. *J Child Adolesc Psychopharmacol*. 2019;29(7):535-544. doi:10.1089/cap.2018.0176.

14. Andersen A, Reimer R, Dawes K, Becker A, Hutchens N, Miller S, Dogan M, Hundley B, A Mills J, D Long J, Philibert R. DNA methylation differentiates smoking from vaping and non-combustible tobacco use. Epigenetics. 2021 Feb 25:1-13. doi: 10.1080/15592294.2021.1890875. Epub ahead of print. PMID: 33588690.
15. Andersen A, Gerrard M, Gibbons FX, Beach SRH, Philibert R. An Examination of Risk Factors for Tobacco and Cannabis Smoke Exposure in Adolescents Using an Epigenetic Biomarker. Front Psychiatry. 2021 Aug 24;12:688384. doi: 10.3389/fpsyt.2021.688384. PMID: 34504443; PMCID: PMC8421639.

16. Richmond RC, Sillero-Rejon C, Khouja JN, Prince C, Board A, Sharp G, Suderman M, Relton CL, Munafò M, Gage SH. Investigating the DNA methylation profile of e-cigarette use. Clin Epigenetics. 2021 Sep 28;13(1):183. doi: 10.1186/s13148-021-01174-7. PMID: 34583751; PMCID: PMC8479883.

17. Shridhar K, Walia GK, Aggarwal A, et al. DNA methylation markers for oral pre-cancer progression: A critical review [published correction appears in Oral Oncol. 2016 Sep;60:e1]. *Oral Oncol*. 2016;53:1-9. doi:10.1016/j.oraloncology.2015.11.012

18. López M, Aguirre JM, Cuevas N, Anzola M, Videgain J, Aguirregaviria J, Martínez de Pancorbo M. Gene promoter hypermethylation in oral rinses of leukoplakia patients--a diagnostic and/or prognostic tool? Eur J Cancer. 2003 Nov;39(16):2306-9. doi: 10.1016/s0959-8049(03)00550-1. PMID: 14556921.

19. Pattani KM, Zhang Z, Demokan S, Glazer C, Loyo M, Goodman S, Sidransky D, Bermudez F, Jean-Charles G, McCaffrey T, Padhya T, Phelan J, Spivakovsky S, Bowne HY, Goldberg JD, Rolnitzky L, Robbins M, Kerr AR, Sirois D, Califano JA. Endothelin receptor type B gene promoter hypermethylation in salivary rinses is independently associated with risk of oral cavity cancer and premalignancy. Cancer Prev Res (Phila). 2010 Sep;3(9):1093-103. doi: 10.1158/1940-6207.CAPR-10-0115. Epub 2010 Aug 26. PMID: 20798208; PMCID: PMC2945229.

20. Liu Y, Zhou ZT, He QB, Jiang WW. DAPK promoter hypermethylation in tissues and body fluids of oral precancer patients. Med Oncol. 2012 Jun;29(2):729-33. doi: 10.1007/s12032-011-9953-5. Epub 2011 Apr 24. PMID: 21516484.
21. Cao J, Zhou J, Gao Y, Gu L, Meng H, Liu H, Deng D. Methylation of p16 CpG island associated with malignant progression of oral epithelial dysplasia: a prospective cohort study. Clin Cancer Res. 2009 Aug 15;15(16):5178-83. doi: 10.1158/1078-0432.CCR-09-0580. Epub 2009 Aug 11. PMID: 19671846.
22. Liu HW, Hu BQ, Cao CF. [The p16 methylation in oral leukoplakia and oral squamous cell carcinoma]. Zhonghua Kou Qiang Yi Xue Za Zhi. 2005 Mar;40(2):94-7. Chinese. PMID: 15842853.
23. Kresty LA, Mallery SR, Knobloch TJ, Song H, Lloyd M, Casto BC, Weghorst CM. Alterations of p16(INK4a) and p14(ARF) in patients with severe oral epithelial dysplasia. Cancer Res. 2002 Sep 15;62(18):5295-300. PMID: 12234999.

24. Kresty LA, Mallery SR, Knobloch TJ, Li J, Lloyd M, Casto BC, Weghorst CM. Frequent alterations of p16INK4a and p14ARF in oral proliferative verrucous leukoplakia. Cancer Epidemiol Biomarkers Prev. 2008 Nov;17(11):3179-87. doi: 10.1158/1055-9965.EPI-08-0574. PMID: 18990760.

25. Bagan L, Sáez GT, Tormos MC, Labaig-Rueda C, Murillo-Cortes J, Bagan JV. Salivary and serum interleukin-6 levels in proliferative verrucous leukoplakia. Clin Oral Investig. 2016 May;20(4):737-43. doi: 10.1007/s00784-015-1551-z. Epub 2015 Aug 9. PMID: 26254143.