**Association of Over-expression of MCM3 with Oral Squamous Cell Carcinoma**

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**Abstract**

**BACKGROUND:** Oral squamous cell carcinoma (OSSC) arises from premalignant oral lesions (PMOL) in majority of the cases. Minichromosome maintenance (MCM) 3 is a proliferative marker that has been investigated as a potential diagnostic biomarker in oral cancer. **OBJECTIVES:**  To evaluate the association between MCM3 expression and clinicopathologic parameters and identify snuff (naswar) as a potential risk factor for changes in MCM3 expression in PMOL and OSCC

**METHODS:** Immunohistochemistry (IHC) of MCM3 was performed on 32 PMOL, 32 OSCC and 16 normal controls after optimization of IHC methodology. Histoscore (0-300) was used as a scoring system and seven different cut-offs were identified for analyses.

**RESULTS:** Among the seven cutoffs, 40% strong positive cells was found a better cut-off as it was associated with many pathological variables (Broder’s, Aneroth’s grade, mitotic activity**).** The differential MCM3 expression in oral lesions (PMOL and OSCC), was statistically significant (p<0.05). Moreover, MCM3 expression is raised with increased duration and frequency of snuff use.

**CONCLUSION**: High MCM3 expression is associated with disease progression and is a potential indicator of the malignant transformations from PMOL to OSCC. Moreover, snuff use is associated with MCM3 over expression.

**Keywords:** Oral squamous cell carcinoma (OSCC), Premalignant oral lesions (PMOL), Minichromosome maintenance (MCM) 3, Naswar (Snuff)

**Background**

Cancer of the oral cavity is one of the most frequent cancer types worldwide. More than 95% of the head and neck cancers are oral squamous cell carcinoma[[1](#_ENREF_1)]. It is the third most common malignancy in Pakistan and tenth worldwide[[2](#_ENREF_2)]. It is the eighth common malignancy in men and fifth in women[[3](#_ENREF_3)]. Males are affected more frequently as compared to females (1.5:1) most probably because of high involvement of men in high risk habits e.g. smoking, etc. as compared to females [[4](#_ENREF_4)]. According to WHO, oral cancer has a death rate of about 45% from 5 years after diagnosis. The chances of survival also depend on the stage of disease with poor prognosis in higher disease stage [[5](#_ENREF_5)].

OSCC has a multifactorial etiology. There is no specific cause but snuff dipping and alcohol consumption is associated with 90% of the patients diagnosed with oral cancer[[6](#_ENREF_6)]. Snuff is the most common smokeless tobacco used in Pakistan. It is made up of sun and heat dried tobacco leaves, slaked lime, tree bark ash, flavoring and coloring agents. [[7](#_ENREF_7)]

Though not all, but most of the oral cancers precede premalignant oral lesions (PMOL). Among all PMOL the most common are leukoplakia and erythroplakia [[8](#_ENREF_8),[9](#_ENREF_9)]. Other potentially malignant conditions which progress to OSCC are lichen planus, submucous fibrosis and tobacco induced keratosis [[10](#_ENREF_10),[11](#_ENREF_11)].

The diagnosis of PMOL and OSCC is established from clinical and pathological assessment of lesions. Higher grade and stage disease predicts worse outcomes [[12](#_ENREF_12)]. However, patients with similar pathological characteristics behave differently which points to the fact that patients are different at molecular level. Molecular biomarker may thus provide a mechanistic insight into the tumour biology. Moreover, changes in the genes or proteins involved in carcinogenesis from risk factors e.g. naswar may provide a further insight into the risk factors associated with the disease.

Cell proliferation has a role in the growth and maintenance of tissue homeostasis. The control of this important process is completely dysregulated in cancer. There are various molecular mechanisms causing excessive proliferation followed by the transformation from normal mucosa to PMOL and consequently to OSCC[[13](#_ENREF_13)]. MCM3 protein is one of the biological markers, used to assess the proliferative activities of cells in neoplastic lesions. It is a subunit of Minichromosome maintenance (MCM) proteins family i.e. MCM2-7, which are the key components of the replication initiation complex that initiates DNA synthesis. MCM proteins bind at DNA replication origin and subsequently interact with origin recognition complex (ORC) and Cdc6 protein to form the prereplicative complex. This DNA synthesis regulation ensures that during each cell cycle DNA replicates only once. MCMs are expressed throughout the cell cycle, but in dormant and differentiating cells its expression is lost. Its expression is up- regulated in proliferating cells, whereas in differentiated and quiescent cells its expression decreases significantly.

Since MCM3 identify both cycling and non-cycling cells with proliferative potential due to its persistence throughout the cell cycle, so antibodies against MCM3 identify more cells in tissue in comparison with other proliferating markers i.e. Ki 67, PCNA[[14](#_ENREF_14)]. Because of its expression in early G1 phase, MCM studies are relevant for determining tumor behavior[[15](#_ENREF_15)].

Thus, we evaluated the potential association of over expression of MCM3 with tumor pathology. In addition, the expression of MCM3 was evaluated in the differential diagnosis of PMOL and OSCC. Finally, snuff was investigated as a potential risk factor for MCM3 over expression. The findings of this study are thus anticipated to have both research and clinical implications.

**Methods**

Histological sections from formalin fixed paraffin embedded (FFPE) tissue blocks were taken from pathology department of Rehman Medical Institute (RMI) and maxillofacial surgery department of Khyber College of Dentistry (KCD). Eighty two cases were selected for the study. The sections include 32 cases of OSCC, 32 cases of PMOL and 16 cases of normal oral mucosa. Patient’s consent and ethical approval was taken from all the concerned institutes and hospitals. Two sets of tissue sections of 4µm were taken from tissue blocks. One set for Hematoxylin and Eosin (H&E) staining and other for immunohistochemistry (IHC) staining in order to investigate the expression of the biomarker.

**Immunostaining:** Before performing the final IHC staining, the antibody (MCM3) was optimized to identify preferred IHC parameters (Table 1). The immunostaining was done on 4µm thick section. After passing through xylene and rehydration in graded alcohol the tissue sections were immersed in Tris EDTA (pH 9.0) and then hydrogen peroxide was used to block peroxidase activity. Monoclonal Mouse Anti-Human; M7263, clone 101(DAKO Ltd) was used followed by secondary antibody. Finally, DAB was used for antibody detection. The optimal staining achieved with IHC conditions was then used on tissue sections of normal oral mucosa, PMOL and OSCC.

**Scoring of tissue sections:** H&E stained sections were evaluated for Broder’s and Aneroth’s grading and other pathological parameters. IHC stained tissue sections were scored for both staining intensity and proportion of cells. A histoscore [0× % positive cells + 1 × positive cells + 2 × positive cells + 3 × positive cells] was generated (range 0-300). Overexpression and underexpression of MCM3 expression was based on a variety of staining cut-offs.

**Statistics and data analysis**: The expression of MCM3 was grouped into underexpression and overexpression according to seven different cutoffs on the basis of mean value of the recorded histoscore i.e. 92±59 [[16](#_ENREF_16)], median histoscore i.e. 100, 10%, 20%, 30% and 40% strong positive cells[[17](#_ENREF_17)] and percentage of the positive tumor cells of any intensity (50%) ( table 2) [[18](#_ENREF_18)].Data was analyzed using different tests i.e. Chi-square test for finding the relation between different variables i.e. oral lesions, Broder’s grade, Aneroth’s grade, TNM staging, mitotic figures, age and gender with MCM3 expression. While spearmen rho was used for the association between snuff use, its duration (in years) and frequency of snuff use per day with MCM3 expression.

The level of significance was set at P value < 0.05. SPSS version 23 was used for statistical analyses.

**Results**

Clinical outcome of the patients having oral lesions are depicted in table 3. The mean age at the time of diagnosis was 60 years. Out of the total n=82 cases 55 were male and 27 were female with a male: female ratio of 2:1. Site distribution in the selected patients showed cheek mucosa (43%) as the most common site.

MCM3 expression was evaluated on the basis of intensity and proportion of staining (Fig. 1).The relationship between different clinical and histopathological parameters and MCM3 expression was evaluated on the basis of seven IHC cutoffs i.e. mean, median, 10% strong positive cells, 20% strong positive cells, 30% strong positive cells, 40% strong positive cells and percentage of positive tumor cells. The cutoff, 40% strong positive cells was found to have better relationship with clinicopathological variables, presented in table 4-11 along with results of other six. On the basis of IHC scoring, 40% and above strong positive tumor cells would be regarded as overexpression and below 40% as under expression.

A statistically significant difference was observed in the expression level of MCM3 among normal, PMOL and OSCC (p= 0.03). Considering the 40% strong positive cells, there is a statistically significant difference in the underexpression and overexpression categories of MCM3 (p= 0.02) regarding Broder’s grade and Aneroth’s grade. MCM3 expression was not statistically significantly associated with TNM stage (p= 0.83). Mitotic figures were statistically associated with expression levels of MCM3 (p= .008)

Finally, a weak to moderate association was found between snuff use, its duration and frequency with MCM3 expression (p= .015, .004 and .027 respectively at 40% strong positive cells cut off)

**DISCUSSION**

The present study focused on the differential expression of MCM3 in normal oral mucosa, premalignant and OSCC tissue samples. The relation between MCM3 expression was assessed with various clinico-pathological parameters (TNM stage, Broder’s grade, Aneroth grade, mitotic figures etc.)and snuff use (duration and frequency). The results showed a statistically significant differential expression of MCM3in various oral lesions. MCM3 expression was also significantly associated with a number of pathological variables including Broder’s grade, Anneroth’s grade and mitotic figures. However no association was observed between MCM3 overexpression and the clinical variables including age, gender and TNM stage. The duration and frequency of snuff use was also significantly correlated with MCM3 overexpression.

The results showed a stepwise increase of MCM3 expression from no staining to very weak staining in normal oral mucosa samples, weak to moderate staining in premalignant oral lesions and moderate to strong staining in oral squamous cell carcinoma samples. These results are in agreement with Rezvani G et al (2015)[[19](#_ENREF_19)]. This differential expression of MCM3 in normal, premalignant and OSCC observed using all 7 cut offs indicates that MCM3 overexpression carries significant diagnostic potential in premalignant lesions and SCC of the oral cavity.

MCM3 overexpression shows significant association with increasing Broder’s histological grade using two out of seven cut offs including40% strong positive cells and percentage of positive cells. Increasing Aneroth’s histological grade in OSCCs was also found to be significantly associated with MCM3 overexpression using 40% strong positive cells as a cut off. The gradual increase in MCM3 expression with disease grade is in agreement with N. Gan et al (2010)[20]These results suggest that the less the differentiated cells, the more will be the proliferative activity and an increased expression.MCM3

Association with TNM staging was found at 20% strong positive cells. The results were in agreement with Verena Karla Monteiro LOPES et al 2017[21], showing no significant relation between TNM staging and MCM3 expression assessed using the remaining cut-offs. Therefore the role of MCM3 expression in evaluating disease severity still remains unclear.

MCM3 overexpression was found to be associated with an increased number of mitotic figures using two cut offs which include 40% strong positive cells and percentage of positive cells. This suggests that MCM3 overexpression might be an indicator of proliferating activity in malignant squamous epithelial cells.

The correlation between clinical parameter i.e. age and gender was tested which shows insignificant results. These results were in agreement with Verena Karla Monteiro LOPES et al 2017[20]. Therefore the role of age and gender in malignant transformation is not clear.

Since snuff is considered as a risk factor for the progression of cancer; thus, it was investigated with MCM3 expression to observe any association between the two variables. Our result show significant association at 20%, 30% and 40% strong positive cells showing that there is a moderate positive association between the naswar and MCM3 expression. This indicates that there is an increase MCM3 expression in snuff users. The association between duration of snuff use and MCM3 expression shows moderate positive and significant results at 10%, 20%, 30% and 40% showing that increased duration of snuff use also increasesMCM3 expression. The result of frequency of snuff use and MCM3 show significant association at 40% strong positive cells. This moderate-strong positive association between MCM3 overexpression in OSCCs and frequency of snuff used per day indicates that snuff use is related to increased proliferative activity via MCM3 overexpression. Therefore snuff use might be considered as a potential risk factor for changes in MCM3 expression in PMOL and OSCC

The main study design limitation is the total number of OSCC cases which affects the data analysis and the results implementation in population. Regarding data, there was very little premalignant and cancer registries in our hospitals. The clinical data especially of snuff use and its type from patients was incomplete. Survival analysis was not possible due to the limited `availability of survival data.

# CONCLUSION

In conclusion the stepwise increasing differential expression of MCM3 from normal oral mucosa to PMOL and OSCC samples in our study points towards the potential role of MCM3 in malignant transformation. Moreover, the association between snuff use and MCM3 overexpression in our OSCC samples reveals the potential molecular role of snuff in the overexpression of MCM3 protein and carcinogenesis. The increased expression level of MCM3 and its association with high mitotic activity further shows that the assessment of this protein could prove useful as a diagnostic marker in OSCCs.

Thus, we conclude from our study that MCM3 could be a valuable marker in differential diagnosis of premalignant oral lesions and oral squamous cell carcinoma. It could be considered as a surrogate marker of malignant transformation. There exists in the study the probability of application of MCM3 for molecular targeted therapy in diseased patients.

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**Conflict of Interests**

None Declared.

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**Table 1: Final immunohistochemistry parameters set on archival tissue sections for MCM**

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | | | | | | | | | |
| Antibody | Company | Clone | Antigen retrieval | PH | Antibody dilution | Retrieval time | Incubation temperature (°Ċ) | Incubation time (hour) | Incubation method |
| MCM3 | DAKO | 101 | Heat induced epitope retrieval (HIER) | Tris EDTA PH=9 | 1/50 | 1 hour | 100 | 1 hour | Oven |
|
|
|
|

**Table 2: Different set cut offs for data analysis**

|  |  |  |  |
| --- | --- | --- | --- |
| **Statistical method** | **Cut-off** | | |
|  | | **Over expression** | **Under expression** |
| Mean | | >92 | ≤92 |
| Median | | >100 | ≤100 |
| 10% strong positive cells | | >10% | ≤10% |
| 20% strong positive cells | | >20% | ≤20% |
| 30% strong positive cells | | >30% | ≤30% |
| 40% strong positive cells | | >40% | ≤40% |
| Percentage of positive cells | | >50% | ≤50% |

**Table 3: Demographic parameters and their observations**

|  |  |  |
| --- | --- | --- |
| **Demographic** | | **Observation (Number of cases)** |
| **oral lesions** | Normal  PMOL  OSCC | 16  34  32 |
| **Age** | ≤ 60  >60 | 58  34 |
| **Gender** | Male  Female | 55  27 |
| **Location of tumors** | Cheek mucosa/ sulcus  Gingival/ alveolar mucosa  Tongue  Lip  Floor of the mouth  Palate | 28  18  14  7  4  2 |

**Table 4: Differential MCM3 expression and oral lesions**

|  |  |  |  |
| --- | --- | --- | --- |
| **Statistical method** | **MCM3**  **Over expression** | **MCM3**  **under expression** | **P-value** |
| **Mean** | Normal: 0  PMOL: 14  OSCC: 29 | Normal: 16  PMOL: 20  OSCC: 03 | .000 |
| **Median** | Normal: 0  PMOL: 12  OSCC: 30 | Normal: 16  PMOL: 22  OSCC: 02 | .000 |
| **10% strong positive cells** | Normal: 0  PMOL: 10  OSCC: 28 | Normal: 16  PMOL: 24  OSCC: 04 | .000 |
| **20% strong positive cells** | Normal: 0  PMOL: 0  OSCC: 25 | Normal: 16  PMOL: 34  OSCC: 07 | .000 |
| **30% strong positive cells** | Normal: 0  PMOL: 0  OSCC: 14 | Normal: 16  PMOL: 34  OSCC: 18 | .000 |
| **40% strong positive cells** | Normal: 0  PMOL: 0  OSCC: 04 | Normal: 16  PMOL: 34  OSCC: 28 | .037 |
| **Percentage of positive cells** | Normal: 0  PMOL: 1  OSCC: 17 | Normal: 16  PMOL: 33  OSCC: 15 | .000 |

**Table 5: MCM3 expression correlation with clinicopathologic parameters at 40% strong positive cells cutoff**

|  |  |  |  |
| --- | --- | --- | --- |
| **Parameters** | **MCM3**  **Over expression** | **MCM3**  **under expression** | **P-value** |
| **Oral lesions**  **Normal**  **PMOL**  **OSCC** | 0  0  04 | 16  34  28 | .03 |
| **Broder’s grade**  **Well diff: OSCC**  **Moderately diff: OSCC**  **Poorly diff: OSCC** | 2  1  1 | 22  6  0 | .02 |
| **Aneroth’s grade**  **Grade I**  **Grade II**  **Grade III** | 1  2  1 | 15  13  0 | .02 |
| **TNM stage**  **Stage I**  **Stage II**  **Stage III**  **Stage IV** | 2  1  1  0 | 15  3  8  2 | .83 |
| **Mitotic figures**  **(0-3)**  **(≥ 4)** | 1  3 | 26  2 | .008 |

**Table 6: MCM3 correlation with Broder’s grade**

|  |  |  |  |
| --- | --- | --- | --- |
| **Statistical method** | **MCM3**  **Over expression** | **MCM3**  **under expression** | **P-value** |
| **Mean** | Well diff SCC: 23  Moderately diff SCC: 6  Poorly diff SCC: 1 | Well diff SCC: 1  Moderately diff SCC: 1  Poorly diff SCC: 0 | .602 |
| **Median** | Well diff SCC: 23  Moderately diff SCC: 6  Poorly diff SCC: 1 | Well diff SCC: 1  Moderately diff SCC: 1  Poorly diff SCC: 0 | .602 |
| **10% strong positive cells** | Well diff SCC: 22  Moderately diff SCC: 5  Poorly diff SCC: 1 | Well diff SCC: 2  Moderately diff SCC: 2  Poorly diff SCC: 0 | .337 |
| **20% strong positive cells** | Well diff SCC: 19  Moderately diff SCC: 5  Poorly diff SCC: 1 | Well diff SCC: 5  Moderately diff SCC: 2  Poorly diff SCC: 0 | .787 |
| **30% strong positive cells** | Well diff SCC: 10  Moderately diff SCC: 3  Poorly diff SCC: 1 | Well diff SCC: 14  Moderately diff SCC: 4  Poorly diff SCC: 0 | .514 |
| **40% strong positive cells** | Well diff SCC: 2  Moderately diff SCC: 1  Poorly diff SCC:1 | Well diff SCC: 22  Moderately diff SCC: 6  Poorly diff SCC: 0 | .025 |
| **Percentage of positive cells** | Well diff SCC: 10  Moderately diff SCC: 6  Poorly diff SCC:1 | Well diff SCC: 14  Moderately diff SCC: 1  Poorly diff SCC: 0 | .077 |

**Table 7: MCM3 expression correlation with Aneroth grade**

|  |  |  |  |
| --- | --- | --- | --- |
| **Statistical method** | **MCM3**  **Over expression** | **MCM3**  **under expression** | **P-value** |
| **Mean** | Grade I: 16  Grade II: 13  Grade III: 1 | Grade I: 0  Grade II: 2  Grade III: 0 | .299 |
| **Median** | Grade I: 16  Grade II: 13  Grade III: 1 | Grade I: 0  Grade II: 2  Grade III: 0 | .299 |
| **10% strong positive cells** | Grade I: 15  Grade II: 12  Grade III: 1 | Grade I: 1  Grade II: 3  Grade III: 0 | .476 |
| **20% strong positive cells** | Grade I: 1  Grade II: 14  Grade III: 10 | Grade I: 2  Grade II: 5  Grade III: 0 | .324 |
| **30% strong positive cells** | Grade I: 8  Grade II: 5  Grade III: 1 | Grade I: 8  Grade II: 10  Grade III: 0 | .333 |
| **40% strong positive cells** | Grade I: 1  Grade II: 2  Grade III: 1 | Grade I: 15  Grade II: 13  Grade III: 0 | .023 |
| **Percentage of positive cells** | Grade I: 7  Grade II: 9  Grade III: 1 | Grade I: 9  Grade II: 6  Grade III: 0 | .421 |

**Table 8: MCM3 expression correlation with TNM stage**

|  |  |  |  |
| --- | --- | --- | --- |
| **Statistical method** | **MCM3**  **Over expression** | **MCM3**  **under expression** | **P-value** |
| **Mean** | Stage I: 17  Stage II: 4  Stage III: 7  Stage IV: 2 | Stage I: 0  Stage II: 0  Stage III:2  Stage IV: 0 | .142 |
| **Median** | Stage I: 17  Stage II: 4  Stage III: 7  Stage IV: 2 | Stage I: 0  Stage II: 0  Stage III: 2  Stage IV: 0 | .142 |
| **10% strong positive cells** | Stage I: 16  Stage II: 4  Stage III: 6  Stage III: 2 | Stage I: 1  Stage II: 0  Stage III: 3  Stage IV: 0 | .164 |
| **20% strong positive cells** | Stage I: 15  Stage II: 4  Stage III: 6  Stage IV: 0 | Stage I: 2  Stage II: 0  Stage III: 3  Stage IV: 2 | .019 |
| **30% strong positive cells** | Stage I: 7  Stage II: 3  Stage III: 4  Stage IV : 0 | Stage I: 10  Stage II: 1  Stage III: 5  Stage IV: 2 | .363 |
| **40% strong positive cells** | Stage I: 2  Stage II: 1  Stage III: 1  Stage IV: 0 | Stage I: 15  Stage II: 3  Stage III: 8  Stage IV: 2 | .830 |
| **Percentage of positive cells** | Stage I: 9  Stage II: 3  Stage III: 3  Stage IV: 2 | Stage I: 8  Stage II: 1  Stage III: 6  Stage IV: 0 | .267 |

**Table 9: MCM3 expression correlation with mitotic figures**

|  |  |  |  |
| --- | --- | --- | --- |
| **Statistical method** | **MCM3**  **Over expression** | **MCM3**  **under expression** | **P-value** |
| **Mean** | Mitotic figure(0-3): 25  Mitotic figure(≥ 4): 5 | Mitotic figure(0-3): 2  Mitotic figure(≥ 4): 0 | 1.000 |
| **Median** | Mitotic figure(0-3): 25  Mitotic figure(≥ 4): 5 | Mitotic figure(0-3): 2  Mitotic figure(≥ 4): 0 | 1.000 |
| **10% strong positive cells** | Mitotic figure(0-3): 24  Mitotic figure(≥ 4): 4 | Mitotic figure(0-3): 3  Mitotic figure(≥ 4): 1 | .512 |
| **20% strong positive cells** | Mitotic figure(0-3): 21  Mitotic figure(≥ 4): 4 | Mitotic figure(0-3): 6  Mitotic figure(≥ 4): 1 | 1.000 |
| **30% strong positive cells** | Mitotic figure(0-3): 11  Mitotic figure(≥ 4): 3 | Mitotic figure(0-3): 16  Mitotic figure(≥ 4): 2 | .631 |
| **40% strong positive cells** | Mitotic figure(0-3): 1  Mitotic figure(≥ 4): 3 | Mitotic figure(0-3): 26  Mitotic figure(≥ 4): 2 | .008 |
| **Percentage of positive cells** | Mitotic figure(0-3): 12  Mitotic figure(≥ 4): 5 | Mitotic figure(0-3): 15  Mitotic figure(≥ 4): 0 | .046 |

**Table 10: Snuff use association with MCM3 expression**

|  |  |  |  |
| --- | --- | --- | --- |
| **Statistical method** | **Correlation coefficient** | | **P-value** |
| **Snuff use (1=Yes, 2=No)** | **MCM3 expression** |
| **Mean** | 1 | .192 | .152 |
|  | .192 | 1 |  |
| **Median** | 1.000 | .159 | .238 |
|  | .159 | 1.000 |  |
| **Percentage of positive cell** | 1.000 | .225 | ..09 |
|  | .225 | 1.000 |  |
| **10% strong positive cells** | 1.000 | .151 | .263 |
|  | .151 | 1.000 |  |
| **20% strong positive cells** | 1.000 | .330 | .012 |
|  | .330 | 1.000 |  |
| **30% strong positive cells** | 1.000 | .364 | .005 |
|  | .364 | 1.000 |  |
| **40% strong positive cells** | 1.000 | .322 | .015 |
|  | .322 | 1.000 |  |

**Table 11: Association of duration of Snuff use with MCM3 expression**

|  |  |  |  |
| --- | --- | --- | --- |
| **Statistical method** | **Correlation coefficient** | | **P-value** |
| **duration of Snuff use (years)** | **MCM3 expression** |
| **Mean** | 1.000 | .301 | .163 |
|  | .301 | 1.000 |  |
| **Median** | 1.000 | .301 | .163 |
|  | .301 | 1.000 |  |
| **Percentage of positive cell** | 1.000 | -.158 | .460 |
|  | -.158 | 1.000 |  |
| **10% strong positive cells** | 1.000 | .360 | ..084 |
|  | .360 | 1.000 |  |
| **20% strong positive cells** | 1.000 | .358 | .094 |
|  | .358 | 1.000 |  |
| **30% strong positive cells** | 1.000 | .457 | ..028 |
|  | .457 | 1.000 |  |
| **40% strong positive cells** | 1.000  .581 | .581  1.000 | .004 |

**Table 13: Association of frequency of Snuff use with MCM3 expression**

|  |  |  |  |
| --- | --- | --- | --- |
| **Statistical method** | **Correlation coefficient** | | **P-value** |
| **Frequency of Snuff use/day** | **MCM3 expression** |
| **Mean** | 1.000 | .277 | .211 |
|  | .277 | 1.000 |  |
| **Median** | 1.000 | .277 | .211 |
|  | .277 | 1.000 |  |
| **Percentage of positive cell** | 1.000 | -.223 | .307 |
|  | -.223 | 1.000 |  |
| **10% strong positive cells** | 1.000 | .227 | .211 |
|  | .227 | 1.000 |  |
| **20% strong positive cells** | 1.000 | .204 | .362 |
|  | .204 | 1.000 |  |
| **30% strong positive cells** | 1.000 | .325 | .139 |
|  | .325 | 1.000 |  |
| **40% strong positive cells** | 1.000  .471 | .471  1.000 | .027 |

**Figure 1: staining intensity of proliferative oral epithelial cells**

|  |  |  |
| --- | --- | --- |
| **Low intensity strong intensity** | | |
| **Normal oral mucosa** | C:\Users\Dr. Rabia\Desktop\pics\normal251.jpg  **AA** | C:\Users\Dr. Rabia\Desktop\pics\(normal 252.jpg  **B** |
| **Premalignant oral lesions** | C:\Users\Dr. Rabia\Desktop\pics\PM 17521.jpg  **C** | C:\Users\Dr. Rabia\Desktop\pics\PM13665.jpg  **D** |
| **Oral Squamous Cell Carcinoma** | C:\Users\Dr. Rabia\Desktop\pics\OSCC 805.jpg  **E** | C:\Users\Dr. Rabia\Desktop\pics\OSCC 1034.jpg  **F** |

**Figure 1:** showing weak and strong staining of proliferating cells of different oral lesions at mean 92 cut off. A & B: low and strong intensity MCM3 expression in normal oral mucosa respectively. C & D: low and strong intensity MCM3 expression in PMOL respectively. E & F: low and strong intensity MCM3 expression OSCC respectively.