(International Print/Online Journal)

SJIF IMPACT FACTOR: 5.565
PUBMED-National Library of Medicine ID-101739732

ISSN (Print): 2209-2870
ISSN (Online): 2209-2862

International Journal of Medical Science and Current Research (IJMSCR)
Available online at: www.ijmscr.com
Volume 4, Issue 3, Page No: 321-329
May-June 2021

Expression of Cancer stem cell marker CD133 with clinicopathogical parameters in Tobacco induced Oral submucosal carcinoma and Oral submucosal fibrosis

Shraddha Awasthi[1]*, Ausaf Ahmad [2] , Anand Narain Srivastava[1]
[1]Department of Pathology, ERA University, Sarfarazganj, Hardoi Road, Lucknow, Uttar Pradesh, India
[2] Amity Institutes of Biotechnology, Amity University Uttar Pradesh, Lucknow, Uttar Pradesh, India

***Corresponding Author:**
Shraddha Awasthi
Department of Pathology, ERA University, Sarfarazganj, Hardoi Road, Lucknow, Uttar Pradesh, India

Type of Publication: Original Research Paper
Conflicts of Interest: Nil

ABSTRACT

Background: The aim of this research was to examin the clinicopathalogical properties of oral squamous carcinoma (OSCC) and oral suqamucous fibrosis (OSMF) and expression level of CD133 in OSCC and OSMF patients. **Methods:** A total 60 cases of OSCC and OSMF were retrived from achieves for analysis. OSCC and OSMF cases were separated on basis of clinocpathological parameters. **Results:** Patients with OSMF and OSCC were categorized into four clinical grades. Overall, 120 (100%) patients were diagnosed for study. Outcomes depicted that in clinical grade I there were 22 (18.3%) patients diagnosed with OSCC and OSMF, among these 4 (6.7%) patients diagnosed with OSCC and 18(30%) patients diagnosed with OSMF. In clinical grade II, there were 30 (25%) patients, among these patients 18 (30%) diagnosed with OSMF and 12 (20%) patients diagnosed with OSCC. In clinical grade, III & IV there were 46 (38.3%) and 22 (18.3%) patients respectively with OSCC & OSMF. Among the all patients (120), 96 (80%) patients had CD33 marker, in overall categorization there were 46(76.7%) individuals with OSMF & 50 (83.3%) Individuals with OSCC had CD133 marker. age intervals of 18 to 35, CD 133 marker were diagnosed in 8 (16.3%) male patients while no CD133 marker were diagnosed in female of same age intervals. males and females of 36 to 50 years old age intervals and consequence depicted that total 27 (45%) patients were diagnosed with CD133 marker among these, there were 2 (18.2%) females and 25 (51%) males had CD133 marker **Conclusion:** The research include 120 patients of which 60 had OSCC and 60 OSMF. Overall, maximum patients were in stage III of OSCC, Surface marker CD133 were prsent in maximum patients of OSCC and OSMF that conclude that CD 133 my be good marker to detect OSCC and OSMF.In stage of invasion, lymphatic system invasion is more common in patints.

Keywords: OSCC, OSMF, cancer, CD133, marker, carcinoma.

INTRODUCTION

Oral cancer, is a massive healthcare issue nationwide owing to its elevated prevalence and poor recovery rate and even the cosmetic and functional disorders that follow the disorder however after diagnosis, . There is an especially high incidence of oral cancer among people, for whom this is the 8 most prevalent cancer[1–3]. The five - year recovery frequency reported by the National Cancer Institute (NCI) for patients with oropharyngeal cancer was 63% between 1999 and 2005[4], with half of these new identified patients being reported in late stages [5]. Strong lesions, particularly oral squamous cell carcinoma (OSCC), are considered to be heterogeneous, primarily because of the ongoing mutations that occur as a result of genomic variability and external factors [6, 7]. More specifically,

functional variability has been speculated to allow for the possibility that not every cancer cells in solid tumors have a similar capacity to drive the development of tumors[8, 9]. This discovery contributed to the so-called 'cancer stem cell (CSC)' concept, that notes that even a tumor may be deemed an irregular organ and acts in a similar way to the usual growth of organs from a stem cell that induces both tumorigenesis, and also gives rise to a wide community of segregated progeny cells that make up the majority of the tumor but do not have a tumor characteristic. A widely used approach for isolating CSCs comprises of filtering them dependent on the presence of cell surface markers. Another of the difficult challenges in identifying CSCs is seeking certain surface markers that unambiguously define the population of certain tumor cells. Almost all of the markers used with the reason so far are focused on information acquired from tissue growth lineage molecules or generated from research into hematopoietic or embryonic stem cells. CD44 and CD133 are the two most widely used surface markers used to classify CSCs. In cell interactions cell adhesion and migration, CD44 is a cell-surface glycoprotein concerned. CD44 is also a hyaluronic acid receptor[11], which stimulates a number of receptor tyrosine kinases in several forms of cancer[12], driving a rise in tyrosine kinase receptors[12]. Tumor cell replication and survival rates by triggering the MAPK and PI3K/AKT pathways [13]. In addition, CD44 also plays a significant role in a number of tumor cells invading and metastasizing[14-16]. CD133 is a component of membrane - spanning pentaspanic glycoproteins explicitly localized to cell protrusions[17], found in hematopoietic stem cells, endothelial progenitor cells, glioblastomas, neuronal stem cells, glial stem cells, and certain other types of cells[18-20]. Although the role of CD133 is not entirely understood, several solid tumors have shown it to be a marker for CSCs[21-25]. The aim of this research was to examine the immunohistochemical expression in OSCC cell populations of surface markers (CD133) and to estimate their usefulness in identifying populations of CSCs from such cancers.

Method and materials

Sample collection and grading:

This research used 120 formalin-fixed, paraffin-embedded tissue blocks from each group, that were classified as well-differentiated, moderately differentiated, or poorly differentiated in the case of OSCC samples, and OSMF samples were classified as well-differentiated, moderately differentiated, or poorly differentiated in the case of OSMF samples Both groups were obtained from Era's Lucknow Medical University records, 60 samples of OSCC patients were obtained from King George Medical University, Oncology Department, and 60 samples of oral submucosal fibrosis were taken from Eras Lucknow Medical University's Department of Dentistry. Samples were categorized into three groups as follows for OSCC samples:

The clinicopathological features of the patients are summarized in Table 1.

Table 1: Clinico-pathological parameters of the patients included in the study

	Characteristics of clicnico-pathology	**No of patients**
1	Group	OSCC 60 (100%) OSMF 60 (100%)
2	Grade	I 22 (18.3 %) II 30 (25%) III 46 (38.3%) IV 22 (18.3%)
3	Gender	Male 49 (100%)

Volume 4, Issue 3; May-June 2021; Page No 321-329

		Female11 (100)
4	Stage of invasion	Invasion deeper than sub mucosa 9 (15%) Less marked border line 12 (20%) Lymph vessel 29 (48.3%) Possible invasion 10 (16.7%)

Statistical analysis:

Statistical research was carried out with the use of the chi square test. version 16 of IBM's Statistical Kit for Social Sciences (SPSS) (Chicago). The OSCC and OSMF groups were graded using a p <0.05 value for statically defined staging. The mean and standard error of the mean were used to summarize the results. Groups were evaluated by the Chi Square/Fisher exact test. One-way ANOVA) study of variance was used to compare groups and search for discrepancies between more than two independent groups, and Pearson's correlation statistics were used to look at correlations.

Results:

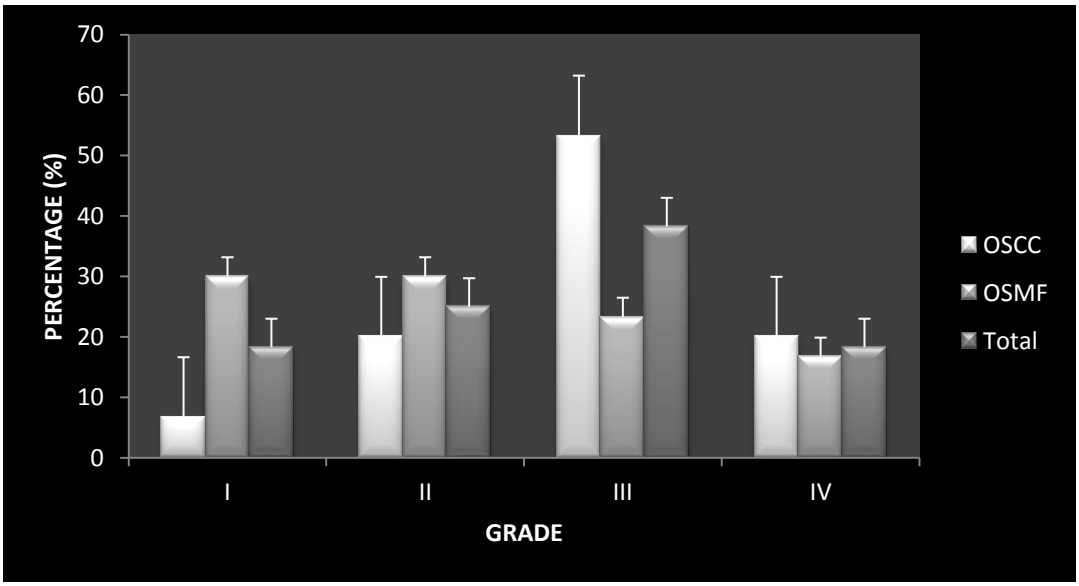

Figure 1: Graphical representation of patients with clinical Grade of OSCC & OSMF

Patients with OSMF and OSCC were categorized into four clinical grades. Overall, 120 (100%) patients were diagnosed for study. Outcomes depicted that in clinical grade I there were 22 (18.3%) patients diagnosed with OSCC and OSMF, among these 4 (6.7%) patients diagnosed with OSCC and 18(30%) patients diagnosed with OSMF. In clinical grade II, there were 30 (25%) patients, among these patients 18 (30%) diagnosed with OSMF and 12 (20%) patients diagnosed with OSCC. In clinical grade, III & IV there were 46 (38.3%) and 22 (18.3%) patients respectively with OSCC & OSMF, among these patients 14 (23.3%) patients diagnosed with clinical grade III of OSMF and 32 (53.3%) patients were with clinical grade III of OSCC. In addition, there were 10 (16.7%) individuals diagnosed with grade IV of OSMF and 12 (20%) individuals were with grade IV of OSCC. The results of interpretation were achieved by chi square test presented in table 2:

Volume 4, Issue 3; May-June 2021; Page No 321-329

Table 2: Results of the chi square test between OSCC and OSMF patients.

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	17.334[a]	3	.001
Likelihood Ratio	18.262	3	.000
N of Valid Cases	120		

a. 0 cells (.0%) have expected count less than 5. The minimum expected count is 11.00.

Remarks: The null hypothesis (H0) is rejected, since the p value (0.001) is less than the level of significance (0.005). Therefore, it can be concluded that there is significant association between OSCC and OSMF.

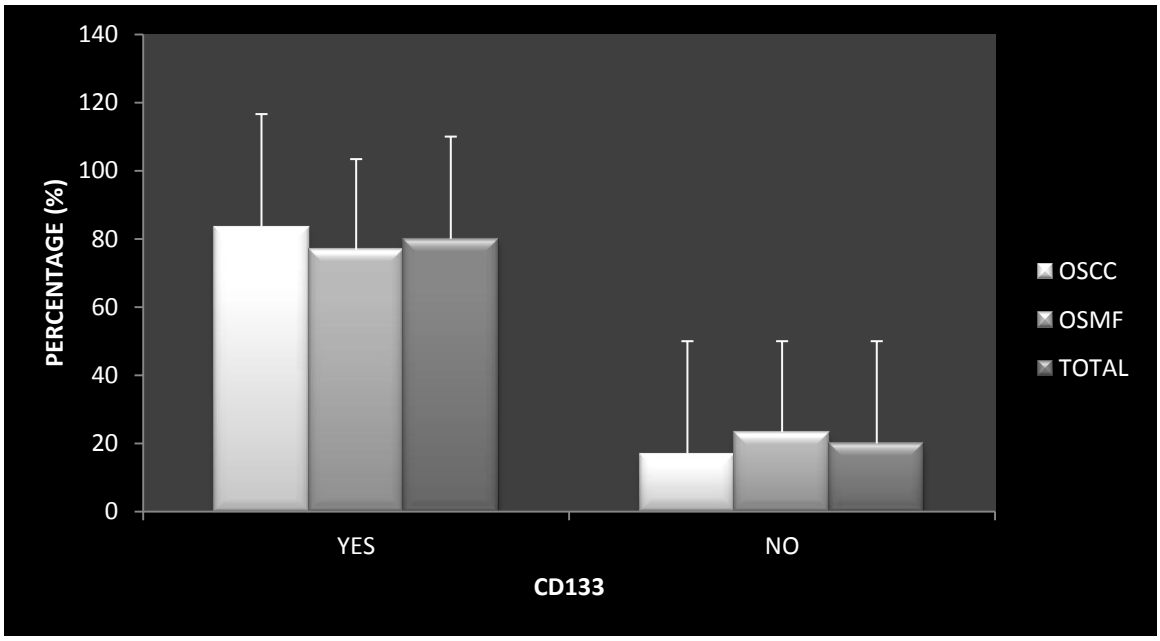

Figure 2: Denotation graph expressing existence of CD133 in patients with OSCC and OSMF.

In this retrospective follow up study, patients were grouped in OSCC and OSMF. The expression level of CD133 was checked. Among the all patients (120), 96 (80%) patients had CD33 marker, in overall categorization there were 46(76.7%) individuals with OSMF & 50 (83.3%) Individuals with OSCC had CD133 marker.

Volume 4, Issue 3; May-June 2021; Page No 321-329

Table 3: Result of chi-square test between surface marker CD133 and cancer cell.

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	.833[a]	1	.361		
Continuity Correction[b]	.469	1	.494		
Likelihood Ratio	.837	1	.360		
Fisher's Exact Test				.494	.247
Linear-by-Linear Association	.826	1	.363		
N of Valid Cases[b]	120				

a. 0 cells (.0%) have expected count less than 5. The minimum expected count is 12.00.

b. Computed only for a 2x2 table

Remark: Since the p value is also more than 0.05, we conclude that there is no significant association between cancer and surface marker CD133

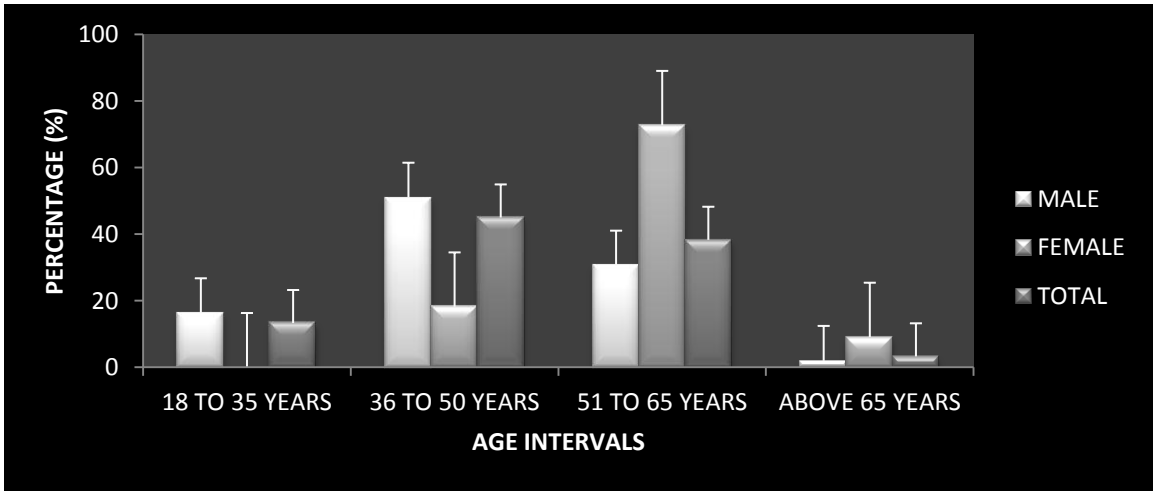

Figure 3: Oral cancer diagnosed in male and female of different age group.

Total 60(10%) individual suffered with oral cancer, were diagnosed for CD133 marker, results illustrated that in age intervals of 18 to 35 , CD 133 marker were diagnosed in 8 (16.3%) male patients while no CD133 marker were diagnosed in female of same age intervals. Like that further diagnosis were performed on males and females of 36 to 50 years old age intervals and consequence depicted that total 27 (45%) patients were diagnosed with CD133 marker among these, there were 2 (18.2%) females and 25 (51%) males had CD 133 marker. Further, same diagnosis was conducted on male and female candidates of age intervals 51 to 65 years, outcomes inferred that 23 (38.3%) patients had CD133 marker, among these, there were 8 (72.7%) females diagnosed with CD133 marker and 15 (30.6%) males were diagnosed with CD 133 marker. In addition, CD133 marker was diagnosed in male and female patients those were above 65 years old above, out turn indicated that between 2 (3.3%) patients 1(9.1%) male and 1(2%) female diagnosed with CD133 marker.

Volume 4, Issue 3; May-June 2021; Page No 321-329

Table 4: Result of the chi-square test between surface marker CD133 and the age of the patients

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	13.745[a]	4	.008
Likelihood Ratio	14.766	4	.005
Linear-by-Linear Association	.352	1	.553
N of Valid Cases	120		

a. 2 cells (20.0%) have expected count less than 5. The minimum expected count is .50.

Remarks: Since the p-value is also more than 0.05, we conclude that there is a no significant Association between the surface marker CD133 and the age of the patients.

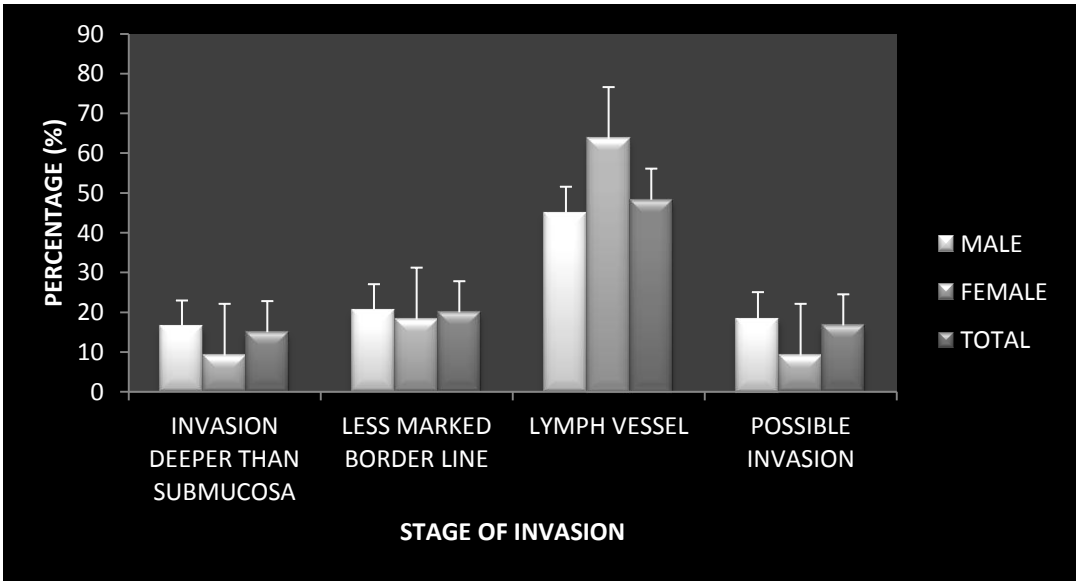

Figure 4: Presents baseline description of stage of invasion in male and female patients.

Total 60 (100%) squamous cell carcinoma patients were taken to estimate the stage of invasion in male and female. Majority of patients were males 8 (16.3%) had invasion deeper than submucosa while only 1 (9.1%) female was found in this stage of invasion. In addition, 10 (20.4%) male were in less marked border line stage of invasion while only 2 (18.2%) female were detected in this stage of inversion. Lymphatic system invasions were seen in 22 (44.9%) males and 7 (63.6%) female patients. Possible invasions were observed in 9 (18.4%) males and only 1 (9.1%) female patients.

Volume 4, Issue 3; May-June 2021; Page No 321-329

Table 5: Result of the chi-square test between stage of invasion and gender

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	1.452[a]	3	.693
Likelihood Ratio	1.521	3	.678
N of Valid Cases	60		

a. 3 cells (37.5%) have expected count less than 5. The minimum expected count is 1.65.

Remarks: Since the p-value is also more than 0.05, we conclude that there is no a significant association between the stage of invasion and gender.

DISCUSSION:

In the Indian subcontinent, the maximum occurrence of oral cancer in the globe was reported primarily owing to the strong incidence of a combination of smokeless tobacco and areca nut chewing (Cannonier et al., 2016; Fidler et al., 2018; Wierzbicka & Napierała, 2017).OSMF is a chronic, disabling disease of the aerodigestive tract, due to permanent fibroelastic shifts in the basal lamina that contribute to oral mucosal dysfunction culminating in persistent trismus, (Wyss et al., 2016) Owing to the rarity of areca nut usage, this is rare in the Western world. In India alone, 5 million people (0.5% of India's population) have OSMFs (Najafi et al., 2019) There are obviously insufficient therapeutic strategies presently established for this extremely malignant condition (Mohajertehran et al., 2018). Around 10% of these can develop into malignancy, have shown (Yu et al., 2016). CD133 is known in epithelial cells and linked with many tumors, comprising those associated to prostate carcinoma, oral cancer (Mohajertehran et al., 2018; Najafi et al., 2019). Resent analysis of colon cancer elaborated that CD133 positive cells expressed strongest tumor colony creation tendency (Ieta et al., 2008). Furthermore, CD133 is less associated with survival prognosis of OSCC patients (Chiou et al., 2008). In addition, positive cell express strong tendency to self-renewable & distinct from CD133 negative cells and undifferentiated cells of tumors indicating that CD133 might be surface marker of CSCs. Prior research of OSCC elaborated that human OSCC cell line like SCC9, show CD133 (Lin et al., 2015). In present study, expression level of CD133 was checked. Among the all patients (120), 96 (80%) patients had CD33 marker, in overall categorization there were 46(76.7%) individuals with OSMF & 50 (83.3%) Individuals with OSCC had CD133 marker. In addition, results showed that in 8 (16.3 %) male patients, CD 133 markers were confirmed at maturity level intervals of 18 to 35 years, whereas no CD133 markers were diagnosed in females of the same age intervals. As every continued diagnosis was made on men and women of 36 to 50 years of age intervals and implication showed that among these, there were 2 (18.2 %) females and 25 (51 %) males with CD 133 marker in a total of 27 (45 %) patients were diagnosed with CD133 marker. In addition, the same diagnosis was performed on male and female candidates aged 51 to 65 years, the findings revealed that 23 (38.3%) patients had a CD133 marker, 8 (72.7%) women were diagnosed with a CD133 marker and 15 (30.6%) males were diagnosed with a CD133 marker. In addition, CD133 marker was identified in male and female patients above 65 years of age, resulting in 1(9.1 %) male and 1(2 %) female diagnosed with CD133 marker among 2 (3.3 %) patients. The depth of invasion has been emphasized in many studies as the sole or most important indicator of prognosis (Ieta et al., 2008; Yu et al., 2016). In this research work, 8 (16.3 %) patients had invasion deeper than sub mucosa, while only 1 (9.1 %) female was present at this level of invasion. The majority of patients were male. Moreover, 10 (20.4 %) males were in a less pronounced invasion boundary line stage, while in this inversion stage only

Volume 4, Issue 3; May-June 2021; Page No 321-329

2 (18.2 %) females were found. In 22 (44.9 %) men and 7 (63.6 %) female patients, lymphatic system invasions were seen. In 9 (18.4 %) men and only 1 (9.1 %) female patients, potential invasions were found.

Conclusion:

Research was conducted on male and female patients, suffered with OSCC and OSMF, patients were of different age intervals, among these patients maximum patients were in stage III of OSCC this conclude that OSCC is more common than OSMF in patients, CD133 were detected in maximum patients of OSCC and OSMF, this wind up that CD133 can be good surface marker to detect OSCC &OSMF. When stage of invasion were analyzed, results came to an end that lymphatic system invasion is more common in patients.

References:

1. Bazarsad, S., Zhang, X., Kim, K., Illeperuma, R., Jayasinghe, R. D., Tilakaratne, W. M., & Kim, J. (2017). Identification of a combined biomarker for malignant transformation in oral submucous fibrosis. Journal of Oral Pathology & Medicine, 46(6), 431–438.
2. Cannonier, S. A., Gonzales, C. B., Ely, K., Guelcher, S. A., & Sterling, J. A. (2016). Hedgehog and TGFβ signaling converge on Gli2 to control bony invasion and bone destruction in oral squamous cell carcinoma. Oncotarget, 7(46), 76062.
3. Chiou, S.-H., Yu, C.-C., Huang, C.-Y., Lin, S.-C., Liu, C.-J., Tsai, T.-H., Chou, S.-H., Chien, C.-S., Ku, H.-H., & Lo, J.-F. (2008). Positive correlations of Oct-4 and Nanog in oral cancer stem-like cells and high-grade oral squamous cell carcinoma. Clinical Cancer Research, 14(13), 4085–4095.
4. Ekanayaka, R P, & Tilakaratne, W. M. (2013). Oral submucous fibrosis: Review on mechanisms of pathogenesis and malignant transformation. J Carcinog Mutagen.
5. Ekanayaka, Rasika Priyadharshani, & Tilakaratne, W. M. (2016). Oral submucous fibrosis: review on mechanisms of malignant transformation. Oral Surgery, Oral Medicine, Oral Pathology and Oral Radiology, 122(2), 192–199.
6. Fidler, M. M., Bray, F., & Soerjomataram, I. (2018). The global cancer burden and human development: A review. Scandinavian Journal of Public Health, 46(1), 27–36.
7. Gadbail, A. R., Chaudhary, M., Gawande, M., Hande, A., Sarode, S., Tekade, S. A., Korde, S., Zade, P., Bhowate, R., & Borle, R. (2017). Oral squamous cell carcinoma in the background of oral submucous fibrosis is a distinct clinicopathological entity with better prognosis. Journal of Oral Pathology & Medicine, 46(6), 448–453.
8. Guo, F., Jian, X. C., Zhou, S. H., Li, N., Hu, Y. J., & Tang, Z. G. (2011). A retrospective study of oral squamous cell carcinomas originated from oral submucous fibrosis. Zhonghua Kou Qiang Yi Xue Za Zhi= Zhonghua Kouqiang Yixue Zazhi= Chinese Journal of Stomatology, 46(8), 494–497.
9. Ieta, K., Tanaka, F., Haraguchi, N., Kita, Y., Sakashita, H., Mimori, K., Matsumoto, T., Inoue, H., Kuwano, H., & Mori, M. (2008). Biological and genetic characteristics of tumor-initiating cells in colon cancer. Annals of Surgical Oncology, 15(2), 638–648.
10. Illeperuma, Rasika P, Kim, D. K., Park, Y. J., Son, H. K., Kim, J. Y., Kim, J., Lee, D. Y., Kim, K., Jung, D., & Tilakaratne, W. M. (2015). Areca nut exposure increases secretion of tumor-promoting cytokines in gingival fibroblasts that trigger DNA damage in oral keratinocytes. International Journal of Cancer, 137(11), 2545–2557.
11. Illeperuma, Rasika Pawiththra, Ryu, M. H., Kim, K. Y., Tilakaratne, W. M., & Kim, J. (2010). Relationship of fibrosis and the expression of TGF-β1, MMP-1, and TIMP-1 with epithelial dysplasia in oral submucous fibrosis. Oral Medicine & Pathology, 15(1), 21–28.
12. Lin, C.-S., Lin, Y.-C., Adebayo, B. O., Wu, A., Chen, J.-H., Peng, Y.-J., Cheng, M.-F., Lee, W.-H., Hsiao, M., & Chao, T.-Y. (2015). Silencing JARID1B suppresses oncogenicity, stemness and increases radiation sensitivity in human oral carcinoma. Cancer Letters, 368(1), 36–45.

Volume 4, Issue 3; May-June 2021; Page No 321-329

13. Lingen, M. W., Pinto, A., Mendes, R. A., Franchini, R., Czerninski, R., Tilakaratne, W. M., Partridge, M., Peterson, D. E., & Woo, S. (2011). Genetics/epigenetics of oral premalignancy: current status and future research. Oral Diseases, 17, 7–22.

14. Mohajertehran, F., Sahebkar, A., Zare, R., & Mohtasham, N. (2018). The promise of stem cell markers in the diagnosis and therapy of epithelial dysplasia and oral squamous cell carcinoma. Journal of Cellular Physiology, 233(11), 8499–8507.

15. Moutasim, K. A., Jenei, V., Sapienza, K., Marsh, D., Weinreb, P. H., Violette, S. M., Lewis, M. P., Marshall, J. F., Fortune, F., & Tilakaratne, W. M. (2011). Betel-derived alkaloid up-regulates keratinocyte alphavbeta6 integrin expression and promotes oral submucous fibrosis. The Journal of Pathology, 223(3), 366–377.

16. Najafi, M., Farhood, B., & Mortezaee, K. (2019). Cancer stem cells (CSCs) in cancer progression and therapy. Journal of Cellular Physiology, 234(6), 8381–8395.

17. Pindborg, J. J., Chawla, T. N., Srivastava, A. N., Gupta, D., & Mehrotra, M. L. (1964). Clinical aspects of oral submucous fibrosis. Acta Odontologica Scandinavica, 22(6), 679–691.

18. Pitiyage, G. N., Lim, K. P., Gemenitzidis, E., Teh, M., Waseem, A., Prime, S. S., Tilakaratne, W. M., Fortune, F., & Parkinson, E. K. (2012). Increased secretion of tissue inhibitors of metalloproteinases 1 and 2 (TIMPs-1 and-2) in fibroblasts are early indictors of oral sub-mucous fibrosis and ageing. Journal of Oral Pathology & Medicine, 41(6), 454–462.

19. Siriwardena, B., Jayawardena, K., Senarath, N. H., & Tilakaratne, W. M. (2018). An evaluation of clinical and histopathological aspects of patients with oral submucous fibrosis in the background of oral squamous cell carcinoma. BioMed Research International, 2018.

20. Teh, M. T., Tilakaratne, W. M., Chaplin, T., Young, B. D., Ariyawardana, A., Pitiyage, G., Lalli, A., Stewart, J. E., Hagi-Pavli, E., & Cruchley, A. (2008). Fingerprinting genomic instability in oral submucous fibrosis. Journal of Oral Pathology & Medicine, 37(7), 430–436.

21. Tilakaratne, W M, Iqbal, Z., Teh, M. T., Ariyawardana, A., Pitiyage, G., Cruchley, A., Stewart, J. E., Hagi-Pavli, E., Lalli, A., & Waseem, A. (2008). Upregulation of HIF-1α in malignant transformation of oral submucous fibrosis. Journal of Oral Pathology & Medicine, 37(6), 372–377.

22. Tilakaratne, Wanninayake Mudiyanselage, Ekanayaka, R. P., & Warnakulasuriya, S. (2016). Oral submucous fibrosis: a historical perspective and a review on etiology and pathogenesis. Oral Surgery, Oral Medicine, Oral Pathology and Oral Radiology, 122(2), 178–191.

23. Utsunomiya, H., Tilakaratne, W. M., Oshiro, K., Maruyama, S., Suzuki, M., Ida-Yonemochi, H., Cheng, J., & Saku, T. (2005). Extracellular matrix remodeling in oral submucous fibrosis: its stage-specific modes revealed by immunohistochemistry and in situ hybridization. Journal of Oral Pathology & Medicine, 34(8), 498–507.

24. Wierzbicka, M., & Napierała, J. (2017). Updated National Comprehensive Cancer Network guidelines for treatment of head and neck cancers 2010-2017. Otolaryngologia Polska= The Polish Otolaryngology, 71(6), 1–6.

25. Wyss, A. B., Hashibe, M., Lee, Y.-C. A., Chuang, S.-C., Muscat, J., Chen, C., Schwartz, S. M., Smith, E., Zhang, Z.-F., & Morgenstern, H. (2016). Smokeless tobacco use and the risk of head and neck cancer: pooled analysis of US studies in the INHANCE consortium. American Journal of Epidemiology, 1–14.

26. Yu, C., Hu, F., Yu, C., & Chou, M. (2016). Targeting CD133 in the enhancement of chemosensitivity in oral squamous cell carcinoma–derived side population cancer stem cells. Head & Neck, 38(S1), E231–E238.

Volume 4, Issue 3; May-June 2021; Page No 321-329