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An Estimation of Salivary Survivin in Oral Leukoplakia & Oral Squamous Cell Carcinoma (Salivary Survivin in OL and OSCC)

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Abstract:-

- ***Background and Objectives***

Oral squamous cell carcinoma (OSCC) is the sixth most common cancer worldwide. It is well known that most OSCCs arise in presence of clinical lesions like oral leukoplakia (OL), lichen planus & submucous fibrosis. During malignant transformation various cellular & biochemical changes occur at tissue level. These changes can be measured with the help of biomarkers. Survivin, an Inhibitor of Apoptosis protein is almost undetectable in normal adult tissues but is broadly expressed in a wide variety of cancers and precancers. Hence in the present study salivary survivin levels were estimated & compared in OL, OSCC and controls in general and as per stages and grades.

- ***Methods***

Salivary Survivin levels were estimated using Enzyme Linked Immunosorbant Assay.

- ***Results***

Results of the study indicated a statistically significant progressive increase in various clinical stages and histological grades of OL and OSCC; in controls, OL and OSCC cases.

- ***Interpretation and Conclusion***

Survivin can be used as an adjunctive diagnostic and prognostic marker for OL and OSCC and also for disease monitoring.

Keywords:- *Oral Leukoplakia, Oral Squamous Cell Carcinoma, Saliva, Survivin.*

I. INTRODUCTION

Oral squamous cell carcinoma (OSCC) is the sixth most common cancer worldwide.[1] It is well known that most OSCCs arise in presence of clinical lesions like oral leukoplakia (OL), lichen planus & submucous fibrosis. In-spite of advancements in treatment protocols, 5-year survival rate of OSCC is 35-50 %.[2, 3] The big reason behind this is the inability to detect the disease at an early stage. Therefore identifying a sensitive & specific molecular marker for oral premalignant lesions & OSCCs will be favourable for assessing the severity grades of the disease and prevention of further progression of the disease as well as for therapeutic monitoring.

Survivin, an Inhibitor-of-Apoptosis, is expressed in fetal development and not present in normal adult tissues.[4] Recent research studies have demonstrated increased survivin levels in different cancers including OSCC, its association with aggressive phenotypes and its role in precancer.[4] Therefore survivin expression could be helpful for evaluating the progressive risk of oral potentially malignant disorders (OPMDs) like OL and prognosis of OSCC.

Hence in the present study salivary survivin levels were evaluated in control, OL and OSCC cases in general and also as per clinical stages and histological grades.

II. MATERIAL AND METHODS

This was a case control study with a study group comprising of 48 patients (28 of histopathologically diagnosed OL and 20 of OSCC) and a control group with 40 age and sex matched healthy subjects. The sample size was calculated using PASS (Power Analysis and Sample Size) Software. Patients with any prior treatment for these lesions, any history of previous malignancy or with any systemic diseases were excluded.

Demographic findings of all study subjects were recorded. After obtaining informed concent, from all the subjects 4 ml of unstimulated saliva was collected, centrifuged and supernatant was separated. Estimation of salivary survivin levels was done using a commercially

available ELISA kit (Cloud Clone Corp, USA). Clinical staging of all OL cases was done according to staging system proposed by van der Waal et al (2000) and OSCC cases according to staging system proposed by AJCC. Histopathological grading of OL cases was done according to WHO grading system (2005) whereas OSCC cases was done according to Anneroth's grading system.

The present study was conducted in the Department of Oral Pathology and Microbiology, Government Dental College & Hospital, Aurangabad in collaboration with Department of General Pathology, Government Medical College and Hospital, Aurangabad with the approval of Institutional Ethical Committee during the period of 2014-2016.

III. STATISTICAL ANALYSIS

Both OL and OSCC groups were compared with controls for salivary survivin levels by independent Student's t test. Categorical variables were compared by chi-square (x^2) test. One way analysis of (ANOVA) test was applied to compare multiple groups in relation to single variable. The significance of mean difference between the groups was evaluated by Tukey's Post Hoc test.

Statistical analysis was performed using Statistical Graph Pad Prism Software.

Group	Age Group (Years)	N (100%)	Range Of Salivary Survivin Levels (pg/ml)	Mean Salivary Survivin Levels (pg/ml)	Std. Deviation
OL (N=28)	21-30	5 (17.85%)	1.099-4.652	3.66	1.46
	31-40	14 (50%)	1.014-4.089	2.69	1.26
	41-50	6 (21.42%)	1.588-5.023	3.29	1.24
	51-60	3 (10.71%)	3.704-5.89	4.79	2.43
	61-70	0			
OSCC (N=20)	21-30	0			
	31-40	6 (30%)	6.074-8.814	7.00	0.95
	41-50	2 (10%)	6.782-8.127	7.45	0.95
	51-60	6 (30%)	6.148-7.002	6.56	0.38
	61-70	6 (30%)	5.972-6.812	6.74	0.89
Controls (N=40)	21-30	2 (50%)	0.063-0.081		
	31-40	16 (40%)	0.013-0.225	0.08	0.06
	41-50	8 (20%)	0.021-0.24	0.09	0.07
	51-60	8 (20%)	0.032-0.423	0.11	0.13
	61-70	6 (15%)	0.035-0.162	0.09	0.04

Table 1:- Age-wise distribution of OL, OSCC, Controls

IV. RESULTS

The age of OL and OSCC cases and Controls ranged from 23-60 years, 32-66 years and 21-70 years respectively with mean (± SD) 37.15 ± 9.66, 53.3 ± 13.48 and 46.77 ± 12.92 respectively (Table I). The peak incidence of OL was noted in 4th decade whereas OSCC was noted in 4th, 6th and 7th decades respectively. Comparing the mean age of two groups i.e. cases (OL+OSCC) and Controls, t test revealed similar ($p>0.05$) age between the two groups ($t=0.67$, $p=0.49$) i.e. no statistical difference. Thus subjects of two groups were age matched and comparable and therefore age may not influence the study outcomes.

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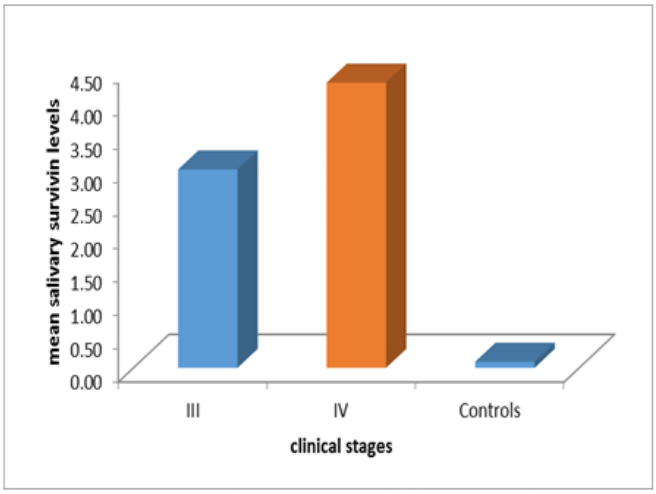
Group	Sex	N (100%)	Range Of Salivary Survivin Levels (pg/ml)	Salivary Survivin Levels (pg/ml)	
				Mean	Std. Deviation
OL N=28	Female	4 (14.28%)	3.981-5.023	4.50	0.58
	Male	24(85.71%)	1.0147-5.89	3.13	1.48
OSCC N=20	Female	7 (35%)	5.972-8.41	6.95	0.97
	Male	13 (65%)	6.074-8.814	6.77	0.69
Controls N=40	Female	10 (25%)	0.021-0.104	0.07	0.05
	Male	30 (75%)	0.013-0.423	0.10	0.08

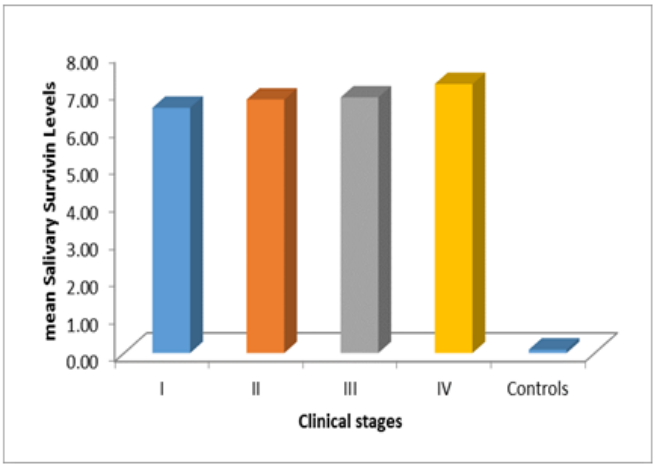
Table 2:- Sex-wise distribution of OL, OSCC, Controls

OL cases comprised of 24 males (85%) and 4 females (15%) with male : female ratio of 5.6 : 1 whereas OSCC cases comprised of 13 males (65%) and 7 females (35%) with male : female ratio of 1.8:1 (Table II). Compairing the proportion of genders of two groups i.e. cases (OL+OSCC) and Controls, X^2 test revealed similar ($p > 0.05$) proportions of genders between the two groups (X^2=0.00, p=0.819) i.e. not differed statistically. In other words, subjects of OSCC cases and Controls were sex matched and comparable and therefore sex may not also influence the study outcomes.



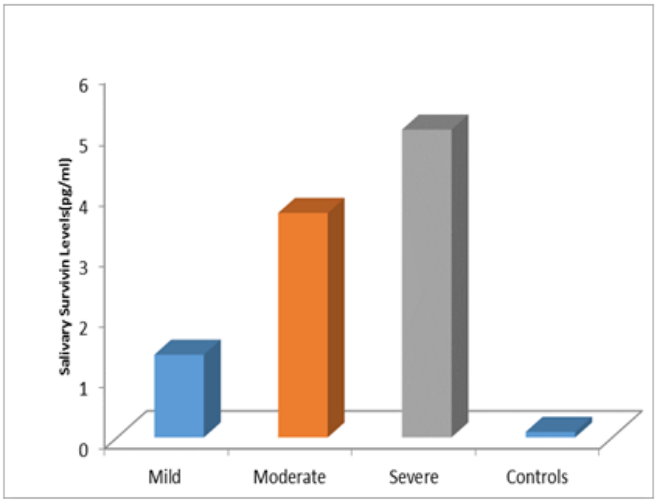
Graph 1:- Comparison of Mean Salivary Survivin Levels in Various Clinical Stages of OL, Controls

Most of the OL cases were in stage III (83.14%) followed by stage IV (17.85%). There was no case in stage I and stage II. The mean salivary survivin level for stage III and stage IV was 2.98±1.28 and 4.29±1.45 respectively. On compairing ANOVA revealed significantly different mean salivary survivin levels among the groups (F=120.781, p<0.001). Post Hoc test revealed significantly different and higher survivin levels in Controls and stages and between stages (p<0.001, p<0.001 and p=0.05 respectively);(Graph I). Most of the OSCC cases were in stage III (40%) followed by stage II (30%), and stage I and stage IV (each with 15%). The mean salivary survivin level for stage I, stage II, stage III and stage IV was 6.56±0.23, 6.78±0.77, 6.84±0.71 and 7.20±1.46 respectively. On compairing ANOVA revealed significantly different mean salivary survivin levels among the groups (F=755.173, p<0.0001). Post Hoc test revealed significantly different and higher survivin levels in Controls and stages and between stages (p<0.01 for controls and stages; and p>0.05 between stages);(Graph II).



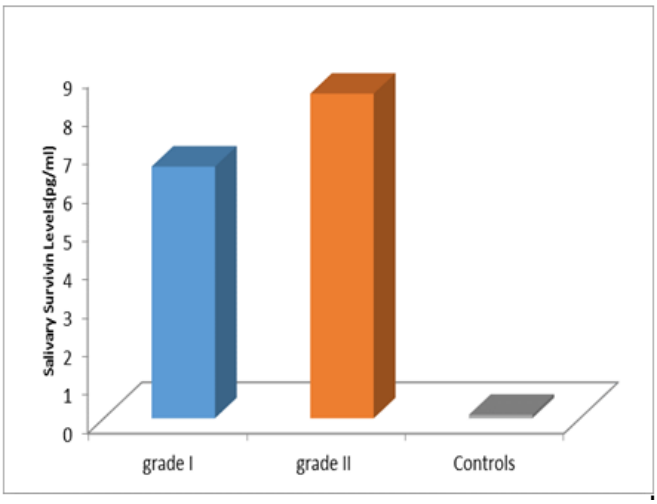
Graph 2:- Comparison of Mean Salivary Survivn Levels in Various Clinical Stages of OSCC, Controls

In OL study-group, 16 (57.14%) cases were exhibiting moderate epithelial dysplasia, 8 (28.57%) cases exhibiting mild epithelial dysplasia and 4 (14.28) cases exhibiting severe epithelial dysplasia histopathologically. Mean salivary survivin levels (pg/ml) were 1.36 ± 0.27, 3.69 ± 0.58 and 5.06±0.59 respectively in mild, moderate and severe epithelial dysplasia. On compairing, ANOVA revealed significantly different mean salivary survivin levels among mild, moderate and severe groups (F = 647.23, p<0.0001). Post Hoc test revealed significant paired differences between Controls vs grades and in between grades (p<0.01);(Graph III). Among OSCC cases there were 17 (85%) cases of grade I, 3 (15%) cases of grade II and no patient of grade III OSCC.



Graph 3:- Comparison of Mean Salivary Survivin Levels in Various Histological Grades of OL, Controls

Mean salivary survivin levels (pg/ml) were 6.55±0.36 and 8.45±0.35 in grade I and grade II respectively. On compairing, ANOVA revealed significantly different mean salivary survivin levels among different grades (F=6993.05, p<0.0001). Post Hoc test revealed significant paired differences between Controls and grades and in between grades (p<0.01);(Graph IV).

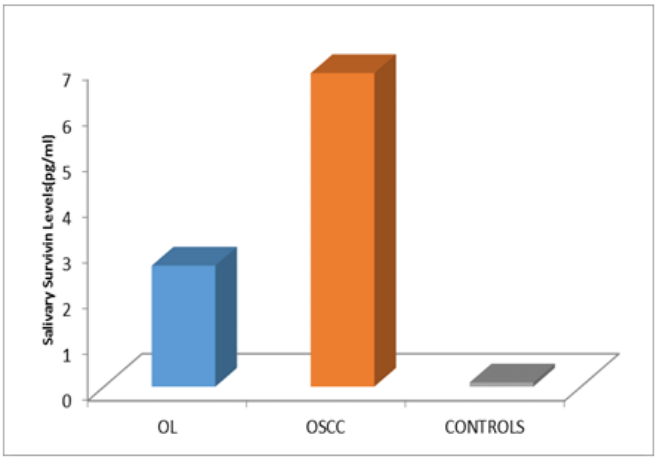


Graph 4:- Comparison of Mean Salivary Survivin Levels in Various Histological Grades of OSCC, Controls

The mean salivary survivin level (pg/ml) of OL, OSCC cases and Controls ranged from (2.64±1.79) pg/ml, (6.84±0.78) pg/ml and (0.09±0.07) pg/ml respectively. The mean salivary survivin levels for OSCC were comparatively higher than OL and Controls. Comparing the salivary survivin levels in OL, OSCC and Controls, ANOVA test revealed significantly different and higher survivin levels in OSCC than in OL and Controls ($F = 271.32$, $p < 0.0001$). Post Hoc test revealed significantly different and higher survivin levels in OSCC than in OL, in OL than in Controls and in OSCC than in Controls ($p < 0.01$);(Graph V).

DISCUSSION

Cancer is the major cause of morbidity and mortality all over the world and is one of the leading causes of death in all societies. Oral and oropharyngeal carcinoma are the most prevalent (6^{th}) cancer worldwide. It is a disfiguring, potentially fatal disease that continues to rise in incidence among younger and older people alike. It is projected that by 2030 there will be ~26 million new cancer cases & 17 million cancer deaths per year.[5] This is attributed to late diagnosis of disease. The current mortality rate, attributed to oral cancer can be reduced greatly if early signs and symptoms are given an adequate attention. [1,6]



Graph 5:- Comparison of Mean Salivary Survivin Levels in OL, OSCC, Controls

OPMD is the latest WHO recommended term for a group of disorders that carry an unpredictable risk of malignant transformation. Amongst OPMDs, OL have greatest malignant potential.[7] Currently risk of progression in OLs is typically determined based on clinical assessment and histopatholgical evaluation of biopsied materials. However, this information can not always predict the potential or risk of the lesion in question becoming malignant. Therefore, there is a need to find predictive biomarkers that can aid in defining progression of a potentially malignant lesion into malignancy.

Conversion from OL to OSCC is linked to multiple genetic and epigenetic alterations. The basic events that occur during carcinogenesis involve alteration of various metabolite levels, dysregulation of proteins associated with cell division and differentiation, stimulation of oncogenes and switching off of the tumor suppressor genes.

It is now well established that, the balance between cell division and apoptosis gets altered during carcinogenesis. Active inhibition of apoptosis occurs due to unchecked

division along with numerous genetic modifications. In recent years, a unique gene known as survivin that encodes for a structurally distinctive suppressor of apoptosis has been recognized.

Survivin is a novel member of inhibitor of apoptosis (IAP) protein family.[4] Although it is expressed during human fetal development, survivin is not detected in normal adult tissues except in thymus and placenta.[4] Survivin reduces caspase activity and apoptosis in cells exposed to diverse apoptosis-triggering stimuli. Survivin is expressed in the cells of all common cancers and studies suggest a relationship between the level of survivin expression and tumor biology, clinical characteristics, and treatment outcome.[4] It also suggests that reactivation of the survivin gene frequently occurs in cancers and hence is being used as a prognostic factor in several human neoplasms.[8]

Demographic data of the present study indicated peak incidence of OL in 4th decade. This finding was consistent with Brokor-Bratic M (2000), Lapthanasupkul P et al (2007), Ambedkar D et al (2015) who reported peak incidence of OL in 4th decade.[9,10] Whereas oral cancer is predominantly a disease of middle-aged men, risk of oral cancer increases with age. In OSCC cases, peak incidence was seen in 4th, 6th and 7th decades which was in accordance with Mathur A et al (2009), Bhugri Y et al (2006) and Sawalani K et al (2014). [11,12,13]

In the present study, in OL cases a male preponderance was noted (5.6:1). This was in accordance with Bokor Bratic M (2003) and, Ambedkar D et al (2015) who reported increased frequency of OL in males as compared to females. [9,10] Similarly in the present study, in OSCC cases a male preponderance was noted (1.8:1). This male predilection of OL and OSCC may be attributable to heavier indulgence in risk habits by men. [14]

Mean salivary survivin levels were found to be increased according to clinical stages and histological grades in both OL and OSCC. Smith S et al demonstrated significant increase in urine samples of different grades of bladder cancer patients.15 Santarelli A et al (2013) evaluated survivin levels in saliva of OSCC cases and observed significantly increased survivin levels in OSCC cases compared to controls.[16] Negi et al (2015) also described the difference in survivin levels in tissues of OL among various histological grades but it was not statistically significant which they attributed to their smaller sample size.[17]

Thus in the present study survivin was expressed in about all OL and OSCC cases, and that its degree of expression correlated with a more aggressive phenotype. These observations were consistent with the observations of IHC studies of Pannone G et al (2007) and Negi A et al (2015) who found significantly higher survivin expression in OL and OSCC tissues than normal oral tissues.[18,17]

These results pointed towards the promising role of salivary survivin as a diagnostic as well as prognostic marker for OL as well as OSCC.

V. CONCLUSION

Hence, in conclusion, our study demonstrated a statistically significant increase in salivary survivin levels in OL and OSCC cases as compared to controls. Thus survivin can be used as an adjunctive salivary marker for OL and OSCC diagnosis. Progressive increase in clinical stages and histological grades of OL and OSCC cases indicate that it may help further in prognostic information about the malignant transformation of OL and also OSCC. As it is a noninvasive technique, it can be also used for disease monitoring. Still further studies with clinical follow-up are required with larger number of samples to confirm the role of survivin as a guide to prognosis of OL and OSCC.

CONFLICTS OF INTEREST: None

REFERENCES

[1]. Warnakulasuriya S. “Global epidemiology of oral and oropharyngeal cancer.” Oral Oncology. 2009;45:309-316.
[2]. Chen Y, Chang J, Liao C, Wang H, Yen T, Chiu c et al. Head and neck cancer in the betel quid chewing area: recent advances in molecular carcinogenesis. Cancer Sci. 2008;99(8):1507-1514.
[3]. Lieu S, Lu C, Chiou C, Yen c, Liaw G, Chen Y et al. Surgical outcomes and prognostic factors of oral cancer associated with betel quid chewing and tobacco smoking in Taiwan. Oral Oncology. 2010;46(4):276-82.
[4]. Muzio L, Pannone G, Staibano S, Mignogna M, Rubini C, Mariggio M et al. Survivin expression in oral squamous cell carcinoma. British journal of cancer 2003;89:2244-2248.
[5]. Thun M, DeLancey J, Center M, Jemal A, Elizabeth M. The global burden of cancer: priorities for prevention. Carcinogenesis. 2010; 31(1): 100–110.
[6]. Mathur A, Jain M, Shiva M, Navlakha M, Prabhu S , Kulkarni S. Tobacco habits and risk of oral cancer: A retrospective study in India. Iranian Journal of Blood and Cancer 2009; 1(3):111-116.
[7]. Brzak B, Stiepetic M, Canjuga I, Barisevic M, Balisevic D, Sikora M et al. “The Frequency and Malignant Transformation Rate of Oral Lichen Planus and Leukoplakia – A Retrospective Study.” Coll. Antropol. 2012;36(3):773-777.
[8]. Johnson M, Howerth E. Survivin: A Bifunctional Inhibitor of Apoptosis Protein. Vet Pathol 2004;41:599–607.
[9]. Bokor-Bratic M. The prevalence of precancerous oral lesions Oral Leukoplakia. Archieve of Oncology. 2000;8(4):169-170.

[10]. Ambekar D, Chaudhary B, Kulkarni V, Yadav M, Singh R. Prevalence Of Oral Leukoplakia In Tobacco Habitual Around Navi Mumbai. DYPJHS. 2014;2(2):25-29.
[11]. Mathur A, Jain M, Shiva M, Navlakha M, Prabhu, Kulkarni S. Tobacco Habits & Risk of Oral Cancer : A Retrospective Study in India. Iranian Journal of Blood and Cancer. 2009;1(3):111-116.
[12]. Bhurgri Y, Bhurgri A, Usman A, Parvez S, Kayani N, Bashir I et al. Epidemiological Review of Head and Neck cancers in Karachi. Asian Pac J Cancer Prev 2006;7:195-200.
[13]. Sawlani K, Kumari N, Mishra A, Agrawal U. Oral Cancer prevalence in a tertiary care hospital in India. J Family Med Community Health 2014;1(4):1022-1027.
[14]. Warnakulasuriya S. Living with oral cancer: epidemiology with particular reference to prevalence and life style changes that influence survival. Oral Oncol 2010;46:407-410.
[15]. Smith S, Wheeler M, Plesica J, Colberg J, Weiss R, Altieri D. Urine detection of survivin is a sensitive marker for the noninvasive diagnosis of bladder cancer. J Urol. 2004;171:626-30.
[16]. Santarelli A, Mascitti M, Russo L, Colella G, Giannatempo G, Bambini F et al. “Detection level of salivary survivin in patients with OSCC” Journal of Carcinogen Mutagen. 2013;S5:1-4.
[17]. Negi A, Puri A, Gupta R, Nangia R, Sachdeva A, Mittal M. Comparison of Immunohistochemical Expression of Antiapoptotic Protein Survivin in Normal Oral Mucosa, Oral Leukoplakia, and Oral Squamous Cell Carcinoma. Pathology Research International 2015; Article ID 840739:1-6.
[18]. Muzio L, Pannone G, Staibano S, Mignogna M, Mariggio M, Salvatore Get al. Expression of the apoptosis inhibitor survivin in aggressive squamous cell carcinoma. Exp Mol Pathol 2001;70:249–254.