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**Research Article**

# EVALUATION OF SERUM AND SALIVARY SURVIVIN LEVEL IN ORAL SQUAMOUS CELL CARCINOMA

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## INTRODUCTION

Oral squamous cell carcinomas (OSCC) are a biologically heterogeneous group of cancers contributing to the major source of cancer morbidity and mortality worldwide, especially in the Indian subcontinent due to high consumption of tobacco and tobacco related products. According to the international agency for research on cancer the incidence of cancer may increase to more than 1.7 million till 2035 in India (Bray *et al* 2013). It is estimated that approximately 7 to 9 lakhs new cases of cancer being identified each year in India (Govt. India Ann Rep 2009).

The delayed diagnosis of OSCC is one of the maincauses for its poor prognosis. Therefore, novel molecular predictors of malignant transformation are crucial to determine oral precancerous lesions at greater risk.

Survivin, one of the most potent tumor markers that is discovered in recent centuries, has been associated with lung cancer, stomach cancer, colon cancer, and oral cancer, and its expression has importantrolein tumor prognosis(Palazzo E. *et al* 2015).

Survivin is anapototic inhibitor, member of Inhibitor apoptosis protein family (IAP) It is a 16.5 kDa protein consisted of 142-amino acid and located on the 17q25 chromosome (Chantalat L*et al* 2000).

The key function of Survivin is to control cell division instead of apoptosis inhibition as the protein regulates the chromosome spindle checkpoint assembly (Okada H. *et al*. 2004). Survivin expression level is low or scanty in most of the normal adult tissues, but is well expressed in various cancer cells (Kelly, R.J. *et al* 2011), suggestingits role inboth cell survival and tumor progression.

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Survivin level is higher in OSCC as compared to normal oral mucosa (Muzio, L, L. *et al* 2001) and its degree of expression is a good indicator of prognosis(Muzio, L.L.*et al*. 2003, & Muzio, L.L. *et al* 2005). Therefore, Survivinestimation can be employed for diagnostic purposes.

In recent years, a rising interest on salivary markers has led to the study of various biomolecules, as it is non-invasive way of diagnosing OSCC at an early stage and patient compliance is also very high. The present study was conducted to evaluate the salivary and serum levels of Survivin in patients with OSCC and healthy subjects, aiming to assess the role of salivary Survivin detection as a diagnostic and prognostic marker for Oral Squamous Cell Carcinoma.

## MATERIALS AND METHODS

The present in vivo study, was conducted in the Department of Oral Medicine and Radiology, Faculty of Dental Sciences, King George's Medical university, Lucknow, Uttar Pradesh. Study was approved from the ethical committee of the institution.

***Study Sample:*** The study consisted of 120 subjects with age group of 18 years and above. The Subjects were divided into two groups of 60 subjects each. **Cases** (Group I) consisted of the subjects, histopathologically diagnosed with Oral Squamous Cell Carcinoma and Controls (Group – II) consisted of the sex and age matched healthy individuals. Detailed history of the subjects was taken according to structured format, after informed consent. A double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) kit (Sunred Biotech) was used to assay the level of Human Survivin in serum and saliva samples.

***Saliva sample collection and Processing:*** 2 ml of unstimulated whole saliva samples were collected by draining method in the morning. Collection tubes were immediately centrifuged at 5000 rpm for 5 minutes at -4°C to remove cell pellets and debris. Aliquot supernatant and stored at -80°C for subsequent analysis.

***Serum sample collection***- 5ml of venous blood was withdrawn from cubital vein by venipuncture method from each subject into a plain sterile vacutainer glass tube. Collection tubes were mounted for coagulation at room temperature for 10-20 minutes, centrifuged at 4000 rpm for 10 minutes at 4◦C.Then immediately aliquot supernatant (serum) and stored samples at -80◦C.

***Assay procedure:*** After standardization of the kit, 50 µl standard solution and 50µl Streptavidin-HRP were loaded into the well of ELISA microplates, (the standard wells had already combined with biotin antibody), then 40µl sample along with 10µl Survivin-antibody and 50µl Streptavidin-HRP were added in test wells and subsequently all experiment sample and standard loading were proceeded in a duplicate set up. Next the plate was swirled gently and covered with sealing membrane and incubated for 60 minutes at $37^0$C.

After incubation, membrane was removed carefully and microplate was washed with 300 µl washing solution for five times and drained, the remaining water was separated away carefully. After that Chromogen solution -A 50µl and Chromogen solution- B 50µl was added to each well and gently mixed then incubated for 10 min at 37℃ away from light. Finally, 50µl stop solution was added in each well and gently mixed. The absorbance reading of standard and sample was observed at 450 nm into the ELISA microplate reader within 15 minutes after addition of stop solution. The results were analyzed on the basis of optical density (OD) of standard and sample. Concentration was represented in the form of pg/ml.

Statistical analysis was done with unpaired students's T test, and one way "ANOVA" analysis.

## RESULTS

Total 120 subjects of age of 18years above were included in the study and divided into cases and control groups of 60 subjects each. Serum and salivary Survivin expression (pg/ml) were analyzed.

### *Sample Statistics*

### *Sample Distribution with Age and Gender*

The age distribution of cases (group I) and control (group II) both showed median age of 55 years, with age ranged from 18-80 years and 27-82 yrs respectively. (Table 1; Fig 1)Gender distribution showed male predominance in both the groups, the M:F ratio of Cases (Group I) was 3:1 and Control (group II)1.2:1. (Table 1; Fig 2)

**Table 1** Intergroup Comparison of age and gender in Study Population

| Age Group (years) | TOTAL (N=120) | | Cases (Group I)(n=60) | | Controls(Group II)(n=60) | |
|---|---|---|---|---|---|---|
| | No. | % | No. | % | No. | % |
| 18 to 40 years | 29 | 24.2 | 14 | 23.3 | 15 | 25 |
| 41 to 60 years | 59 | 49.2 | 31 | 51.7 | 28 | 46.7 |
| Above 60 years | 32 | 26.7 | 15 | 25 | 17 | 28.3 |
| | | | | $\chi^2$=0.312; p=0.856 | | |
| **Gender** | | | | | | |
| Male | 78 | 65 | 45 | 75 | 33 | 55 |
| Female | 42 | 35 | 15 | 25 | 27 | 45 |
| | | | | $\chi^2$=5.275 ; p=0.022 | | |

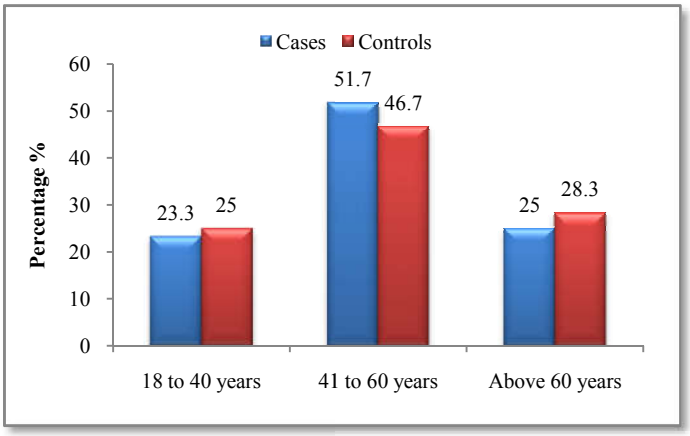


**Fig 1** Showing mean age distribution in Cases (Group I) and Control (Group II)

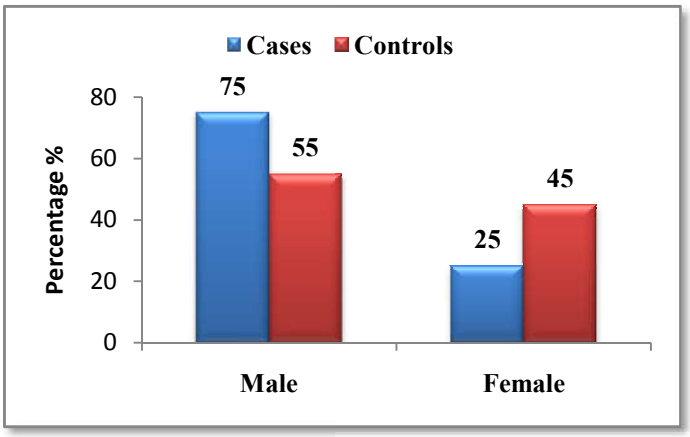


**Fig 2** showing male and female % distribution in Cases (Group I) and Control (Group II)

### *Sample Distribution with Site of Carcinoma*

Most common site of occurrence for OSCC was border of tongue (26.7%) and posterior bucco-alveolus region (26.7%) and it was non-significant.

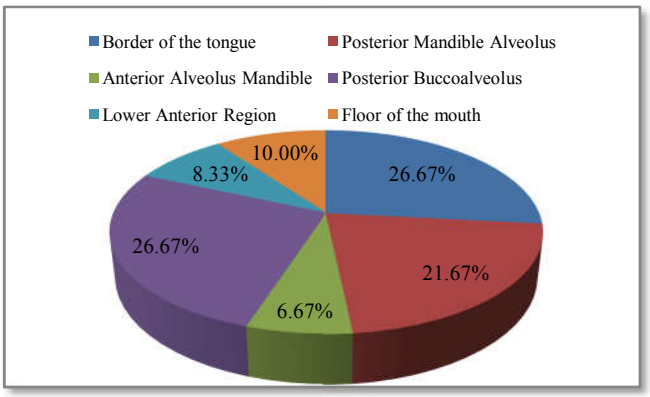


**Fig 3** Pie chart showing frequency distribution of site of OSCC

### *Survivin expression*

### *Serum and Salivary Survivin level*

It was observed that the mean serum level of Survivin in subjects of Cases (Group I) was significantly higher than Control (group II) subjects (Table 2, Fig 4). The mean salivary level of Survivin in Cases (Group I) and Control (Group II) subjects were13.37 pg /ml and 5.28pg/ml respectively. It was observed that the mean salivary level of Survivin in cases

subjects was significantly higher than control subjects. (Table 2, Fig 4).

**Table 2** Mean comparison of Serum and Salivary Survivin according to their groups.

| | Cases (n=60) | Controls (n=60) | P Value |
|---|---|---|---|
| | Mean ± SD | Mean ± SD | |
| SER SUR | 22.47±2.520 | 15.10±1.062 | <0.001* |
| SAL SUR | 13.37±2.178 | 5.28±0.960 | <0.001* |

(Unpaired t test for significance. *Significant.)

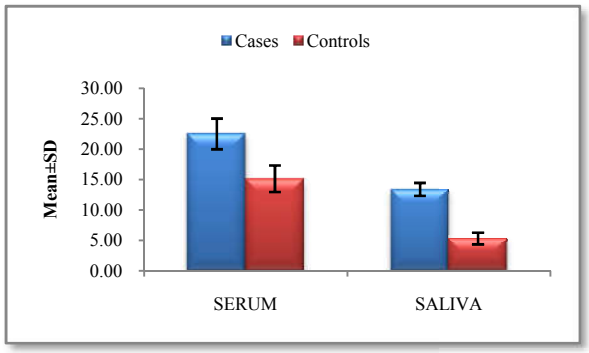


**Fig 4** Showing Mean comparison of serum and salivary Survivin according to their groups

### *Survivin Expression according to Gender and Age*

### *Gender*

Serum and salivary Survivin level were found high in males in both the groups Case (Group I) and Control (Group II) though it was statistically insignificant (Table 3).

**Table 3** Mean comparison of Serum and Salivary Survivin according to their gender in Case (Group I) and Controls (Group II)

| | Cases (Group I) | | |
|---|---|---|---|
| | Male (n=45) | Female (n=15) | P Value |
| | Mean ± SD | Mean ± SD | |
| SER SUR | 22.46±2.772 | 22.40±1.611 | 0.950 |
| SAL SUR | 13.44±2.358 | 13.15±1.567 | 0.657 |
| | CONTOLS (Group II) | | |
| Gender | Male (n=33) | Female (n=27) | P Value |
| | Mean ±SD | Mean ±SD | |
| SER SUR | 15.22±1.162 | 14.95±0.924 | 0.332 |
| SAL SUR | 5.35±0.977 | 5.20±0.953 | 0.555 |

### *Age*

On correlating serum Survivin level with age, it was maximum in the age group of 60 years above in both the groups. **Cases** (Group I)showed Survivin expression 25.21pg/ml and Controls (Group II)showed 16.37pg/ml. Minimum values were found in subjects between 20-40 years of age in both the groups, Cases (Group I) showed Survivin expression 19.24pg/ml and Controls (Group II) showed 13.77pg/ml, it was statistically significant.

On correlating salivary Survivin level with age, it was maximum in the age group of 60 years above in both the groups. Cases (Group I) showed Survivin expression 15.87pg/ml and Controls (Group II) showed 6.34pg/ml. Minimum values were found in subjects between 20-40 years of age in both the groups, Cases (Group I) showed Survivin expression 10.49pg/mland Controls (Group II) showed 4.05pg/ml, it was statistically significant.

**Table 5** Mean comparison of serum and salivary Survivin according to their age intervals in Cases (Group I) and Controls (Group II). (Applied one way ANOVA for significance. *Significant)

| | CASES (Group I) | | | |
|---|---|---|---|---|
| | 20 to 40 years (n=14) | 41 to 60 years (n=31) | Above 60 years (n=15) | P value |
| | Mean ± SD | Mean ± SD | Mean ± SD | |
| SER SUR | 19.24±1.440 | 22.60±1.635 | 25.21±0.753 | 0.001* |
| SAL SUR | 10.49±.912 | 13.45±1.379 | 15.87±0.342 | 0.001* |
| | CONTROL (Group II) | | | |
| | 20 to 40 years (n=15) | 41 to 60 years (n=28) | Above 60 years (n=17) | P value |
| | Mean±SD | Mean±SD | Mean±SD | |
| SER SUR | 13.77±0.523 | 15.04±0.434 | 16.37±0.472 | 0.010* |
| SAL SUR | 4.05±0.417 | 5.30±0.482 | 6.34±0.500 | 0.010* |

## DISCUSSION

OSCC is the most common malignant tumor of the oral cavity, having 5-years survival rate of less than 50% (Parkin *et al*., 2002). Most of oral cancer cases occur in 4$^{th}$ and 5$^{th}$ decade of life, in our study also we found that both cases (group I) and control (group II) showed median age of 55 years. Continuous exposer to some physical and chemical changes with genetic predisposition could be the cause of this carcinogenic process. The most commonly OSCC occur in buccal mucosa and gingivo-buccal sulcus (Tandon A, 2018) whereas in our study the most common site was border of tongue (26.7%) followed by posterior bucco-alveolus region (26.7%). This variation may be attributed to the aetiological factors and geographical distribution.

Despite advances in treatment modalities of cancer, the life expectancy is the same as last decades primarily due to delayed diagnosis (Mcdowell *et al*., 2006). Various biomarkers found in body fluids (as blood, saliva or urine) would be desirablerather tissue markers as they are affordable as low cost, easy for sampling, require low-invasive procedure, and repetitive (Nunes *et al*.,2000).

Survivin is an antiapoptotic protein, of Inhibitor of Apoptosis Protein (IAP) family, it is expressed during embryogenesis and in tumor cells but insignificant in normal mature tissue (Li *et al*., 2012). It decreases caspase activity and apoptosis in cells exposed to diverse apoptosis-triggering stimuli, mainly expressed in cells of most of the cancers (Tamm *et al*., 1998), (Jattella *et al*., 1999).

There are controversial opinions for the expression of Survivinin normal mucosa and potentially malignant lesions. According to some author the Survivin expression is present at a low level in both, the normal oral mucosa and the premalignant lesions (Lodi *et al*., 2010). Whereas other studies emphasize a substantial difference between malignant disorders and normal mucosa (Tanaka *et al*., 2003). Lots of studies have been done regarding over expression of Survivin in urine (Srivastava *et al*., 2013), peripheral blood, tissue (Kapellos *et al*., 2013) and pleural fluid (Park *et al*., 2012). Muzio *et al*. 2003concluded Survivinas a potential early prognosticator for tumor progression in oral mucosa. Very exiguous studies have been done in serum and saliva regarding over expression of Survivin in OSCC (Muzio *et al*., 2001; 2003, Tanaka *et al*., 2003).

In this study, serum and salivary Survivinevaluation has been done in control and OSCC patients and tried to find the possibility of using it as a potential serum and salivary

biomarker. This pioneer study has emphasized on the comparative evaluation of salivary and serum Survivin in OSCC patients. The previous Survivin based studies were focused mainly on other body fluids like urine, concluded that cancer patients might have higher levels of Survivinas compare to healthy control (Shariat *et al*., 2004).

In our study we found significant correlation between serum and salivary Survivin expression level with patient's gender and age whereas Li *et al* (2016) reported that the Survivin expression showed no obvious correlation with patient's age, gender, lesion site, or course of the diseases. The maximum mean value of serum and salivary Survivin was found in patients above 60 years. The reason behind this could be that, at this age group multiple factors aggravate the carcinogenesis process such as low immunity, longer exposure to carcinogens like tobacco products and alcohol. This was also supported by Pelucchi *et al*. (2006) who reported that alcohol was considered an autonomous risk factor for many types of cancer.

On analyzing the serum and salivary Survivin, the results of the present study indicatethat Survivin levels in patients with OSCC are significantly higher than those of healthy subjects. This study finds a positive co-relation between the Survivin expression and malignant transformation. In our study we did not considered the degree of malignant transformation with Survivin expression. It was a cross-sectional analytical study, focused only one particular population with low sample size with only prospective data on serum and salivary Survivin. These could be considered as the drawback of the study.

## CONCLUSION

Presentstudy may be the milestone in the field of serum and salivary Survivin expression. The study emphasizes that salivary Survivin expression may be a good noninvasive diagnostic aid for early detection of oral cancer hence increasing patients' survival rate.

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