10.5005/jp-journals-10015-1415

ORIGINAL RESEARCH

Assessment and Comparison of Salivary Survivin Biomarker in Oral Leukoplakia, Oral Lichen Planus, and Oral Cancer: A Comparative Study

[1]Ruchika Garg, [2]Arvind V Shetti, [3]Anjana S Bagewadi

ABSTRACT

Introduction: Oral squamous cell carcinoma (OSCC) is the most common malignant tumor of the oral cavity. It is preceded by various potentially malignant conditions like oral leukoplakia and oral lichen planus. Survivin is an inhibitor of apoptosis whose levels have been found increased in oral cancer as well as various potentially malignant conditions. Thus survivin can act as a biomarker and help in early detection of potentially malignant conditions which can prevent its transformation into malignancy.

Aim: To assess and compare the level of total human salivary survivin in oral leukoplakia, oral lichen planus, oral cancer, and control group.

Materials and methods: A total of 96 subjects were included in the study, which were further grouped into 24 in each group. The saliva was analyzed for survivin level among all the four groups. Survivin concentration (pg/mL) was studied in relation to clinical data.

The results were analyzed using Mann–Whitney U test to derive the statistical difference.

Results: The average of levels of survivin in control group was 0.199 pg/mL, in oral leukoplakia group 0.312 pg/mL, in oral lichen planus group 0.380 pg/mL, and in oral cancer group 0.430 pg/mL. A comparison of all these groups revealed statistically significant difference among the groups.

Conclusion: Survivin may not be considered as an independent predictor of the malignant transformation for premalignant lesions but it can be an indicator for an increased risk of malignant transformation.

Keywords: Oral cancer, Oral leukoplakia, Oral lichen planus, Potentially malignant conditions, Survivin.

How to cite this article: Garg R, Shetti AV, Bagewadi AS. Assessment and Comparison of Salivary Survivin Biomarker in Oral Leukoplakia, Oral Lichen Planus, and Oral Cancer: A Comparative Study. World J Dent 2017;8(2):73-76.

Source of support: Nil

Conflict of interest: None

[1-3]Department of Oral Medicine and Radiology, KLE VK Institute of Dental Sciences, Belgaum, Karnataka, India

Corresponding Author: Ruchika Garg, Department of Oral Medicine and Radiology, KLE VK Institute of Dental Sciences Belgaum, Karnataka, India, Phone: +91-9535619586, e-mail: drruchikamcods@gmail.com

INTRODUCTION

Oral squamous cell carcinoma (OSCC) is the most common malignant tumor of the oral cavity representing more than 90% of all cancers of this district and being the 8th most common cancer worldwide.[1,2] In spite of so many advances in the field oncology, the mortality rates in the last 20 years remains almost unchanged.[2,3] As we all know, cancer develops much more slowly than has been thought, with the timelines from when it forms to the final stage which may be spanning up to months or years. It is preceded by potentially malignant lesion like oral leukoplakia and, though potential for malignant transformation of oral lichen planus, is debatable; however, few cases have been reported of its transformation to oral carcinoma. So if they are left untreated, they have an increased probability of transformation to OSCC. Thus, it is important to monitor and check them in their early stages. The early diagnosis of these lesions can prevent them from malignant transformation, and the delay represents one of the principal reasons for its transformation and poor prognosis.[2]

Its development is a multi-step process involving activation of oncogenes and inactivation of tumor suppressor.[4,5] Recently, there has been discovery of sensitive and specific tumor markers for early detection of cancer which may improve the survival rates of patients with OSCC.[2] Survivin is a member of inhibitor of apoptosis, and it exhibits an undetectable expression levels in most of the nonproliferating adult tissues, but is majorly expressed in a wide range of cancers suggesting that it is associated with both control of cell survival and regulation of mitosis in tumor cells and its degree of expression is directly related to poor prognosis.[2,4,6] Thus, it may represent a reliable universal marker for OSCC because of which survivin detection could be employed for diagnostic purposes.[2]

However, one of the major limitations of salivary survivin biomarker to be used solely as a biomarker is that there is no biologically justified cutoff value for levels of survivin in patients with cancer, including oral cancer, as it is also present though in minimal levels in normal patients. Further studies in larger cohorts of patients is necessary to establish a standard biological and clinically relevant cutoff values.[2] In humans, the mechanism of carcinogenesis delineate by loss of mechanisms which

regulate cell-cycle progression, cell viability/death balance, and apoptosis.[5] Apoptosis preserves normal homeostasis and organ morphogenesis.[5,7] An aberration of this process may contribute to carcinogenesis by prolonging the cell viability.[5,8] The serial changes in the process of apoptosis are an essential feature of cancer cells.[4,6]

So, survivin here comes into role play.

This is the first of its own kind of study as no studies have been done to assess the levels total human salivary survivin biomarker in oral leukoplakia with a sample size, as taken under the present study and oral lichen planus. Also, it has never been compared to the levels in oral cancer.

So, the aim of this study was to assess the levels of total human salivary survivin biomarker in the potentially malignant disorders like oral leukoplakia, oral lichen planus, and oral cancer. The objectives of the study were to assess these levels and to compare them in all the above groups.

Since the study gave an estimation of levels of survivin in potentially malignant conditions, so it can be correlated to the levels in oral cancer and can form a reliable marker for its malignant transformation to OSCC.

Hypothesis can be given as:

- *Null hypothesis*: There is no significant difference in the levels of survivin in the potentially malignant lesions and oral cancer.
- *Research hypothesis*: There is significant difference in the levels of survivin in the potentially malignant lesions and oral cancer.

MATERIALS AND METHODS

Ethics statement: The protocol used for the study was reviewed and approved by the institutional research and ethical committee.

Subjects of either sex with clinically confirmed cases of oral leukoplakia, oral lichen planus, and oral cancer with age group above 18 years and willing to participate in the study and sign an informed consent reporting to the Department of Oral Medicine and Radiology, KLE VK Institute of Dental Sciences, Belgaum, were included in the study after obtaining the consent. Clinical confirmation of cases of oral leukoplakia were done according to classification by Van der Waal, cases of oral lichen planus were done according to classification by JO Andreasen, and cases of oral cancer were done according to modified TNM staging classification for cancer of oral cavity by Howaldt et al.[9-11] In the current study, all the types and stages of cases of oral leukoplakia, oral lichen planus, and oral cancer were included.

Patients with potentially malignant disorders other than leukoplakia, lichen planus, salivary gland disorders, long-term drug history and medically compromised patients, and also patients presenting with any other carcinoma other than oral cavity were excluded.

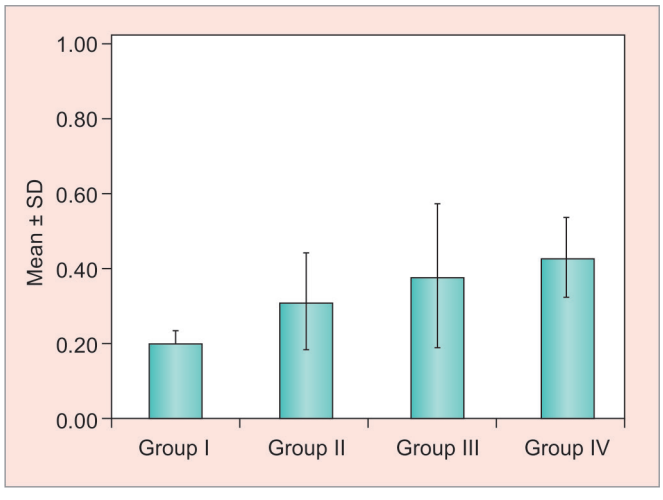
The included patients were a consecutive series of patients presenting at the study center which fulfilled the inclusion criteria. It was a prospective study.

METHODOLOGY

The study included 96 subjects divided into four groups: (1) Control group (Group I), (2) oral leukoplakia group (Group II), (3) oral lichen planus group (Group III), and (4) oral cancer group (Group IV). Patients were given the following instructions (David T Wong): (1) Not to brush their teeth within 45 minutes prior to sample collection; (2) dental work should not be performed within 24 hours prior to sample collection; (3) saliva samples visibly contaminated with blood should be discarded and recollected; (4) document consumption of alcohol, caffeine, nicotine, and prescription/over-the-counter medications within the prior 12 hours; (5) document vigorous physical activity and the presence of oral diseases or injury; and (6) do not eat a major meal within 60 minutes of sample collection; (7) rinse mouth with water to remove food residue and wait at least 10 minutes after rinsing to avoid sample dilution before collecting saliva. Saliva was collected by asking the patient to sit erect on the dental chair and allow saliva to collect in the floor of mouth for 3 to 5 minutes. Then they were asked to spit in the vials. Later, the collected saliva was sent to basic research laboratory for its analysis by Human Total Survivin DuoSet IC Enzyme Assay Kit.

Statistical Analysis

The results were analyzed as follows: Pairwise comparison was done by Mann–Whitney U test, as shown in Graph 1.



Graph 1: Graphical representation of the mean levels of survivin in all four groups

RESULTS

A total of 96 patients participated in the study which extended from December 2014 to February 2015. These were divided into four groups—Groups I, II, III, and IV. All the patients gave their consent for the study. The average of levels of survivin was 0.199 pg/mL in control group, 0.312 pg/mL in leukoplakia group, 0.380 pg/mL in oral lichen planus group, and 0.430 pg/mL in oral cancer group (Table 1). Then the comparison of all these groups was done to find the statistical difference between them. It was found that there was statistically significant difference present in between

- Groups I and II
- Groups I and III
- Groups I and IV
- Groups II and IV, and
- Groups III and IV (Table 2).

DISCUSSION

Oral squamous cell carcinoma is the leading malignant tumor in the oral cavity, with a 5-year survival rate of less than 50%.[1,2,4] Despite many advances in oncology, the trend has remained unchanged over the last few decades mainly because of diagnostic delay, which leads to making the diagnosis at an advanced stage thus drastically reducing effectiveness of therapies and life expectancy.[2] So, the identification of molecular markers for early cancer detection is very mandatory. Salivary lactate dehydrogenase (LDH), a metabolic enzyme catalyzing the anerobic glycolysis, has been a nonspecific indicator of various systemic diseases. It is an intracellular enzyme detectable in the cellular cytoplasm of all the cells in the human body, which becomes extracellular upon the cell death. Therefore, its extracellular presence is related to the cell death and tissue destruction. The LDH concentration in saliva is an expression of cellular necrosis and could be a more specific indicator of the oral lesions that affect the integrity of the oral mucosa. The levels of LDH can be measured, and screening of oral lichen planus can be performed, which is feasible, simple, and a convenient approach that does not require expert examiners.[12] Moreover, salivary markers are preferred because of saliva's ease of collection, noninvasive procedure, low cost, and multiple sampling for monitoring over time.[13] Studies have shown that there was overexpression of survivin in tumors like neuroblastoma, colorectal, bladder, and breast cancers.[14] So in the current study cases with malignancies other than oral cancer were excluded to avoid any bias. Several studies have shown that saliva from patients with OSCC contain high levels of survivin.[15-20] Hence, in the current study possibility of using survivin as a biomarker for oral leukoplakia and oral lichen planus and its role in involvement in the process of carcinogenesis was evaluated.

Table 1: Mean value of salivary survivin in all four groups

Groups	*Mean*
I	0.20
II	0.31
III	0.38
IV	0.43

Table 2: Statistical difference between all the groups which are significant

Pair-wise comparison by Mann–Whitney U test	
Group I *vs* II	p = 0.00001*
Group I *vs* III	p = 0.00001*
Group I *vs* IV	p = 0.00001*
Group II *vs* III	p = 0.1578
Group II *vs* IV	p = 0.00001*
Group III *vs* IV	p = 0.0260*

*p < 0.05; Where Group I is normal group; Group II is oral leukoplakia group; Group III is oral lichen planus group; Group IV is oral cancer group

In the present study, a total of 96 subjects were included and the Human Total Survivin DuoSet IC Enzyme Assay analysis was performed which revealed that there was statistically significant difference present between all the groups confirming its functionality as an early diagnostic tool and also its role in the early involvement in carcinogenesis.[5] Evidence/literature reveals that this is the first study which is estimating the salivary survivin level in the patients with oral lichen planus. In the current study, there was increased level of survivin found in the premalignant lesion like oral leukoplakia as compared to the control group which was in agreement with the study done by Santarelli et al.[2] In previous studies, there is a continuing debate regarding the potential of oral lichen planus undergoing malignant transformation, but the current study showed an increased level of survivin in oral lichen planus compared to the control group which was slightly higher than the levels of survivin in oral leukoplakia, which indicates an increased risk of malignant transformation in oral lichen planus. So, further studies have to be done with a larger sample size. The limitation of the current study was that all the types and stages of oral leukoplakia and oral lichen planus were included in the study without segregating them into groups which would further provide a more precise analysis of the rate of each type of these lesions undergoing malignant transformation. Even taking into account the above-mentioned limitation of the present study, survivin seems to be remarkable salivary biomarker for detection of an increased risk of malignant transformation of oral leukoplakia and oral lichen planus. However, present data should be considered preliminary, and thus the use of survivin as a single marker may be not

sufficient for confirmation of malignant transformation of oral leukoplakia and oral lichen planus.

CONCLUSION

The study explains that survivin may not be considered an independent predictor of malignant transformation of potentially malignant lesion like oral leukoplakia, but can be considered as a marker for the increased risk of these lesions to undergo malignant transformation as well as demonstrate its role in the early process of carcinogenesis. Also elevated levels in lichen planus compared to leukoplakia point toward an increased state of risk in lichen planus patients for malignant transformation which has to be confirmed by further studies in a controlled setup.

REFERENCES

1. Lo WY, Chang NW. An indirubin derivative, indirubin-3-monoxime suppresses oral cancer tumorigenesis through the downregulation of survivin. PLoS One 2013 Aug;8(8):e70198.
2. Santarelli A, Mascitti M, Lo Russo L, Colella G, Giannatempo G, Bambini F, Emanuelli M, Procaccini Ml. Detection level of salivary survivin in patients with OSCC. J Carcinogene Mutagene 2013 Oct. doi:10.4172/2157-2518.S5-004.
3. Chianeh YR, Prabhu K. Biochemical markers in saliva of patients with oral squamous cell carcinoma. Asian Pac J Trop Dis 2014 Dec;4(Suppl 1):S33-S40.
4. Li SX, Chai L, Cai ZG, Jin LJ, Chen Y, Wu HR, Sun Z. Expression of survivin and caspase 3 in oral squamous cell carcinoma and peritumoral tissue. Asian Pac J Cancer Prev 2012;13(10):5027-5031.
5. Khan Z, Tiwari RP, Mulherkar R, Sah NK, Prasad GB, Shrivastava BR, Bisen PS. Detection of survivin and p53 in human oral cancer: correlation with clinicopathologic findings. Head Neck 2009 Aug;31(8):1039-1048.
6. Stenner M, Weinell A, Ponert T, Hardt A, Hahn M, Preuss SF, Guntinas-Lichius O, Klussmann JP. Cytoplasmic expression of survivin is an independent predictor of poor prognosis in patients with salivary gland cancer. Histopathology 2010 Nov;57(5):699-706.
7. Nagata S. Apoptosis and autoimmune diseases. IUBMB Life 2006 May-Jun;58(5-6):358-362.
8. Thompson CB. Apoptosis in the pathogenesis and treatment of disease. Science 1995 Mar;267(5203):1456-1462.
9. Warnakulasuriya S, Johnson NW, van der Waal I. Nomenclature and classification of potentially malignant disorders of the oral mucosa. J Oral Pathol Med 2007 Nov;36(10):575-580.
10. Andreasen JO. Oral lichen planus - a clinical evaluation of 115 cases. J Oral Surg Oral Med Oral Pathol 1968 Jan;25(1):31-42.
11. Howaldt HP, Kainz M, Euler B, Vorast H. Proposal for modification of the TNM staging classification for cancer of the oral cavity. J Craniomaxillofac Surg 1999 Oct;27(5):275-288.
12. Lokesh K, Jayanthi K. Lactate dehydrogenase: an enzymatic biomarker in oral health and disease. Int J Contemp Dent Med Rev 2015 Sep;2015:040815.
13. Hu S, Arellano M, Boontheung P, Wang J, Zhou H, Jiang J, Elashoff D, Wei R, Loo JA, Wong DT. Salivary proteomics for oral cancer biomarker discovery. Clin Cancer Res 2008 Oct;14(19):6246-6252.
14. Shariat SF, Casella R, Khoddami SM, Hernandez G, Sulser T, Gasser TC, Lerner SP. Urine detection of survivin is a sensitive marker for the noninvasive diagnosis of bladder cancer. J Urol 2004 Feb;171(2 Pt 1):626-630.
15. Tanaka C, Uzawa K, Shibahara T, Yokoe H, Noma H, Tanzawa H. Expression of an inhibitor of apoptosis, survivin in oral carcinogenesis. J Dent Res 2003 Aug;82(8):607-611.
16. Jinbu Y, Tsukinoki K, Miyagi N, Senna T, Obi Y, Matsumoto K, Noguchi T, Kusama M. Expression of survivin in oral squamous cell carcinoma. Oral Med Pathol 2006;11(2):41-44.
17. Muzio LL, Pannone G, Staibano S, Mignogna MD, Rubini C, Mariggio MA, Procaccini M, Ferrari F, De Rosa G, Altieri DC. Survivin expression in oral squamous cell carcinoma. Br J Cancer 2003 Dec;89(12):2244-2248.
18. Su L, Wang Y, Xiao M, Lin Y, Yu L. Up-regulation of survivin in oral squamous cell carcinoma correlates with poor prognosis and chemoresistance. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2010 Oct;110(4):484-491.
19. Nambiar KS, Haragannavar VC, Augustine D, Sowmya SV, Rao RS. Diagnostic aids in detection of oral precancer and cancer: past to present. Int Dent Med J Adv Res 2016;2:1-7.
20. Lokesh K, Jayanthi K. Saliva: a mirror to health. Int J Contemp Dent Med Rev 2015;2015:030715.