ORIGINAL ARTICLE

INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACEUTICAL SCIENCES

Published by JK Welfare & Pharmascope Foundation Journal Home Page: www.ijrps.com

Estimation of Total and Lipid Bound Sialic acid in oral leukoplakia and oral squamous cell carcinoma patients

Archana Sonone*, Alka Hande, Madhuri Gawande, Swati Patil

Department of Oral Pathology and Microbiology, Sharad Pawar Dental College, Datta Meghe Institute of Medical Sciences (Deemed to be University), Sawangi (Meghe), Wardha, Maharashtra, India

Article History:

Received on: 11 Aug 2020
Revised on: 12 Sep 2020
Accepted on: 14 Sep 2020

Keywords:

Oral Squamous Cell Carcinoma,
Oral Leukoplakia,
Sialic Acid

ABSTRACT

Tumour markers are biochemical substances released through tumour cells. They are considered as the rationale or consequence of the carcinogenesis process. Neoplasms often have an increased concentration of sialic acid on the tumour cell surface and are shed or secreted by some of these cells, which increase the concentration in blood. To determine serum levels of total sialic acid (TSA), lipid-bound sialic acid (LBSA), in patients of oral Leukoplakia (LP) and oral squamous cell carcinoma (OSCC). The study comprises 75 subjects which include 25 cases of LP, 25 cases of OSCC and 25 cases of healthy individuals as control. 10 ml intravenous blood was collected under aseptic condition, and biochemical analysis of total sialic acid and lipid-bound sialic acid was carried out by spectrophotometer. We observed levels of TSA and LBSA significantly increased in LP and OSCC as compared to a healthy control group. The increased level of TSA and LBSA in LP helps to determine the early stage of the disease. Further differentiation in grades of OSCC is also possible by these biochemical markers. Thus serum levels of TAS, LBSA can be used as diagnostic and prognostic markers.

*Corresponding Author

Name: Archana Sonone
Phone: 9145229353
Email: archanasonone169@gmail.com

ISSN: 0975-7538

DOI: https://doi.org/10.26452/ijrps.v11iSPL4.4317

Production and Hosted by

IJRPS | www.ijrps.com

INTRODUCTION

Oral squamous cell carcinoma (OSCC) is one of the life-threatening and mutilating disease affecting humanity. It is a prime health hazard in the Indian subcontinent. It is amongst the first three types of cancers like carcinoma of the breast, cervical carcinoma, and oral squamous cell carcinoma in the country (Elango *et al.*, 2006). Most of the OSCC are preceded by asymptomatic clinical lesions together called oral potentially malignant disorders (OPMD) or a precancerous lesion. The prevalence of precancerous lesions in the oral cavity is about 2.5% in the general population (Petti, 2003). About 15%–48% of the OSCC are developed from innocent appearing precancerous lesions, and approximately 60% of them are clinically present as white keratotic lesions (Ali *et al.*, 2004). Oral Leukoplakia (LP) is the most common white keratotic lesion amongst OPMD. It is defined as "a predominantly white lesion or plaque of questionable behaviour having excluded clinically and histopathologically, any other definable white disease or disorder" as stated by van der Waal (Brouns *et al.*, 2013). The overall frequency of malignant transformation rate of LP varies between 15.6% and 39.2% (Neville and Day, 2002) which is the highest of other OPMDs.

Despite the vast majority of research and advances in the field of surgical oncology, the mortality rate remains unchanged (Massano *et al.*, 2006). The five-

year survival rate of OSCC is estimated to be approximately 50% (Elango *et al.*, 2006). OSCC is usually detected at advanced clinical stages which results in unfortunate prognosis and causes substantial financial constraint. Thus early detection and treatment of OSCC offer a better outcome and so affordable to patients. Thus inexpensive and accessible diagnostic tools could be the game-changer to decrease the mortality and morbidity of cancer.

Thus markers with diagnostic and prognostic relevance are the need of the hour. There are various biological markers which may be applicable to observe progression as well as predict the therapeutic response and prognostic outcome of cancer. They may also aid in the diagnosis of the disease. These biological markers, which are commonly referred to as tumour markers, are involved in physiological processes and altered during the pathological changes which take place in cancer. They can be estimated in plasma, serum, as well as other body fluid (Romppanen, 2003).

The carbohydrate, which is covalently linked with protein, lipid, peptides and saccharides are called glycoconjugates (Boston, 2007). These glycoconjugates play a significant role in the pathophysiology of cancer. Terminal epitopes of carbohydrates are essential in cell-cell interactions and loss of cell adhesion during malignant transformation. Sialic acids are non-reducing, negatively charged, terminal carbohydrate residues of oligosaccharide chain of many glycoproteins and glycolipids. Due to their exposed position, they play a significant role in cell to cell adhesion, identification, tumour invasion and immunogenicity. In the neoplastic transformation, these glycoconjugates get altered and released into circulation through their increased turn over by secretions and shedding of cancerous cells. This leads to increased levels of sialic acid in the blood due to its non-reducing termini. Thus estimation of sialic acids may act as an effective diagnostic and prognostic markers and may be useful in mass screening of LP and OSCC cases. (Rao *et al.*, 1998). In this research, efforts were made to ascertain the efficacy of TSA and LBSA as tumour markers in a patient suffering from LP as well as OSCC.

MATERIALS AND METHODS

The present observational cross-sectional research was carried out on subjects reporting in the Department of Oral Pathology and Microbiology Sharad Pawar Dental College and Hospital, Wardha, Maharashtra, India. The study comprised of 75 subjects reported to the open patient door (OPD), which included 25 each clinically diagnosed and histopathologically confirmed cases with LP (Group I), and 25 OSCC, 25 age and sex-matched healthy cases were selected as controls (Group III). Informed written consent was procured from all the cases. Ethical clearance was obtained from the Ethical Committee of the Institute. The detailed case history was recorded along with a thorough examination of the soft and hard tissues of the oral cavity. In Group I and II, subjects with a history of any systemic diseases such as diabetes mellitus, hypertension, endocrine disorders, and cardiovascular disorders, pregnant and lactating women and subjects diagnosed with malignancies in sites other than the oral cavity were excluded. Subjects who were on any medications, diagnosed with any other oral mucosal lesions were also excluded. Subjects in Group III were healthy without any oral or systemic diseases, who were not under any medications and did not have any oral adverse habits.

The histopathological grading of OSCC was done according to the malignancy grading system proposed by Broders (1983). LP was graded according to the status of presence or absence of dysplasia as per Smith-Pindborg criteria (Pindborg *et al.*, 1985). Under all aseptic precautions, venous blood was drawn from midcubital/ antecubital vein with the help of disposable 5ml sterile syringe and 22 gauge needle. The blood was transferred to a sterile glass bulb and allowed to clot for an hour. Serum was separated and centrifuged at 4000 RPM. Serum samples were stored at -20 ^{0}C in the deep freezer until tested. Protocol for estimation of serum TSA was followed as per Plucinsky *et al.* (1986), and LBSA by Katopodis *et al.* (1982). Resorcinol reagent method was used for the evaluation of TSA and LBSA.

Statistical analysis

To compare the mean of TSA and LBSA, analysis of variance (one way ANOVA) was carried out for the three groups. Comparison of the mean values between groups was made using multiple comparison test by Tukey –HSD procedure, using the statistic package of SPSS10.0 for windows.

OBSERVATIONS & RESULTS

In the present study on clinicopathological analysis in LP, the patient's ages were in the range of 31 to 80 years with an average of 45.8 years. Amongst 25 patients of LP, 13 (37.3%) patients showed the presence of dysplasia. Estimation of serum TSA and LBSA was carried out in LP. The mean value of TSA, LBSA in LP patients, was 65.24 mg/dl, 22.29 mg/dl, respectively. On comparison of these values with the control group, it was found statistically significant

Table 1: Comparison of TSA and LBSA in group I in group II and group III

Parameter	Groups	N	Mean	SD	'p' value
TSA mg/dl	Group I (LP)	25	65.224	6.1840	0.000
	Group II (OSCC)	25	85.938	13.1784	
	Group III (CG)	25	53.897	5.9861	
LBSA mg/dl	Group I (LP)	25	22.2916	3.65129	0.000
	Group III (OSCC)	25	33.2016	6.20393	
	Group III (CG)	25	18.1740	3.36466	

The T-test is used to compare the means between groups. ANOVA test is used to see the difference among the means of three groups; significant results are highlighted

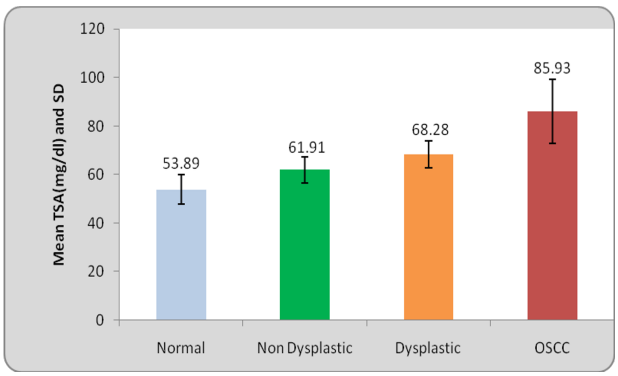
Table 2: Comparison of TSA and LBSA in non-dysplastic to dysplastic cases of LP

Parameters	Grades	N	Mean	Std. Deviation	'p' value
TSA	Non-Dysplastic	12	61.913	5.2327	.013
	Dysplastic	12	67.897	5.5659	significant
LBSA	Non-Dysplastic	12	22.1675	3.91172	.835
	Dysplastic	12	22.4950	3.69350	NS

Table 3: Comparison of TSA and LBSA in different histological grades of OSCC cases

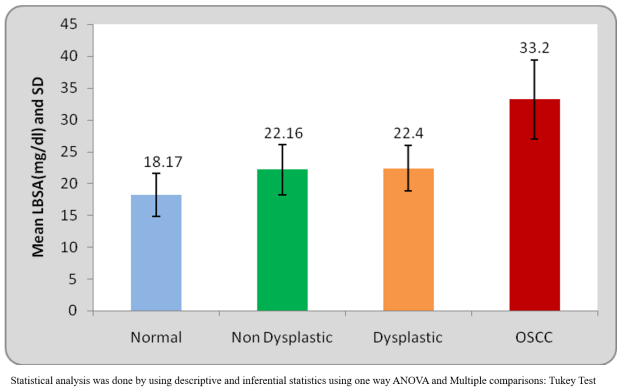
Parameters	Grades	N	Mean	SD	'p' value
TSA	WDSCC	18	84.506	12.2444	.032 significant
	MDSCC	5	38.6280	3.98977	
LBSA	WDSCC	18	32.4567	5.98368	.043 significant
	MDSCC	5	38.6280	3.98977	

(p=0.001) (Table 1).



Graph 1: Comparison of TSA (mg/dl) in four groups Descriptive Statistics

Further on comparison of serum TSA amongst dysplastic and non-dysplastic cases of LP, TSA was increased significantly from normal (53.89 ± 5.98 mg/dl,) to no dysplasia (61.91 ± 5.23 mg/dl) to (67.897 ± 5.56 mg/dl) dysplasia(p=0.001). On the evaluation of LBSA, levels and their comparison from normal to no dysplasia to dysplasia, we observed no significant difference. (p> 0.001)



Graph 2: Comparison of LBSA (mg/dl) in four groups Descriptive Statistics

(Table 2).

On the evaluation of clinicopathological analysis of OSCC cases, patient's ages were in the range of 39 to 86 years with an average of 54.36 years. In general, OSCC was observed mostly in males (56%) as compared to females (44%) with a ratio of 1.5:1. Further on the evaluation of histopathological grading of OSCC, 80 % cases were in "well-differentiated oral squamous cell carcinoma (WDOSCC)" grade

whereas 20 % were in "moderately differentiated oral squamous cell carcinoma (MDOSCC)" grade. No case was there in "poorly differentiated oral squamous cell carcinoma (PDOSCC)" grade.

On the evaluation of TSA and LBSA in OSCC, TSA and LBSA were found to increase significantly from average (53.897 ± 5.9861 mg/dl and 18.1740 ± 3.36466 mg/dl) to OSCC (85.938 ± 13.1784 mg/dl and 33.2016 ± 6.20393 mg/dl) (Table 1).

There were twenty cases in WDOSCC grade of OSCC whereas five were in MDOSCC grade. On comparison of TSA in WDOSCC and MDOSCC cases it was increased significantly from WDOSCC (84.506 ± 12.2444 mg/dl,) to MDOSCC (97.734 ± 3.98977 mg/dl,) Similarly LBSA was also found to increase significantly from WDOSCC (32.4567 ± 5.98368 mg/dl,) to MDOSCC (38.6280 ± 3.98977 mg/dl,) (Table 3).

It is observed that on comparison of TSA and LBSA in non-dysplastic to dysplastic cases LP to OSCC, TSA was found to increase from non-dysplastic (61.91 ± 5.23 mg/dl) to dysplastic cases (67.897 ± 5.56 mg/dl) of LP to OSCC (85.938 ± 13.1784 mg/dl) (Graph 1).

On the evaluation of LBSA it was found to increase from non- dysplastic (22.1675 ± 3.91172 mg/dl) to dysplastic (22.4950 ± 3.69350 mg/dl) cases of LP and other cases of OSCC (33.2016 ± 6.20393 mg/dl). However, the difference between non- dysplastic to dysplastic cases of LP was not significant (Graph 2).

DISCUSSION

Early detection and diagnosis are significant in reducing the mortality and morbidity of oral cancer. Clinically appreciable precancerous lesions precede most oral cancer; however, sometimes the precancerous or cancerous alterations are not always discernible clinically as well as histologically. Estimation of various serum biomarkers can act as an adjunct to the conventional biopsy procedure. The inference of these biomarkers may be significant in mass screening of oral precancerous and cancerous patients. The technique of estimation of these biomarkers is noninvasive as well as cost-effective.

Tumour markers are biochemical substances released by tumour cells either due to the reason or consequence of the carcinogenesis process. Thus, these biomarkers could be of substantial diagnostic and prognostic importance in cancer patients. Cell membrane becomes altered during the process of carcinogenesis and is vital for uncontrolled growth and malignant behaviour of the neoplastic cells. Certain glycoproteins and glycolipids are considered to be specific tumour markers as these are the major constituents of the cell membrane. Increased levels of glycopeptides containing galactose, mannose, fructose and sialic acid are observed in altered cells of several types of solid tumours, suggesting a relationship among malignant transformation and alterations in cell-surface glycoconjugates (Romppanen, 2003).

Literature search has revealed the estimation of serum sialic acid in various types of malignancies like colorectal carcinoma (Tewarson *et al.*, 1993), lung carcinoma (Dnistrian *et al.*, 1982) and OSCC (Rajpura *et al.*, 2005).

The present study included 25 clinically and histologically diagnosed cases of LP. The mean of TSA and LBSA in LP cases were 65.24 mg/dl, 22.29 mg/dl, and 10.76 mg/dl respectively and was statistically significant as compared to control group patients (Table 1).

Concerning TSA, we observed significantly increased levels in LP as compared to control group patients. (p=0.001). Our findings were consistent with Rajpura *et al.* (2005); Joshi and Patil (2010); Taqi (2012). We also observed significantly increased level of LBSA in LP (p=0.001). Our observations are in line with Baxi *et al.* (1991); Taqi (2012). Further on comparison of TSA, LBSA amongst dysplastic and non-dysplastic cases of LP. We observed significantly increased levels from normal to nondysplastic to dysplastic cases of LP (Table 2). Although morphological and cellular alterations in dysplastic cells are discernible in histological examination of tissue, molecular modifications cannot be perceived at much earlier phase of cellular transformation. Alteration of sialic acids, the terminal carbohydrate residues of oligosaccharide chain of many glycoproteins is observed during neoplastic transformation. Further, these get released into circulation through the shedding of dysplastic cells. Thus significantly increased levels of TSA, and LBSA suggest the alterations in glycoproteins during the process of cellular and molecular changes of the transformation of normal into dysplastic cells and their release into the serum.

Regarding OSCC, we observed significantly increased levels of TSA as compared to normal (Table 1). Our results are in accordance with Shashikanth and Rao (1994); Joshi and Patil (2010), similarly LBSA was also found to increase which is in agreement with Rajpura *et al.* (2005); Taqi (2012). It is observed that both the parameters, ie. TSA, LBSA was increased significantly from regular to non – dysplastic to dysplastic cases of LP to

OSCC (Graphs 1 and 2). Sialic acid is non-reducing, negatively charged, terminal carbohydrate residues of oligosaccharide chain of many glycoproteins and glycolipids. Due to their exposed position, they play a significant role in cell to cell adhesion identification, cell invasiveness and immunogenicity. In the neoplastic transformation, these glycoconjugates get altered and released into circulation through increased turnover by secretions and shedding from malignant cells. This leads to increased levels of sialic acid in the blood due to its non-reducing termini.

In the present study, we also attempt to compare both parameters according to histopathological grading of OSCC. Rajpura *et al.* (2005); Taqi (2012) compared levels of TSA and LBSA in OSCC according to histopathological grading. Regarding histological grading of OSCC, the levels of TSA and LBSA increased in MDOSCC as compared to WDOSCC (Table 3). In MDOSCC, cellular activity is more as compared to WDOSCC, which further results in increased turnover of tumour cells and ultimately due to increased sialic acid concentration in blood.

CONCLUSION

We observed they increased in TSA and LBSA in patients of LP, which helps in determining the early stage of the disease. The level of serum TSA increased from normal to non-dysplastic to dysplastic cases in LP, suggesting its association with malignant transformation. There is a progressive elevation in serum levels of TSA and LBSA in grades of OSCC that is from WDOSCC to MDOSCC. Thus serum levels of TSA and LBSA can be used as diagnostic and prognostic markers in LP and OSCC.

Funding Support

The authors declare that they have no funding support for this study.

Conflict of Interest

The authors declare that there is no conflict of interest for this study.

REFERENCES

Ali, A. A., Al-Sharabi, A. K., Aguirre, J. M., Nahas, R. 2004. A study of 342 oral keratotic white lesions induced by qat chewing among 2500 Yemeni. *Journal of Oral Pathology & Medicine*, 33(6):368–372.

Baxi, B. R., Patel, P. S., Adhvaryu, S. G., Dayal, P. K. 1991. Usefulness of serum glycoconjugates in precancerous and cancerous diseases of the oral cavity. *Cancer*, 67(1):135–140.

Boston, M. A. 2007. Glycoconjugates. In: Synthesis and Characterization of Glycosides. *Springer*, pages 272–303.

Broders 1983. A Textbook of oral pathology. volume 2, pages 115–117. 4th edn. Saunders Philadelphia, Chapter 2.

Brouns, E., Baart, J., Bloemena, E., Karagozoglu, H., van der Waal, I. 2013. The relevance of uniform reporting in oral leukoplakia: Definition, certainty factor and staging based on experience with 275 patients. *Medicina Oral Patología Oral y Cirugia Bucal*, 18(1):e19–e26.

Dnistrian, A. M., Schwartz, M. K., Katopodis, N., Fracchia, A. A., Stock, C. C. 1982. Serum lipid-bound sialic acid as a marker in breast cancer. *Cancer*, 50(9):1815–1819.

Elango, J. K., Gangadharan, P., Sumithra, S., Kuriakose, M. A. 2006. Trends of head and neck cancers in urban and rural India. *Asian Pacific Journal of Cancer Prevention*, 7(1):108–112.

Joshi, M., Patil, R. 2010. Estimation and comparative study of serum total sialic acid levels as tumor markers in oral cancer and precancer. *Journal of Cancer Research and Therapeutics*, 6(3):263–263.

Katopodis, N., Hirshaut, Y., Geller, N. L., Stock, C. C. 1982. Lipid-associated Sialic Acid Test for the Detection of Human Cancer. *Cancer Research*, (12):5270–5275.

Massano, J., Regateiro, F. S., Januário, G., Ferreira, A. 2006. Oral squamous cell carcinoma: Review of prognostic and predictive factors.

Neville, B. W., Day, T. A. 2002. Oral Cancer and Precancerous Lesions. *CA: A Cancer Journal for Clinicians*, 52(4):195–215.

Petti, S. 2003. Pooled estimate of world leukoplakia prevalence: a systematic review. *Oral Oncology*, 39(8):770–780.

Pindborg, J. J., Reibel, J., Holmstrup, P. 1985. Subjectivity in evaluating oral epithelial dysplasia, carcinoma in situ and initial carcinoma. *Journal of Oral Pathology and Medicine*, 14(9):698–708.

Plucinsky, M. C., Riley, W. M., Prorok, J. J., Alhadeff, J. A. 1986. Total and lipid-associated serum sialic acid levels in cancer patients with different primary sites and differing degrees of metastatic involvement.

Rajpura, K. B., Patel, P. S., Chawda, J. G., Shah, R. M. 2005. Clinical significance of total and lipid bound sialic acid levels in oral pre-cancerous conditions and oral cancer. *Journal of Oral Pathology and Medicine*, 34(5):263–267.

Rao, V. R., Krishnamoorthy, L., Kumaraswamy,

Ramaswamy, G. 1998. Circulating Levels in Serum of Total Sialic Acid, Lipid-Associated Sialic Acid, and Fucose in Precancerous Lesion and Cancer of the Oral Cavity. *Cancer Detection & Prevention*, 22(3):237–240.

Romppanen, J. 2003. Serum sialic acid in clinical diagnostics. *Kuopio University Publ D. Med Sc*.

Shashikanth, M. C., Rao, B. B. 1994. Study of serum fucose and serum sialic acid levels in oral squamous cell carcinoma. *Indian J Dent Res*, 5(4):119–143.

Taqi, S. A. 2012. Clinical evaluation of total and lipid bound sialic acid levels in oral precancer and oral cancer. *Indian Journal of Medical and Paediatric Oncology*, 33(1):36–36.

Tewarson, S. L., Mittal, V. P., Singh, M., Gupta, G. P. 1993. Serum sialic acid - an important cancer marker. *Indian Journal of Cancer*, 30(3):125–131.