Section A-Research paper

Evaluation of serum and salivary stathmin levels in oral leukoplakia and oral squamous cell carcinoma: A case control study

Running title: Serum and Salivary stathmin levels in oral leukoplakia and OSCC

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Abstract:

Aim:

To evaluate the salivary and serum stathmin levels in patients with leukoplakia and Oral squamous cell carcinoma and to histopathologically correlate with different grades of dysplasia in patients with leukoplakia and Oral squamous cell carcinoma.

Materials and methods:

Three groups were analysed for stathmin levels using ELISA kit.GROUP A-Healthy controls(n=15) ,GROUP B-Leukoplakia patients (n=15) ,GROUP C-15 oral squamous cell

carcinoma patients(n=15) saliva and serum samples collected from each group. Incised tissue samples of all three groups were processed and assessed for histopathological grades of oral epithelial dysplasia and grades of oral squamous cell carcinoma

Results:

The mean salivary and serum stathmin levels among three groups was statistically significant. There was a moderate positive correlation between the mean serum and mean salivary stathmin levels among the leukoplakia and oral squamous cell carcinoma. The correlation of salivary and stathmin levels in leukoplakia and oral squamous cell carcinoma with grades of oral epithelial dysplasia and grades of oral squamous cell carcinoma were found not to be statistically significant.

Conclusion:

It was speculated that the salivary and serum stathmin assessment could be a potent biomarker in the early diagnosis of the oral leukoplakia and oral squamous cell carcinoma.

Clinical significance:

Salivary stathmin levels can be used for the assessment of diagnostic and prognostic parameters of oral leukoplakia and Oral squamous cell carcinoma in their early diagnosis and better prognosis. This could be used as a prognostic marker in the high-grade cases of dysplasia and oral squamous cell carcinoma.

Keywords: Stathmin, Serum stathmin, Salivary stathmin, Oral leukoplakia, Oral squamous cell carcinoma

Introduction:

Oral squamous cell carcinomas (OSCC) are a diverse group of malignancies that arise from the oral cavity's mucosal lining. It is originating from the alveolar ridge, buccal mucosa, mouth floor, palate, tongue, and other regions of the oral cavity accounted for 350 000 new cases and 170 000 deaths in 2018.[1]

According to Surveillance, Epidemiology, and End Results (SEER) Program, there were 11.5 percent new cases of pharynx and oral cavity cancer for every 100,000 men and women per year. The annual mortality rate was 2.5 per 100,000 men and women. The 5-year annual survival rate is 68 percent. Globally, lip and oral cavity cancer accounted for 1.96 percent of new cases and 1.79 percent of mortality in GLOBOCAN 2020. [2] Oral cancer is the most frequent disease among men in the Indian subcontinent, despite the fact that it is only the sixth most common cancer worldwide.Early diagnosis of oral cancer is important to decrease the morbidity and mortality of oral cavity tumors and to improve the quality of life of patients with oral cancers. Detecting oral cancer at an early stage, when lesions are less invasive, is believed to be the most effective means to reduce mortality and to increase survival rate.

Oral potentially malignant disorders (OPMDs) are a set of disorders that damage the oral mucosa and are associated with a higher risk of cancer. The most frequent OPMD seen in clinical practice is oral leukoplakia.[3] Early detection and prevention of OPMD are considered essential because its incidence and prevalence are rapidly rising in India is leukoplakia. Comprehensive research determined that leukoplakia has an estimated 2% prevalence rate

globally. Lesions that appear mostly on the tongue, lip, or vermilion have a significant chance of developing into cancer.[4]

The most reliable independent risk factor for malignant transformation of Oral leukoplakia is now thought to be the prevalence and severity of oral epithelial dysplasia.[5] The rate of malignant transformation (range) in the 24 studies for analysis ranged between 0.13% and 34.0%. All investigations on Oral leukoplakia patients had a mean rate of transformation of 14.9%.[6] The percentage of malignant transformation ranged from 1.1% to 40.8%. Malignant transformation was significantly correlated with female gender, non-homogeneous clinical type, and epithelial dysplasia contrastingly our study reported all 3 cases with severe dysplasia in men.[7]

The goal of early cancer diagnosis is to identify symptomatic individuals as soon as possible to give them the best chance of a successful course of treatment. A reduced chance of survival, increased treatment associated issues, and expensive care are all consequences of delayed or inaccessible cancer therapy. By providing care at the earliest possible stage, early diagnosis improves better outcomes, making it an important public health approach in all contexts

A cell's growth and division are part of a series of processes called a cell cycle. Interphase, prophase, metaphase, anaphase, and telophase are the five stages of mitosis. A cell has most of its time in interphase, and during this time it grows, replicates its chromosomes, and prepares for cell division. After completing mitosis, the cell leaves interphase, and divides to completion. The resulting cells, known as daughter cells, each enters its own interphase and begin a new round of the cell cycle.[8]

Microtubules are protein polymers comprising of a/b tubulin heterodimers, which are essential for the structure and function of the cell. These processes include cell motility, polarity, and intracellular transport. The easiest way to explain the dynamics of microtubule function is as an alternating pattern of stabilization and destabilization.

Stathmin, also called oncoprotein 18, primarily recognized in neuroendocrine cells, plays a critical role during signal transduction in modulation and control of microtubule polymerization dynamics. The word "stathmos," which is Greek for "relay," is where the name "stathmin" comes from. It illustrates stathmin's function as a crucial mediator in the modulation and regulation of microtubule polymerization during signal transduction. Tubulin sequestration or "catastrophe" can both cause stathmin-mediated instability. The latter results from microtubule depolymerization and is counterbalanced by ‘rescue’, which is affected by polymerization.

Stathmin protein plays a major role in the progression of the cell cycle which is entirely dependent on the phosphorated/unphosphorylated status. So, it was suggested that the phosphorated levels can influence on the cells entering into mitotic phase of the cell cycle with increased stathmin levels.[9]

Early diagnosis of oral cancer is important to decrease the morbidity and mortality of oral cavity tumors and to improve the quality of life of patients with oral cancers. Detecting oral

cancer at an early stage, when lesions are less invasive, is believed to be the most effective means to reduce mortality and to increase survival rate. The importance of early detection of cancer or oral potentially malignant disorders is essential by using variable biomarkers which should be assessed using the body fluids to evaluate the disease progression. Using Overexpression of stathmin as a novel biomarker in cancer tissues has been correlated with poor prognosis of numerous cancers. Numerous studies have reported that stathmin is associated with poor prognosis and chemoresistance in a variety of human malignancies like acute leukemia, prostate, gastric, breast and ovarian cancer.[10]

There are limited studies regarding the expression of stathmin in body fluids, to overcome the lacunae of use of body fluids for the evaluation of stathmin levels in detection of progression of cancer.

Taking this into account, the present study was designed to evaluate and compare the expression of Salivary and Serum Stathmin in oral leukoplakia and Oral Squamous cell Carcinoma (OSCC) using Enzyme linked immunosorbent assay method and to correlate with different histopathological grades of oral epithelial dysplasia and oral squamous cell carcinoma.

Subjects and Methods:

The present study was conducted after obtaining approval from the institutional review board, SRM dental college [SRMDC/IRB/2020/MDS/No.601] and carried out in accordance with the 1964 Helsinki Declaration and its later amendments. The sample size was calculated using openepi version 3.0 for unmatched case control study. The sample size was estimated to be 15 in each case and control group based on the following measures: Probability of exposure in control = 40%;Probability of exposure in cases = 90%;Power = 80%;Error = 5% were considered. Therefore, the sample size was estimated to be 15 in each group.This case control study included 15 healthy control subjects (Group I), 15 clinically diagnosed leukoplakia and histopathological diagnosis of oral epithelial dysplasiabased on WHO classification of oral epithelial dysplasia 2017[11]as mild, moderate and severe epithelial dysplasia (Group II) and 15 patients with clinically diagnosed Oral squamous cell carcinoma and histopathological diagnosis of Broder's classification of oral squamous cell carcinoma[12]as well differentiated, moderately differentiated, poorly differentiated oral squamous cell carcinoma (Group III). All the patients belonged to the age group between 18 to 75 years.In Group I, healthy controls were included based on clinically no evidence of lesions or inflammation, Group II in which subjects were included based on the clinically evident white, homogenousnon-scrapable white lesion in the oral cavity with clinical diagnosis of oral leukoplakia and histopathological diagnosisof oral epithelial dysplasia with different grades (mild /moderate or severe oral epithelial dysplasia). Group III included subjects with ulceroproliferativegrowth or ulceroinfiltrative lesion evident on the oral cavity with clinical diagnosis of oral squamous cell carcinomaand histopathological diagnosis of oral squamous cell carcinoma with different histopathological grades (Well differentiated/ Moderately differentiated or Poorly differentiated oral squamous cell carcinoma). The exclusion criteria for all the groups were individuals with history of any other systemic illness and those involving the immune systems and individuals with gingival diseases or any

oral lesions were excluded. The patients with previous history of other oral lesions and previous history of radiation therapy were excluded from the study. A deliberate attempt was made to compare the grades of oral epithelial dysplasia and grades of OSCC in group II &III and it is used to analyse and correlate the grades of dysplasia and grades of oral squamous cell carcinoma with the stathmin levels.

Collection of samples:

The need of the study was explained to all the participants followed by obtaining case history and written informed consent forms the tissues were collected from the participants which were subjected for histopathological diagnosis.

Tissue sample collection for histopathological examination:

Procedure is initiated by applying topical and local anaesthesia to the biopsy site. 3 to 5 mm of tissue is collected from the abnormal appearing site within a lesion with the help of disposable Punch Biopsy Punches - 3/5mm (Punch Biopsy) or with the help of BP blade-No.15 (Wedge biopsy) and black silk sutures placed at the site of biopsy. The obtained tissue is stored in 10% formalin for Histopathological examination. The study samples are then subjected for tissue processing for histopathological examination with haematoxylin and eosin staining procedure for the assessment of grades of oral epithelial dysplasia and grades of oral squamous cell carcinoma using Olympus microscope.

Patients were reported for their suture removal and collecting their histopathological report, during that time patient were explained about the study and explained about the procedure with written consent forms followed by intravenous blood and saliva were collected from the participants.

Saliva and blood collection for stathmin estimation:

After incisional biopsy followed by histopathological diagnosis, patients were assessed for serum and saliva collection before the initiation of management.

Intravenous blood collection: Two ml of intravenous blood was drawn from subjects using standardized phlebotomy procedures and allowed to rest and coagulate for 60 minutes at 37ºC. Sera were separated by centrifugation in a cooling centrifuge at 3000 rpm for past 10 min at 4°C and were immediately aliquoted.

Saliva collection: To collect whole saliva, the participants were instructed to swallow first, tilt their head forward and then expectorate the saliva into the centrifuge tubes and subjected to centrifugation for 10 mins at 1500 rpm. Resulting supernatant was stored into 1 ml aliquots at — 80 °C freezer for further biochemical analysis.

Serum and salivary supernatant samples segregation:

Serum and salivary stathmin levels were determined using ELISA kit (Elabscienceslaboratory). 100 µl of 1:10 diluted sample and 100 µl of the standard solutions in different concentrations (5,2.5,1.25,0.63ng/ml) were pipetted in pre designated wells in duplicates. Following incubation at room temperature for 60 minutes, the wells were washed four times with the wash buffer. 100 µl appropriately enzyme antibody conjugate was pipetted to each well, incubated at room temperature for 20 minutes and then washed four

times with the wash buffer. 100 µl of hydrogen peroxidase substrate solution was pipetted into each well, incubated in the dark at room temperature for five minutes and then 100 µl of stop solution was added to each well. The absorbance of the contents was determined for each well using the ELISA reader.

Following derivation of the optical density value and subsequent calculation of the quantity of stathmin in saliva and serum, the resultant values were subjected to statistical analysis. KruskalWallis test, followed by post hoc Tukey analysis and Pearson's correlation method were used to analyse the results.

Similarly, based on the grades of oral epithelial dysplasia and grades of oral squamous cell carcinoma, the intergroup comparison of the serum and salivary stathmin levels are statistically analysed using Kruskal Wallis test.

Results:

The age and gender of the study population is given in the table1.

For statistical analysis, KruskalWallis test used to analyse serum stathmin levels and salivary stathmin levels among three groups respectively among three groups. Post hoc Tukey test was used to analyse pair wise comparison among the group and Pearson's correlation was used for the assessment of the correlation between salivary and serum stathmin levels.

Salivary stathmin levels was higher in the Oral squamous cell carcinoma (OSCC) group (8.48ng/ml) when compared to the leukoplakia group (4.17ng/ml) and healthy controls (2.21ng/ml).(Graph 1) Serum stathmin levels was higher in the OSCC group (20.06ng/ml) when compared to the leukoplakia group (14.03ng/ml) and healthy controls (4.64ng/ml).(Graph 2) This difference in the serum and salivary stathmin levels was found to be statistically highly significant between three groups ($P<0.001$)(Table 2)

On post hoc Tukey analysis for the pair wise comparison, the mean difference of salivary stathmin levels was statically significant between normal and OSCC group(6.26 ng/ml) ($P<0.001$) and between leukoplakia and OSCC group (4.30ng/ml) ($P=0.027$). Serum stathmin levels, the pair wise comparison shows the mean difference of serum stathmin levels was statistically significant normal group and leukoplakia group (9.38ng/ml) ($P=0.005$); normal group and OSCC group (15.41 ng/ml)($P< 0.001$) and between leukoplakia and OSCC group (6.03ng/ml) ($P=0.005$). On Pearson's correlation, moderate positive correlation was found between saliva and serum stathmin levels which are statistically significant($r=0.719$; $P<0.001$).

Intragroup comparison of serum and salivary stathmin levels with subgroups in oral epithelial dysplasia: The serum and salivary stathmin levels were assessed based on grades of oral epithelial dysplasia in group II and grades of oral squamous cell carcinoma in group III. The Serum stathmin levels was highest among subjects with severe dysplasia followed by subjects with moderate dysplasia and least among subjects with mild dysplasia. However, the difference in serum stathmin levels within various groups were not found to be significant statistically. The salivary stathmin levels was highest among subjects with severe dysplasia followed by subjects with moderate dysplasia and least among subjects with mild dysplasia.

However, the difference in salivary stathmin levels between various groups were not found to be statistically significant.(Table 3, Graph 3)

Intragroup comparison of serum and salivary stathmin levels with subgroups in oral squamous cell carcinoma: The Serum stathmin levels was highest among subjects with moderately differentiated OSCC followed by subjects with poorly differentiated OSCC and least among subjects with Well differentiated OSCC. However, the difference in serum stathmin levels among various groups were not found to be significant statistically. The Salivary stathmin levels was highest among subjects with moderately differentiated OSCC followed by subjects with Well differentiated OSCC & poorly differentiated OSCC and least among subjects with poorly differentiated OSCC. However, the difference in salivary stathmin levels among various groups were not found to be significant statistically.(Table 4, Graph 4)

Discussion:

Oral cancer prevention and treatment must focus on early detection of premalignant conditions (OPMDs), extensive education about risk factors, comprehension of the mechanisms underlying malignant progression, and the development of targeted, more effective treatments.[13]

Carcinogenesis results from various or multiple genetic alterations that controls various aspects of cell proliferation, cell differentiation and programmed cell death. Many aspects of genes get altered or mutated in human cancer development which are involved directly in cell division cycle regulation, controls machinery that responsible for cell proliferation.[14]

In eukaryotic cells, during cell division , microtubules form the important components of mitotic spindle that are required for chromatid segregation. These microtubules are extremely dynamic, it grows and shrinks alternatively between the phases of cell cycle. Individual microtubules may elongate and shrink simultaneously on the opposite ends at given point of time. Whether microtubule growing or shrinking is determined by 'catastrophe' and 'rescue' rates. Catastrophe is when growing microtubule begins to shorten rapidly. Rescue is the shift of shrinking microtubule to elongate rapidly.

Stathmin is an important marker in the family of proteins that critically play an important role in microtubule cytoskeleton regulation. Stathmin /oncoprotein 18/ megablastin is a cytosolic protein which helps by depolymerising or by preventing polymerisation of tubulin heterodimers of microtubules in microtubular dynamics regulation. When the cells enter into mitosis phase, these microtubules polymerise to form mitotic spindle which is essential feature for chromatid segregation and cell division. These phosphorylated stathmin is reactivated by depolymerisation before the cells enter into new interphase of cell cycle. In normal cells, stathmin helps in regulating cell cycle and microtubule dynamics. When there is interference/alteration in the stathmin, it leads to accumulation of cells in G2/M phases or arrest cells in early stages of mitosis leading to chromosomal aberrations namely aneuploidy. Numerous studies postulated that overexpression of stathmin in various malignancies is associated with poor prognosis.[15]

To best of our knowledge, this is the first study to estimate the stathmin levels in saliva of oral leukoplakia and oral squamous cell carcinoma and to compare with the histopathological grades. The goal of the present study is to evaluate the serum and salivary stathmin levels in normal healthy controls, leukoplakia group, Oral squamous cell carcinoma patients using enzyme linked immunosorbent assay and to correlate the levels with differing histopathological grades of oral epithelial dysplasia in clinically diagnosed leukoplakia group and differing grades of oral squamous cell carcinoma in oral squamous cell carcinoma group.

In recent times, use of biomarkers in various body fluids for assessment of various clinical conditions. Saliva is produced by salivary glands which is used as diagnostic tool in many clinical and systemic conditions. It contains various proteins, cellular by products ,exfoliated cells and genetic materials that can be used in identifying disease behaviour and progression levels. Salivonomics is considered to be one of the emerging fields in which it is a study of salivary contents in assessment of clinical condition and also helps in understanding about salivary biomarkers in early detection of clinical conditions. Tumor usually shed their by-products in saliva and blood which can be used in screening method for diagnosis of clinical conditions. Several salivary biomarkers like S100P, CD44, COL5A1[16]; can Interleukin(IL-6)[17] ;IL-8 [18]&Tumornecrotising factor alpha [19]; be used in assessment of leukoplakia and oral epithelial dysplasia.

Radhika et al reported that various salivary biomarkers like p53, IL[6,8,10]; Matrix metalloproteinases [2,9]; Transforming growth factor beta, Cancer antigen (CA 125); Ki 67; can be used as assessment for early diagnosis in oral squamous cell carcinoma.[20]

Saliva can be considered as reliable source in diagnosis. As salivary biomarker is a non-invasive tool for assessing the early detection of potentially oral malignant disorders and oral squamous cell carcinoma.[16]

As mentioned before, cell cycle is a series of synchronized events. When the cells are upon enter into mitosis phase, these microtubules polymerise to form mitotic spindle which is essential feature for chromatid segregation and cell division. This occurs due to phosphorylation and dephosphorylation of stathmin. Stathmin is an important protein These phosphorylated stathmin is reactivated by depolymerisation before the cells entering into new interphase of cell cycle. In normal cells, stathmin helps in regulating cell cycle and microtubule dynamics. When there is interference/alteration in the stathmin leads to accumulation of cells in G2/M phases or arrest cells in early stages of mitosis leading to chromosomal aberrations namely aneuploidy. Numerous studies postulated that overexpression of stathmin in various malignancies associated with poor prognosis.[10]

The goal of the present study is to evaluate the serum and salivary stathmin levels in normal healthy controls, leukoplakia group, Oral squamous cell carcinoma patients using enzyme linked immunosorbent assay.

In our study, we found that the salivary stathmin and serum stathmin levels were increased; mean salivary stathmin level was 8.48 ng/ml and mean serum stathminlevels was 20.06ng/ml in Oral squamous cell carcinoma and mean salivary stathmin level was 4.17ng/ml and mean serum stathmin levels was 14.03 ng/ml in oral leukoplakia compared to mean salivary

stathmin level was 2.21ng/ml and mean serum stathmin levels was 4.64 ng/ml in healthy controls which were statistically significant. This could be probably due to altered microtubule dynamics, accumulation of cells in early mitosis of cell cycle leading to overexpression of stathmin. There was also evidence of significant increase of salivary and serum stathmin levels in the order of highest in oral squamous cell carcinoma than leukoplakia ; it is higher compared to the healthy controls. The possible reason for overexpression of stathmin could be due to abnormal proliferation of cells in oral squamous cell carcinoma compared to oral leukoplakia. We attempted different histopathological grades we could not get significant results due to unequal distribution of cases in each histopathological group.

On comparing within the leukoplakia group; the mean salivary stathmin level was 15.05 ng/ml and mean serum stathmin level was 6.63 ng/ml in severe dysplasia compared to mean salivary stathmin level of 14.84 ng/ml and mean serum stathmin level of 5.34ng/ml in moderate dysplasia. The mean salivary stathmin level was 13.41 ng/ml and mean serum stathmin level was 1.99 ng/ml in mild dysplasia. This shows that there was an increase in stathmin levels in severe dysplasia followed by moderate dysplasia and mild dysplasia. Over expression of serum and salivary stathmin levels in severe dysplasia could be due to increased or abnormal proliferation of cells leading to increased cell survival and decreased apoptosis in the epithelium and potential for malignant transformation rate is higher in severe dysplasia compared to moderate dysplasia and severe dysplasia.

On comparing the levels of stathmin expression in the oral squamous cell carcinoma(OSCC) group; it was found that in moderately differentiated OSCC, the mean salivary stathmin level was 20.66 ng/ml and mean serum stathmin level was 11.04ng/ml. The mean salivary stathmin level was 19.99 ng/ml and mean serum stathmin level was 7.83 ng/ml in well differentiated OSCC. The mean salivary stathmin level was 19.53 ng/ml and mean serum stathmin level was 10.99 ng/ml in poorly differentiated OSCC. This shows that there is an increase in serum and salivary stathmin levels in moderately differentiated oral squamous cell carcinoma followed by poorly differentiated OSCC and well differentiated OSCC. The probable reason for increase in stathmin levels in moderately differentiated squamous cell carcinoma than that of poorly differentiated squamous cell carcinoma could be because of inclusion of only one case of poorly differentiated carcinoma which we observe as a limitation of the study. However, the overall trend of stathmin expression in our study suggests an increase in both saliva and serum stathmin with increasing grades of dysplasia and oral squamous cell carcinoma.

Similar to our study, Vadla et al conducted an immunohistochemical study where he suggested that overexpression of stathmin in oral leukoplakia and oral squamous cell carcinoma with increase in histopathological grades of oral epithelial dysplasia in oral leukoplakia and oral squamous cell carcinoma.[21]

Kouzu et al reported the stathmin expression in Oral squamous cell carcinoma tissues using immunohistochemistry and western blot analysis, there was evidence of differential expression of stathmin in different stages of Oral squamous cell carcinoma was found to be

correlated with TNM staging. There was significant increased expression of stathmin were observed in stage III and stage IV Oral squamous cell carcinoma other than early stages I and II oral squamous cell carcinoma.[22]

Similarly, serum stathmin levels were assessed in oesophageal cancer and lung adenocarcinoma. Yan et al suggested that Increased Serum stathmin levels were observed in Oesophageal cancer and Stathmin promotes to tumour development and malignant transformation. Elevated stathmin expression has been linked to poor pathological grade, lymph node metastases, advanced stage, and poor prognosis in ESCC.[23]Biaoxue reported increased serum stathmin levels in lung adenocarcinoma were substantially correlated with advanced tumor stages, lymph node metastasis. [24]

Similar to our study, Nie et al suggested stathmin overexpression in ovarian cancer tissues. The level of stathmin was closely associated with pathological differentiation and clinical stages with ovarian cancer; higher expression of stathmin was associated with lower overall survival rate.[25]Leiprakaram et al investigated stathmin levels in metastatic colorectal cancer in which there was increased overexpression of stathmin levels associated with poor overall prognosis.[26] Liu et al reported increased stathmin levels associated with liver cancer progression and shorter survival rate and decreased apoptosis with advanced clinical stages.[27]Hsu et al suggested that overexpression of stathmin in nasopharyngeal carcinoma is associated with poor histological differentiation, advanced clinical stages and poor prognosis.[28]Akhtar et al conducted the study on distal oesophageal adenocarcinoma; higher stathmin levels was observed in lymph node metastasis.[29] Akhtar et al investigated higher stathmin levels with poor prognosis and overall survival rate in oesophageal squamous cell carcinoma.[30]Obayashi et al reported higher stathmin levels with lower survival rate; highly expressed in basal cell lines than luminal cell lines which was associated with aggressiveness of breast cancer.[31]Watanabe et al reported that higher stathmin expression in extrahepatic Cholangiosarcoma which was associated with poor prognosis and cancer progression.[32]Nie et al suggested high stathmin levels in non-small cell lung cancer (NSCLC) associated with poor tumor differentiation, increased tumor size, advanced TNM staging and poor prognosis.[33]

Overexpression of stathmin levels can be associated with increased cell proliferation due to mitotic activity. Due to abnormal mitotic activity causes alterations in DNA i.e., aneuploidy which is considered to be classic hallmark of carcinogenesis.

The future prospective of study would be having robust literature to comprehend the molecular mechanisms by which the stathmin controls tumour growth, migration, motility, and metastasis. But the aberrant expression of stathmin in human cancers has made the development of stathmin-dependent molecular diagnosis and targeting therapy a definite prospect. In future, the study with increased sample size in differing grades of dysplasia and oral squamous cell carcinoma would help us to assess the exact role of stathmin so that it can be potential therapeutic agent in cell cycle progression and improving the survival rate

The limitation of study is considered to be minimal cases used in the study. However, the role of stathmin as an ideal biomarker in oral leukoplakia and oral squamous cell carcinoma can

be ascertained by doing this study with a greater number of cases correlating with grades of dysplasia and grades of squamous cell carcinoma can be done

Conclusion:

The present study aimed to evaluate and compare the salivary and serum stathmin levels in healthy, leukoplakia and oral squamous cell carcinoma patients.

Although, as per the literature there were less studies that analyzed and reported the molecular pathogenesis of stathmin. Owing to the lack of robust literature, comparative evaluation of stathmin levels in saliva and serum was done for leukoplakia and Oral squamous cell carcinoma.

Collectively, the observed findings suggest that there is increased stathmin levels in saliva and serum in the leukoplakia and oral squamous cell carcinoma when compared to the healthy controls which was statistically significant. Also, there was increase in salivary and serum stathmin levels in oral squamous cell carcinoma when compared to the leukoplakia patients which was statistically significant.

Therefore, we speculate that the use of salivary or serum stathmin as a potent biomarker in the assessment of diagnosis and prognosis of the oral leukoplakia and oral squamous cell carcinoma.

Declaration of patient consent:

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Conflicts of interest:

Nil

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Tables:

Table 1: Demographic data of study population(Three groups)

Table 2: Comparison of serum and salivary stathmin levels among three groups using Kruskal Wallis test

Table 3: Intragroup comparison of Serum and salivary stathmin levels in different grades of oral epithelial dysplasia:

Table 4: Intragroup comparison of salivary and serum stathmin levels in grades of Oral squamous cell carcinoma.

Graphs:

Graph 1: Mean salivary stathmin levels among three groups.

Graph 2: Mean serum stathmin levels among three groups.

Graph 3: Correlation of serum and salivary stathmin levels in grades of oral epithelial dysplasia (Mild epithelial dysplasia, Moderate epithelial dysplasia, Severe epithelial dysplasia)

Graph 4: Correlation of serum and salivary stathmin levels in grades of Oral squamous cell carcinoma (Well differentiated squamous cell carcinoma, moderately differentiated squamous cell carcinoma, poorly differentiated squamous cell carcinoma)

Table 1: Demographic data of study population(Three groups)

Study groups	Mean age (in years) ± Standard deviation	Gender	
		Female (n)	Male (n)
Group I (n=15)	35.6±15.6	10	5
Group II (n=15)	50.9± 8.5	3	12
Group III (n=15)	58.2± 11.6	6	9

Table 2: Comparison of serum and salivary stathmin levels among three groups using Kruskal Wallis test

Parameter	Group	N	Mean	Std. Deviation	P value
Serum stathmin levels	Normal	15	4.64	1.72	<0.001
	Leukoplakia	15	14.03	1.94	
	OSCC	15	20.06	1.64	
Salivary stathmin levels	Normal	15	2.21	2.81	<0.001
	Leukoplakia	15	4.17	2.94	
	OSCC	15	8.48	3.94	

Table 3: Intragroup Comparison of Serum and salivary stathmin levels in different grades of oral epithelial dysplasia:

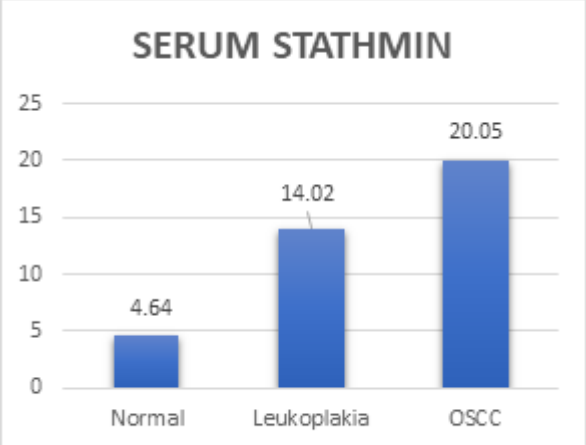
Groups	Subgroups	Number of cases	Mean	SD	P value
Serum stathmin	Sub Group II A-Mild Dysplasia	9	2.9558	1.99587	0.119
	Sub Group II B-Moderate Dysplasia	3	5.3470	5.03058	
	Sub Group II C-Severe Dysplasia	3	6.6310	1.12680	
Salivary stathmin	Sub Group II A-Mild Dysplasia	9	13.4110	2.26951	0.641
	Sub Group II B-Moderate Dysplasia	3	14.8413	.52858	
	Sub Group II C-Severe Dysplasia	3	15.0577	1.11080	

Table 4: Intragroup Comparison of salivary and serum stathmin levels in grades of Oral squamous cell carcinoma.

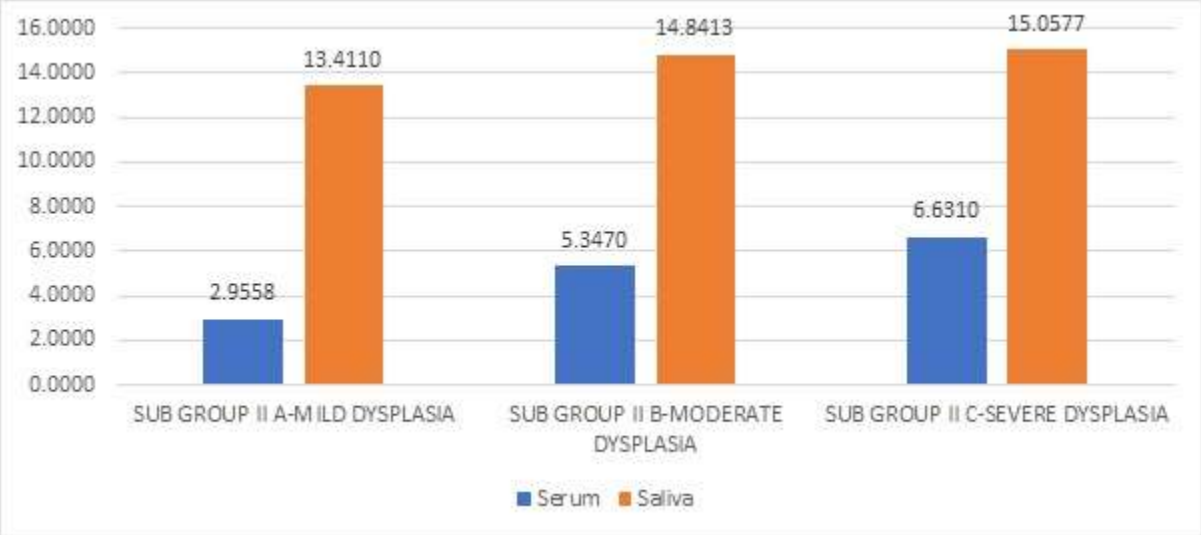
Groups	Subgroups	Number of cases	Mean	SD	P value
Serum stathmin	Subgroup -IIIA Well Differentiated	12	7.8389	4.11689	0.194
	Subgroup-III B Moderately Differentiated	2	11.0440	2.47346	
	Subgroup-III C Poorly Differentiated	1	10.9940		
Salivary stathmin	Subgroup-III A Well Differentiated	12	19.9998	1.72766	0.867
	Subgroup-III B Moderately Differentiated	2	20.6620	1.94313	
	Subgroup-III C Poorly Differentiated	1	19.5380		

Graph 1: Mean salivary stathmin levels among three groups.

Graph 2: Mean serum stathmin levels among three groups.



Graph 3: Correlation of serum and salivary stathmin levels in grades of oral epithelial dysplasia (Mild epithelial dysplasia, Moderate epithelial dysplasia, Severe epithelial dysplasia)



Graph 4: Correlation of serum and salivary stathmin levels in grades of Oral squamous cell carcinoma (Well differentiated squamous cell carcinoma, moderately differentiated squamous cell carcinoma, poorly differentiated squamous cell carcinoma)