Evaluation of magnesium levels in blood and saliva of oral squamous cell carcinoma and potentially malignant disorders by xylidyl blue method

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INTRODUCTION
The term precancer refers to a clinically visible, benign, morphologically altered tissue that has a greater than normal risk of malignant transformation. They precede

Abstract
Background: Cancer is Latinized from Greek word ‘karkinos’ meaning crab, denoting how carcinoma extends its claws like a crab into adjacent tissues. It has been well established by researchers that virtually all oral cancer are preceded by visible clinical changes in the oral mucosa usually in the form of white or red patch (two-step process of cancer development). Mg is an essential mineral that is needed for a broad variety of physiological functions. Imbalances in Mg metabolism are common and are associated with different pathological conditions. The purpose of this study was to evaluate and compare the magnesium concentration in blood serum and saliva of oral squamous cell carcinoma, potentially malignant disorders and healthy subjects to serve as a positive marker or indicator in the process of carcinogenesis.

Materials and Methods: The study includes 17 precancerous (OSMF + Leukoplakia) patients, 17 OSCC and 17 control group. Blood and saliva was collected; serum and saliva was extracted from both the groups and was biochemically evaluated for magnesium levels. Statistical analysis was performed using ANOVA.

Results: The Salivary magnesium Mean ± SD of Healthy group is higher 1.6681 ± 0.0207 mmol mg/l followed by Potentially Malignant Disorder group 1.5532 ± 0.0283 and Oral Squamous Cell Carcinoma 0.5979 ± 0.0659. The mean values differ significantly between 3 groups (P < 0.001) The Serum magnesium Mean ± SD of Healthy group is higher 1.9188 ± 0.0550 mmol mg/l followed by Potentially Malignant Disorder group 1.6951 ± 0.0949 and Oral Squamous Cell Carcinoma 0.7329 ± 0.1561. The mean values differ significantly between 3 groups (P < 0.001) The study revealed decreased serum and salivary magnesium in oral precancerous patients and an Oral Squamous cell carcinoma patients compared to healthy individuals.

Conclusion: The magnesium concentration was low in both blood plasma and saliva of oral squamous cell carcinoma as compared to potentially malignant disorders and healthy subjects. Thus the magnesium ion concentration in blood plasma and saliva could be considered as tumor marker, playing an important role in carcinogenesis.

Keywords: Magnesium, oral squamous cell carcinoma, xylidyl blue method

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the emergence of invasive cancers.\textsuperscript{[1,2]} Oral premalignancy is considered as intermediate stage, and there are two types of oral premalignancies; premalignant lesions and premalignant conditions. However, recently, WHO considered both premalignant lesions and premalignant conditions under a single group of disorders named as “potentially malignant disorders (PMDs).”\textsuperscript{[3,4]} Oral leukoplasia is the best known PMD which is defined as “A white plaque of questionable risk having excluded other known diseases or disorders that carry no increased risk of cancer.” Presence of epithelial dysplasia may be even more important in predicting carcinogenesis than the clinical characteristics.\textsuperscript{[5,6]}

The human body contains abundant elements and trace elements which are involved in the formation of covalent bonds that are important constituents of tissues and semi-major elements, which often exist in the ionic state and play a major role in the living body by maintenance of osmotic pressure and membrane potentials. Trace elements or micronutrients are chemical elements required in minute amounts, usually as part of a vital element.\textsuperscript{[7]}

Essential trace elements such as zinc (Zn), copper (Cu), selenium (Se), chromium (Cr), cobalt (Co), iodine (I), manganese (Mn) and molybdenum (Mo). Although they account for only 0.02% of the total body weight, they play a significant role.

Enzymes of trace elements play an important role in certain biological and chemical reactions. They work in harmony with proteins and certain other coenzymes. They attract substrate molecules and enable their conversion and specific end product. Some trace elements are involved in redox reactions, for example, in the burning of food products. Some have structural roles by helping to impart stability and three-dimensional structures to vital biological molecules.\textsuperscript{[8]}

Several studies on diet and cancer links suggest that micronutrients, particularly antioxidants and minerals such as Se, Cu, Zn, magnesium (Mg) and iron have a role as cancer risk modifiers. Many elements perform functions indispensable to maintenance of growth and reproduction. Inadequate levels of some elements may impair cellular and physiological functions. Many metabolic disorders, oral precancerous conditions and oral cancer are accompanied by alterations in the concentration of one or more trace elements such as Mg, Cu, iron and Zn in body fluids, especially blood serum or plasma. Serum trace elements also give an idea about necessary elements to be given as supplements to counteract the oxidative stress induced by free radicals which can cause serious damage to cells in oral precancerous and cancerous conditions. Assessment and evaluation of biochemical alterations in the serum and saliva of patients with precancerous and oral cancer can help not only in the early diagnosis and treatment but also in prognosis; it can also be used to assess the etiology of various diseases, especially cancer.\textsuperscript{[9]}

Mg is one of the most abundant cations present in living cells which is an essential element that is needed for a broad variety of physiological functions.\textsuperscript{[10,11]} It is considered as physiological calcium antagonist. At a cellular level, it may act as an important regulator of cell functions. Its serum concentration of Mg is remarkably constant in healthy participants and protects the body against various diseases. Mg exerts

**Graph 1:** Graphical representation of mean values of salivary magnesium ion concentration in oral squamous cell carcinoma, potentially malignant disorder and healthy controls.

**Graph 2:** Graphical representation of mean values of magnesium ion concentration in oral squamous cell carcinoma, potentially malignant disorder and healthy participants in serum.
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Table 1: Details of magnesium ion concentration in oral squamous cell carcinoma, potentially malignant disorder and healthy participants in saliva

| n   | Mean±SD | 95% CI for mean | Minimum | Maximum |
|-----|---------|-----------------|---------|---------|
|     |         | Lower bound     | Upper bound |         |
| Healthy | 17 | 1.6681±0.0207 | 1.6050 | 1.6919 |
| Potentially malignant disorder | 17 | 1.5532±0.0283 | 1.5040 | 1.5941 |
| Oral squamous cell carcinoma | 17 | 0.5979±0.0659 | 0.5310 | 0.8121 |

F=3156.835, P<0.001. SD: Standard deviation, CI: Confidence interval

Table 2: Tukey's honestly significant difference multiple comparisons

| Group (I) | Group (J) | Mean difference (I−J) | P | 95% CI Lower bound | Upper bound |
|-----------|-----------|-----------------------|---|-------------------|-------------|
| Healthy controls | Potentially malignant disorder | 0.1149 | <0.001 | 0.0792 | 0.1507 |
| Oral squamous cell carcinoma | Potentially malignant disorder | 1.0702 | <0.001 | 1.0344 | 1.1059 |
| Oral squamous cell carcinoma | Potentially malignant disorder | 0.9553 | <0.001 | 0.9195 | 0.9910 |

CI: Confidence interval

Table 3: Details of magnesium ion concentration in oral squamous cell carcinoma, potentially malignant disorder and healthy participants in blood serum mean and standard deviation of Serum magnesium

| n   | Mean±SD | 95% CI for mean | Minimum | Maximum |
|-----|---------|-----------------|---------|---------|
|     |         | Lower bound     | Upper bound |         |
| Healthy controls | 17 | 1.9188±0.0550 | 1.8905 | 1.9471 |
| Potentially malignant disorder | 17 | 1.6951±0.0949 | 1.6463 | 1.7439 |
| Oral squamous cell carcinoma | 17 | 0.7329±0.1561 | 0.6527 | 0.8132 |

F=556.132, P<0.001. SD: Standard deviation, CI: Confidence interval

Table 4: Tukey’s honestly significant difference multiple comparisons

| Group (I) | Group (J) | Mean difference (I−J) | P | 95% CI Lower bound | Upper bound |
|-----------|-----------|-----------------------|---|-------------------|-------------|
| Healthy controls | Potentially malignant disorder | 0.2236 | <0.001 | 0.1323 | 0.3150 |
| Oral squamous cell carcinoma | Potentially malignant disorder | 1.1859 | <0.001 | 1.0945 | 1.2772 |
| Oral squamous cell carcinoma | Potentially malignant disorder | 0.9622 | <0.001 | 0.8708 | 1.0536 |

CI: Confidence interval

a large variety of biological functions, ranging from structural roles by complexing negatively charged groups such as phosphates in nucleic acids, a control role in enzyme activation or inhibition and regulatory role by modulating cell proliferation, cell cycle progression and differentiation.[12] Different pathological conditions are associated with Mg ion imbalances.[10,11]

Over 300 enzymes that influence the metabolism of carbohydrate, amino acids, nucleic acids and protein and ion transport and require Mg.[13] It has been proposed that Mg has a key role in cell cycle and that its deficiency is an important conditioner in precancerous cell transformation. Optimal Mg intake is thought to be prophylactic against the initiation of some neoplasms.[14] Relationships between Mg and cancer are complex: both Mg load and Mg deficit may produce either carcinogenic or anticarcinogenic effect. Carcinogenesis modifies the Mg status inducing Mg distribution disturbances which may frequently associate a tumor Mg load with Mg depletion in nonneoplastic tissues.[15,16] Thus, we aimed at colorimetric estimation of Mg in blood and saliva in oral squamous cell carcinoma (OSCC) and PMDs so as to serve as a positive marker or indicator in the process of carcinogenesis.

METHODOLOGY

The study included 17 precancerous (OSMF + leukoplakia) patients, 17 OSCC and 17 control group. Blood and saliva were collected; serum and saliva were extracted from both the groups and were biochemically evaluated for Mg levels.

- Under all aseptic precautions, about 3 ml venous blood was collected from antecubital vein of the study and control group into plain sterile bulb. The sample was then allowed to clot at room temperature for about 2 h and then centrifuged at 3000 rpm for 10 min, to separate the serum. Immediately, this serum was used for the estimation of Mg
- Five milliliters of saliva sample was collected from the study and control group and centrifuged at 5000 rpm to remove excess mucous and unwanted particles. After the centrifugation process, the supernatants were harvested to determine the amounts of Mg
- The estimation of Mg in serum and saliva was done using xylidyl blue method. The results are expressed in mg/dl
- The obtained mean values were compared between the OSCC, PMDs and control groups.
RESULTS

Data were analyzed using one-way ANOVA. Tukey's honestly significant difference (HSD) multiple comparison test was done to find out which means differed significantly. Software Microsoft Excel and SPSS 20, IBM, (Armonk, NY, USA) were used to analyze the data with $P < 0.05$, $P$ value being statistically significant.

Salivary magnesium
The salivary Mg mean ± standard deviation (SD) in healthy control was highest, i.e., 1.6681 ± 0.0207 mmol mg/l followed by PMD group, i.e., 1.5532 ± 0.0283 OSCC showed least with a mean value of 0.5979 ± 0.0659.

The mean values differ significantly between three groups ($P < 0.001$).

The salivary Mg levels differ significantly between healthy controls and PMD group ($P < 0.001$), healthy controls and OSCC ($P < 0.001$) and PMD and OSCC group ($P < 0.001$) [Tables 1 and 2, Graph 1].

Serum magnesium
The serum Mg level mean ± SD of healthy control group was higher, i.e.,1.9188 ± 0.0550 mmol mg/l followed by PMD group, i.e., 1.6951 ± 0.0949 and least in OSCC 0.7329 ± 0.1561.

The mean values differ significantly between three groups ($P < 0.001$).

The salivary Mg levels differ significantly between healthy controls and PMD group ($P < 0.001$), healthy controls and OSCC ($P < 0.001$) and PMD and OSCC group ($P < 0.001$) [Tables 3 and 4, Graph 2].

DISCUSSION

Oral diseases and conditions have a broad impact on health and well-being of an individual. Oral cancer, being the tenth most common cancer worldwide, continues to be the most prevalent cancer related to high consumption of tobacco in various forms. The incidence of oral cancer is high in industrialized countries as related to the use of tobacco and excessive consumption of alcohol.[17]

Nevertheless, a significant reduction in mortality can be achieved by advances in early diagnosis and implementation of multidisciplinary treatment programs, leading to improvement of survivorship and better quality of life.[17]

One such step toward early diagnosis is by assessing the biochemical alterations occurring in the body fluids. Microelements such as Cu, Zn, Mg and iron in the serum of tobacco users have shown alterations in their levels.

Trace elements, directly or indirectly, play an important role in various physiological metabolic processes in humans. More than 25% of the enzymes in the body need to be activated by metal ions to carry out their metabolic functions.

Mg is one of the most abundant cations present in living cells. It is an essential mineral that is needed for a broad variety of physiological functions. Mg exerts a large variety of biological functions, ranging from structural roles by complexing negatively charged groups such as phosphates in nucleic acids, a control role in enzyme activation or inhibition and regulatory role by modulating cell proliferation, cell cycle progression and differentiation.[10,12]

Mg has a central regulatory role in the cell cycle that affects transphorylation and DNA synthesis. It has been postulated that Mg controls the timing of spindle chromosome cycles by bringing about changes in intracellular concentration during the cell cycle, i.e., Mg level drops as cells enlarge, until it reaches a level that allows for spindle formation. Mg influx then causes spindle breakdown and cell division. Thus, 1) Mg controls key rate-limiting steps in the cell cycle at the onset of DNA synthesis and mitosis, a function that may be lost in transformed cells and processes thought to be regulated by Ca/calmodulin are Mg dependent since low Ca levels can be regulators only when there is adequate free Mg. The metabolic effects of Ca are achieved indirectly through its competition with Mg for membrane sites.[18]

The purpose of this study was to evaluate and compare the Mg concentration in blood serum and saliva of OSCC, PMD and health controls to serve as a positive marker or indicator in the process of carcinogenesis.

In this study, blood and saliva samples were drawn from 17 healthy individuals, 17 patients with PMD and 17 patients with OSCC.

The data was analyzed using one-way ANOVA and Tukey’s HSD test. The Mg content in the saliva of healthy patients had a mean value of 1.668 mmol Mg, whereas, in patients with PMDs, it was 1.533 mmol Mg. In OSCC, it was 0.598 mmol Mg. There was a significant variation in the values between PMDs and OSCC. The mean value differs significantly between the three groups with $P < 0.001$ being statistically significant.
The Mg content in the serum of healthy patients had a mean value of 1.918 mmol Mg, whereas, in patients with PMDs, it was 1.695 mmol Mg and in OSCC it was 0.732 mmol Mg. Significant variation in the values was noticed between PMDs and OSCC. The mean value differed significantly between the three groups with \( P < 0.001 \) being statistically significant.

Normal Mg level in blood serum by calorimetry xylidyl blue method was 1.6888–2 mmol Mg, whereas normal Mg level in saliva by calorimetry xylidyl blue method was 1.60–2 mmol Mg.

The study results showed that the Mg concentration was low in both blood serum and saliva of patients with OSCC as compared to PMDs and healthy controls. Thus, the Mg concentration in blood serum and saliva could be considered as a tumor marker, playing an important role in carcinogenesis.

Saliva has advanced exponentially as a diagnostic tool in the past decade as it can be easily harvested and monitored for the purpose of diagnosis and thus makes it a convenient method for mass screening.

Thus, we hypothesize that decrease in Mg content could serve as a positive marker or indicator in the process of carcinogenesis. The changes in Mg value concentration could act as a screening marker to indicate the progress of potentially malignant disorders.

**CONCLUSION**

Increasing interest in the use of saliva to diagnose systemic diseases has developed due to the simplicity in the collection of saliva with minimal discomfort to the patient, and more importantly, saliva contains constituents that are altered in the presence of cancer.

The use of saliva for the detection of malignancies deals primarily in the identification and quantification of cancer-related proteins and elements which are elevated in the serum of affected individuals.

Mg is one such abundant cation which plays an important regulatory role in cell cycle progression and differentiation.

In the present study, we evaluated and compared the Mg concentration in blood serum and saliva of PMD and OSCC, and the results were compared with healthy controls. Our study proved a significant decrease in the level of serum Mg in oral precancerous patients when compared to the normal healthy individuals.

There was decrease in the level of serum Mg in oral cancer patients when compared to the normal healthy individuals and oral precancer patients with a statistically significant \( P \) value.

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**Conflicts of interest**

There are no conflicts of interest.

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