ORIGINAL RESEARCH

Colorimetric Determination of Magnesium in Blood and Saliva in Oral Squamous Cell Carcinoma and Potentially Malignant Disorders by Titan Yellow Method

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ABSTRACT

Background: The purpose of this study to evaluate and compare the magnesium concentration in blood plasma and saliva of oral squamous cell carcinoma, potentially malignant disorders and health subjects to serve as a positive marker or indicator in the process of carcinogenesis.

Materials and methods: The study consisted of 30 cases each of oral squamous cell carcinoma, potentially malignant disorders and healthy subjects of age and sex matched. The estimation of magnesium in blood plasma and saliva was carried out by colorimeter by using titan yellow method.

Results: There was significantly low concentration of magnesium in blood plasma of oral squamous cell carcinoma was noted as compared to potentially malignant disorders ($p = 0.000$) and healthy subjects ($p = 0.000$). However, there was no significant difference in magnesium concentration between potentially malignant disorders and healthy subjects.

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 concentration in blood plasma and saliva could be considered as tumor marker, playing an important role in carcinogenesis. Future studies should be carried out for further clarification.

Keywords: Magnesium, Oral squamous cell carcinoma, Potentially malignant disorders, Blood plasma and saliva.

Abbreviations: Oral squamous cell carcinoma (OSCC), Potentially malignant disorders (PMD), Healthy subjects (HS), Well-differentiated squamous cell carcinoma (WDSCC), Moderately differentiated squamous cell carcinoma (MDSCC) and Poorly differentiated squamous cell carcinoma (PDSCC).

How to cite this article: Bagulkar BB, Chaudhary M, Gawande M, Patil S, Gadbail A, Bagulkar S. Colorimetric Determination of Magnesium in Blood and Saliva in Oral Squamous Cell Carcinoma and Potentially Malignant Disorders by Titan Yellow Method. J Orofac Res 2013;3(4):240-244.

Source of support: Nil

Conflict of interest: None

INTRODUCTION

Magnesium (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. Magnesium is considered the physiological calcium antagonist. At a cellular level, it may act as an important regulator of cell functions. Its serum concentration is remarkably constant in healthy subjects. The normal Mg serum concentrations are protective against various diseases.[1] Imbalances in magnesium metabolism are common and are associated with different pathological conditions.[2]

Magnesium 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.[3]

Over 300 enzymes that influence the metabolism of carbohydrate, amino acids, nucleic acids and protein, and ion transport, require Mg.[4,5] It has been proposed that Mg is central in the cell cycle, and that its deficiency is an important conditioner in precancerous cell transformation.[6-8] Optimal Mg intake is thought to be prophylactic against initiation of some neoplasms.[9]

Relationships between magnesium and cancer are complex: both Mg load and Mg deficit may produce either carcinogenic or anticarcinogenic effects. Carcinogenesis modifies the Mg status inducing Mg distribution disturbances which may frequently associate a tumor Mg load with Mg depletion in non-neoplastic tissues.[10-12] Thus, we aim at colorimetric estimation of magnesium in blood and saliva in oral squamous cell carcinoma and potentially malignant disorders so as to serve as a positive marker or indicator in the process of carcinogenesis. Also an attempt is made for showing how these data can bring about new promising developments in cancer research as well as in anticancer treatment.

MATERIALS AND METHODS

The present study was carried out after getting the approval of the institutional ethical committee. This study consists of 30 patients each of oral squamous cell carcinoma and potentially malignant disorders. Thirty healthy subjects (HS) were matched according to age, sex and ethnicity were included in the study. A detailed case history was recorded on a case history proforma with special reference to habits of betel chewing, smoking and alcohol consumption. An informed consent of each patient was obtained after

explaining the intended procedure. The HS, comprised of healthy individuals with age and sex matched and free from any systemic illness or oral habits. Whole blood and unstimulated saliva was collected under all aseptic condition. Centrifugation of blood was done for plasma collection.

Magnesium Estimation was done with the Titan Yellow Method

Principle

A trichloroacetic acid filtrate of serum and saliva is treated with the dye titan yellow in alkaline solution. The red lake that forms is a dye, adsorbed on the surface of colloidal particles of magnesium hydroxide, which are kept 'in solution with the aid of polyvinyl alcohol. This last reagent also increases the sensitivity of the method by a factor of approximately 2.[13]

Procedure

In this study, reagents used were trichloroacetic acid, 5.0 g/100 ml, sodium hydroxide, 5.0 mol/I, polyvinyl alcohol, 0.1 g/100 ml, titan yellow (stock solution), titan yellow (working solution) and stock standard, 20 mmol Mg~+2/1 and following steps were followed:

1. Place 1 ml of the unknown blood plasma and saliva sample into a 15 × 150 mm test tube and blow in 5 ml of trichloroacetic acid, (5 g/l00 ml). Some prefer to use trichloroacetic acid, 7.5 g/100 ml.
2. Mix tubes gently but thoroughly, let stand for 5 min, and centrifuge for 5 min at 2000 rpm.
3. Transfer 3 ml of the clear supernatant to a Coleman or other suitable cuvet.
4. Prepare a standard set by pipetting 0.5 ml of each magnesium working standard into a separate cuvet, followed by 2.5 ml of trichloroacetic acid (5 g/l00 ml). Prepare a reagent blank by substituting distilled water for the magnesium standard.
5. Add to all cuvets 2 ml of titan yellow working solution and 1 ml of 5.0 molar NaOH. Mix tubes thoroughly and read after 5 minutes, but not later than 30 minutes at a wavelength of 540 nm with the reagent blank set to 100 percent T or zero absorbance.[13]

The instrument used for the measuring of the wavelength was digital photo calorimeter (Model no. 312, Electronic instrumentation, Bhopal, Madhya Pradesh).

Table 1: Details of histopathological diagnosis of leukoplakia and erythroplakia

	Mild dysplasia	*Moderate dysplasia*	*Severe dysplasia*
Leukoplakia	09	06	06
Erythroplakia	00	06	03

CALCULATION

Construct a standard curve or employ the following formula:

Au

As × Cs = mmol $Mg^{2}_{1/2}$ + /1

Where A = absorbance readings

Cs = concentration of magnesium standard most nearly corresponding to the value of the unknown.

The normal concentration range of magnesium in blood plasma and saliva using the Titan yellow method is from 1.4 to 2.3 mmol Mg/1. But for saliva, the concentration is more toward the lower limit of the normal range and that for blood plasma; it's more toward the higher side of the normal range.[13]

STATISTICAL ANALYSIS

Statistical analysis was carried out by using SPSS software version 16. Kruskalvalis test and Mann-Whitney test was done.

RESULTS

OSCC: A total of 30 of OSCC patients comprising of 21 males (70%) and 09 female (30%) with an age range of 40 to 60 years. Out of 30 patients of OSCC, 15 were well-differentiated squamous cell carcinoma, 09 were moderately differentiated squamous cell carcinoma and 06 were poorly differentiated squamous cell carcinoma. When gender-wise data for occurrence of OSCC was analyzed, strikingly these lesions were found to be more prevalent in males as compared to females.

PMD: A total of 30 of PMD patients comprising of 18 males (60%) and 12 female (40%) with an age range of 40 to 60 years. Out of 30 patients, 21 were diagnosed as leukoplakia and 09 were diagnosed as erythroplakia. Details of histopathological diagnosis are summarized in

Table 2: Details of magnesium ion concentration in OSCC, PMD and healthy subjects in blood plasma

Groups	*N*	*Mean*	*Std. deviation*	*Std. error*	*95% confidence interval for mean*		*Minimum*	*Maximum*
					Lower bound	*Upper bound*		
1	30	1.9970	0.23796	0.07525	1.8268	2.1672	1.61	2.30
2	30	1.9620	0.26786	0.08471	1.7704	2.1536	1.61	2.30
3	30	0.9210	0.40465	0.12796	0.6315	1.2105	0.38	1.68

Group 1: Healthy subjects; Group 2: PMD; Group 3: OSCC

Table 3: Details of magnesium ion concentration in OSCC, PMD and healthy subjects in saliva

Groups	N	Mean	Std. deviation	Std. error	95% confidence interval for mean		Minimum	Maximum
					Lower bound	Upper bound		
1	30	1.6850	0.26146	0.08268	1.4980	1.8720	1.32	2.04
2	30	1.5940	0.27814	0.08795	1.3950	1.7930	1.19	2.04
3	30	0.5880	0.32903	0.10405	0.3526	0.8234	0.20	1.23

Group 1: Healthy subjects; Group 2: PMD; Group 3: OSCC

Table 1. When gender-wise data for occurrence of PMD was analyzed, strikingly these lesions were found to be more prevalent in males as compared to females.

HS: The HS consists of apparently healthy individuals within age groups of 40 to 60 years. Healthy subjects had 15 males (50%) and 15 females (50%). None of them had acute oral infection or had received any medication.

Normal magnesium level in blood plasma by calorimetry Titan yellow method: –1.4 to 2.3 mmol $Mg^{2}_{1/2}$ + /1. It was found significantly low in oral squamous cell carcinoma patients but was within normal limits in potentially malignant disorders and healthy subjects (Table 2).

Normal magnesium level in Saliva by calorimeter Titan yellow method: –1.4 to 2.3 mmol $Mg^{2}_{1/2}$ + /1. It was found significantly low in oral squamous cell carcinoma patients but was within normal limits in potentially malignant disorders and healthy subjects (Table 3).

DISCUSSION

In this study, there was a significant decrease in concentration of magnesium noted in blood plasma and saliva in oral squamous cell carcinoma than in potentially malignant disorder and normal patients, i.e. below 1.4 mmol Mg/l carried out by colorimetric Titan yellow method. Studies in animal models have found that Mg deficiency has caused lymphopoietic neoplasms, osteomyelosclerosis and subperiosteal desmoids tumors and intestinal tumor-like overgrowth in young rats.[14-19] Shpitzer T et al studied salivary concentrations of Mg was higher in oral cancer by 28% (p = 0.12), which is not in accordance to this present study. This might be due to local therapeutic agents which can be easily applied to the oral mucosa, altering its 'bathing medium'-the saliva.[20]

This is the only study present in the literature carried out on human blood plasma and saliva by colorimetric Titan yellow method. There are also other studies present in the literature which are carried by other methods, however comparison cannot be made. Mg has a central regulatory role in the cell cycle, that affects transphorylation and DNA synthesis, has been proposed as the controller of cell growth, rather than Ca.[21] This has been related to mitosis and division.[6-8] It is postulated that Mg controls the timing of spindle and chromosome cycles by changes in intracellular concentration during the cell cycle, i.e. Mg falls 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, not Ca, 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 (2) processes thought to be regulated by Ca/calmodulin are Mg-dependent, since low, i.e. Ca levels can be regulators only when there is adequate free Mg. The metabolic effects of Ca are produced indirectly through its competition with Mg for membrane sites.[22]

Evaluation of the data on the role of Mg in cell proliferation indicates absolute requirements in crucial steps of cell activation that can trigger normal and neoplastic cell division, but the precise phase(s) of cell cycle where Mg exerts its regulatory effects is in need of further study. The Mg, which is present in a small amount as the free cation, or bound to ligands (i.e. the many enzymes it activates), is compartmented to a different degree in different types of cells; it is not influenced by extracellular Mg concentration.[18] In some cell types rapidly and slowly or nonexchangeable compartments are detectable. In gradually Mg depleted cells, Mg-dependent metabolic functions are inhibited in the following order: glycolysis < RNA and DNA synthesis < respiration < protein synthesis protein synthesis appears to be the most sensitive function affected.[23]

Altered membrane phospholipids influence the viscosity of membranes, which is decreased both in Mg deficient and in cancer cells. Membrane abnormalities of erythrocytes of Mg deficient rodents were shown to be responsible for their reduced survival time.[20] Mg low cell membranes have recently been shown to be characterized by increased fluidity and permeability.[24,25] Changed lipid membrane components can reflect changes in lipid metabolism caused by Mg deficiency.[26] Anghileri et al propose that modifications of cell membranes are principal triggering factors in cell transformation leading to cancer. Using cells from induced cancers, they found that there is much less Mg binding to membrane phospholipids of cancer cells, than to normal cell membranes. This might be involved in precancerous changes; in the preneoplastic phase, binding of Mg to

membranes is decreased at the same time that cytosolic Mg increases. There is drastic change in ionic flux from the outer and inner cell membranes (higher Ca and Na; lower Mg and K levels), both in the impaired membranes of cancer, and of Mg deficiency. It has been suggested that Mg deficiency may trigger carcinogenesis by altering fidelity of DNA replication, and increasing membrane permeability.[27-29] A mechanism proposed is competition of Mg with oncogens for DNA binding sites, and its prevention of incorporation of incorrect nucleotides during DNA synthesis.

Mg deficiency depresses cell-mediated immunity, impairs phagocytic activity as well as lymphocytic function.[30] Mg plays following role in apoptosis –it causes increase in DNA fragmentation, cleavage of substrates associated with caspase activation and tissue shrinkage.[3]

Thus, we hypothesize that decrease in Mg content could serve as a positive marker or indicator in the process of carcinogenesis. This change in values of Mg concentration could acts as a screening marker to indicate the progress of potential malignant lesions to malignancy. Ease of use of saliva as a tool for the use of diagnosis makes this a convenient method for mass screening.

CONCLUSION

Our study finding suggests that Mg deficiency might be implicated in aspects of pathogenesis and treatment of neoplasms, there are many unknowns. Investigation of these questions might lead to means to prevent oral squamous cell carcinoma or possibly of immuno-incompetence. Whether higher Mg intakes might be protective against oncogens in humans as it is in some animal models deserves study.

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