Study of Biochemical Level for Mg and Ca-Mg Imbalance in Patients with Oral Cancer and Potentially Malignant Disorder and their Prostetical and DSSS Treatment

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In the last decade it has been noticed a significant increase of indicators of oral cancer and oral potentially malignant disorders frequency, which led to the integration of this pathology among the primary problems of public health regarding dental medicine. It seems that besides the essential role of magnesium and calcium in the functions of human body, the changes of serum and salivary levels of magnesium and calcium may play a role in the pathogenesis of oral cancer and oral potentially malignant disorders.The aim of the study was to measure the serum levels of magnesium and calcium in patients with oral cancer and oral potentially malignant disorders.The serum and saliva levels of magnesium in oral cancer were higher than in healthy controls subjects. There are no significant statistic differences between the serum variations of total calcium and salivary calcium in the studied groups, compared to the controls.

Keywords: public health dentistry, magnesium, saliva, oral cancer, oral potentially malignant disorders, prosthetical and DSSS treatment

The WHO Global Oral Health Programme has established a global surveillance system of oral cancer in order to assess risk factors and to help the planning of effective intervention programmes. Data on oral cancer (ICD-10: C00-C09) morbidity and mortality are stored in the Global Oral Health Data Bank [1]. An estimated 300,400 new cases and 145,400 deaths from oral cancer (including lip cancer) occurred worldwide, in 2012 [2].

In Romania, the number of new cases of oral cancer and deaths caused by it registers a more increased growth rate than the regional and global average; this is explained by the high prevalence of certain risk factors like smoking and heavy alcohol consumption [3].

The quantity of magnesium (Mg) in human body is estimated at 23-25g, respectively 0.035% of an adult body mass; Mg is mainly intracellular (70% being bound by proteins), its plasma concentration being 14-33 mg/l [4].

Although Mg acts as a competitive with Ca in the human body, one must not disregard that Mg and Ca are interdependent, they often work in a *team*, because they have similar chemical properties and share the same homeostatic system in the human body, including gutabsorption and kidney reabsorption to maintain a normal balance of Ca and Mg (as an example, the changes in blood or colon lumen concentrations for Ca and Mg are monitored by the same receptor, the CaSR) [5]. Magnesium and Ca potentially antagonise each other in many other pathophysiological mechanisms, such as oxidative stress, cell differentiation and proliferation, apoptosis and angiogenesis, DNA repair, which may also be involved in cancer development [6]. Carcinogenesis induces Mg disturbances, which cause Mg mobilization through blood cells and Mg depletion in non-neoplastic tissues [7]. Neo-angiogenesis is one of the main culprits of tumour growth and spreading. The carcinogenesis process involves a highly complex and co-ordinated series of events, including increased vascular permeability, matrix degradation, endothelial cell proliferation, migration, survival and differentiation. Many of these steps are affected by Mg in different ways [8]. Without a comprehensive understanding of these factors, efforts to prevention, diagnosis and manage diseases such as oral cancer and oral potentially malignant disorders are likely to be ineffective in terms of outcomes and use of resources [9].

Experimental part

Motivation, purpose and objectives

Oral cancer became a highly important public health problem in the field of dental medicine in the last decade, due to the increasing prevalence rate of this pathology, high rate of fatality, the decrease in the age of debut, adverse prognosis, and last but not least, high costs for diagnostic and treatment [10]. In this framework, oral cancer represents a current and a much debated public health problem for Romania too, with large perspectives in the research regarding morbidity, etiologic factors, pathophysiology, risk factors and, not in the least important, the treatment [11]. On the other hand, the estimation of serum levels of trace elements like Mg and Ca in oral submucosal fibrosis and oral squamous cell carcinoma may help us in detection of early cancerous changes or even the occurrence of oral potentially malignant lesions in such a population. These elements represent a great value for public health because they may be considered as potential markers of disease [12].

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The elaboration of the present study was motivated by the need of finding proof that would confirm the significant modification of Mg and Ca homeostasis in patients having this pathology, proof that would contribute to a better knowledge of the disease.

From this perspective, the purpose of the study was to highlight changes of the serum and salivary biochemical levels of Mg and implicitly of Ca in patients belonging to the two studied groups, compared to the control group having healthy subjects, and revealing any possible correlations between the analysed biochemical parameters and the studied demographic variables (age and gender).

Our study followed several *specific objectives* in order to accomplish the target aim:

-determining the biochemical levels of total Mg and total Ca in serum, and investigating the balance between these two trace elements using serum Ca/Mg *ratio*;

-determining the salivary concentrations for Mg, Ca and salivary Ca/Mg *ratio*;

-statistical significance testing of the noticed differences related to the variation of the biochemical parameters analysed.

Material and method

The study design respected the methodology of the control-case studies [13].

Out of the total of 342 new cases of oral cancer reported in 2015, a representative group was chosen for the Moldavian area (cases group 1), which comprised of 35 adult patients.

The inclusion criteria were:

-in patients from whom it was possible to collect biological samples (blood and saliva) prior any treatment and the informed agreement signed before the participation in the study.

The average age calculated for group cases 1 was 55.07 ± 16.35, the minimum age was 32, and the maximum age was 78 years.

Comparisons were made with cases group 2, which included 28 patients, having oral potentially malignant disorders, and a control group, which comprised of 43 healthy individuals. The average age for group cases 2 was 52.07 ± 15.45, the minimum age was 30, the maximum age was 80, and the average age for the control group was 51.95 ± 17.13 years.

The exclusion criteria were:

-ill persons who received, by any means, medication containing Mg and/or Ca, diuretics or medication/substances (*for ex.*, patients treated with EDTA or patients who received a radiology contrast agent) which can significantly alter Mg and Ca homeostasis (one month before the onset of the study);

-patients having diseases (acute or chronic) which evolve together with oncological pathology and which can significantly alter Mg or/and Ca homeostasis (kidney failure, heart failure, diabetes mellitus, suppuration and bone fracture in the last year).

For each and every case, the initial diagnostic was established based on the medical history and physical exam, the certainty diagnostic being confirmed by the results of the histopathology exam. The diagnostic was established in agreement with the clinical, radiological and laboratory criteria for all the cases.

The control group comprised 43 healthy adults who expressed their free consent, not without previous being informed about it, regarding the participation to this study, and in the case of which a good collaboration with the clinician was noticed. The recommendation towards participating to the study and expressing agreement for the collection of biological material was made to persons who went to the dental medicine private practice in 2015 in order to solve other health problems than the pathology argued in the study.

The main statistic indicators (mean value, standard deviation, confidence interval 95%) [13], were calculated in order to highlight the comparability between the studied groups; their values revealed a proper correlation regarding the structure of the studied groups for the main characteristics that are being studied.

The process of collecting the biological material was conducted according to the standard procedures [14, 19]. Blood and saliva samples were collected before any therapeutic intervention. For all subjects from the studied groups, saliva was collected using the Holmes method, which involves aspiration (for 5 minutes).

The serum assays were conducted in an accredited laboratory. Evaluating Mg levels is challenging because Mg is largely intracellular; a deficit of total Mg can easily be present with normal serum [18, 21]. Atomic absorption spectroscopy (AAS) was used in order to perform the quantitative automatic test for the biochemical levels of total Mg and total Ca in serum.

The database was created using Microsoft Excel 2010 for Windows and the computer statistic processing was performed using SPSS 18.0 for Windows. The descriptive statistics module was used, which allowed the calculation of the main statistic indicators (mean value, standard deviation and confidence interval CI 95%). Statistical significance tests (*t* Student test) were applied in order to check the statistical significance of the noticed differences.

Ethical clearance for the study was obtained from the institutional ethical committee.

Results and discussions

Recent literature describes a role of Mg in carcinogenesis. The relationship between Mg and carcinogenesis is complex and multifactorial. Mg is necessary in the neoplastic cellular processes, malignant disorders biology is characterized by high energy requirements due to rapid proliferation, dedifferentiation, and cell immortality [15].

Study of biochemical levels of serum magnesium and serum calcium

Determination of biochemical levels of serum Mg and serum Ca was followed by a calculation of the main statistic indicators, the results being presented for the groups taken for study, by comparison to the control group (table 1).

A significant increase ($p=0.0027$) was highlighted for patients suffering from oral cancer, regarding serum total Mg (of +11.53%) by comparison to the control group. There is also a statistically significant increase ($p=0.019$) regarding serum total Mg (of +11.48%) in the case of the group comprised of patients suffering from oral potentially malignant disorders, by comparison to the control group.

One needs to take into consideration that although these increases of serum Mg are statistical significant by comparison to the control group, they are still relatively close to the upper limit of the normal reference values in the laboratory (the comparison to the upper limit not being statistical significant $p=0.09$).

A close attention must be given when interpreting this data since the increase of the Mg serum level can be correlated either with a decrease in the renal excretion of the Mg or with an increase in its tubular reabsorption.

Although an increase of 2.64% was highlighted, regarding

Table 1

SERUM BIOCHEMICAL LEVELS FOR MAGNESIUM AND CALCIUM

Indicators	**Magnesium** (in mg/dl)			**Calcium** (in mg/dl)			**Calcium / Magnesium** *ratio*		
	Cases 1 Oral cancer	Cases 2 Oral potentially malignant disorders	Controls	Cases 1 Oral cancer	Cases 2 Oral potentially malignant disorders	Controls	Cases 1 Oral cancer	Cases 2 Oral potentially malignant disorders	Controls
Absolute no. (n)	35	28	43	35	28	43	35	28	43
Mean value	2.448	2.362	2.119	8.305	8.449	8.602	3.49	3.58	3.35
Standard deviation	±0.159	±0.207	±0.211	±0.697	±0.703	±0.621	±0.37	±0.46	±0.43
Minimum value	2.157	2.123	1.782	7.091	7.416	8.031	2.65	2.57	2.73
Maximum value	2.927	2.634	2.482	9.896	9.874	10.017	3.98	4.02	4.27
Coefficient of variation (%)	6.49	8.76	9.96	8.39	8.32	7.22	10.91	12.85	12.81
95% Confidence Interval	2.237-2.574	2.124-2.415	2.016-2.203	7.402-8.735	8.015-8.862	8.017-9.713	3.025-3.596	3.191-3.602	3.278-3.691
p value (*Statistically Significant)	Cases 1 *vs.* Controls $p=0.0027$* Cases 2 *vs.* Controls $p=0.019$* Cases 1 *vs.* Cases 2 $p=0.15$			Cases 1 *vs.* Controls $p=0.10$ Cases 2 *vs.* Controls $p=0.053$ Cases 1 *vs.* Cases 2 $p=0.60$			Cases 1 *vs.* Controls $p=0.017$ Cases 2 *vs.* Controls $p=0.012$ Cases 1 *vs.* Cases 2 $p=0.11$		

Conversion factor serum Mg: mmol/l x 2.43 = mg/dl; mEq/l x 0.5 = mmol/l; mEq/l x 1.2 = mg/dl.
Reference value: 1.7-2.2 mg/dl (18-20 years old); 1.6-2.6 mg/dl (21-60 years old); 1.6-2.4 mg/dl (60 years old and over). Conversion factor serum Ca: nmol/l x 4 = mg/dl; mg/dl x 0.25 = mmol/l. Reference value: 8.6-10 mg/dL (18-60 years old).

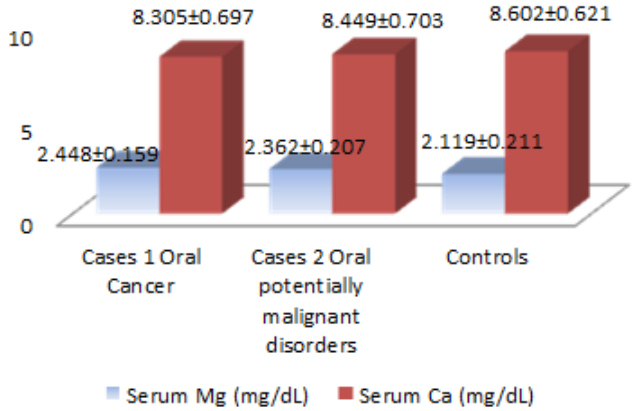

Fig.. 1. Mean Values of Serum Magnesium and Serum Calcium

total Mg in patients suffering from oral cancer *vs.* oral potentially malignant disorders, this increase was not registered as having any statistical significance (p=0.15). There are no significant statistical differences regarding serum variation of total Ca in the studied groups by comparison to the control group (fig. 1).

Study of salivary biochemical levels of magnesium and calcium

The results of the quantitative assay of Mg and Ca in saliva, and the calculated statistic indicators are displayed in table 2.

The presented data reveal a statistically significant increase (p=0.001) regarding salivary biochemical level of Mg (of +10.75%) in patients with oral cancer by comparison to the controls. Also, there is a statistically significant increase (p=0.045) regarding salivary level of Mg (of +6.10%) in patients with oral potentially malignant disorders as well, by comparison to the control group.

Therefore, an increase of 2.38 % was noticed, regarding salivary level of Mg in patients with oral cancer *vs.* patients with oral potentially malignant disorders. This has not proved to be statistically significant (p=0.11).

Magnesium salivary excretion does not statistically correlate at a significant level with the serum level of Mg (p=0.09).

There are no statistically significant differences regarding the variations of salivary Ca in studied groups by comparison to the control group (fig.2).

Table 2

SALIVA BIOCHEMICAL LEVELS FOR MAGNESIUM AND CALCIUM

Indicators	**Magnesium** (in mg/dl)			**Calcium** (in mg/dl)			**Calcium / Magnesium** *ratio*		
	Cases 1 Oral cancer	Cases 2 Oral potentially malignant disorders	Controls	Cases 1 Oral cancer	Cases 2 Oral potentially malignant disorders	Controls	Cases 1 Oral cancer	Cases 2 Oral potentially malignant disorders	Controls
Absolute no. (n)	35	28	43	35	28	43	35	28	43
Mean value	0.381	0.365	0.344	5.401	5.314	5.362	14.07	14.55	15.58
Standard deviation	±0.041	±0.043	±0.036	±0.487	±0.472	±0.461	±2.462	±2.343	±2.011
Minimum value	0.311	0.317	0.301	4.016	4.029	4.508	9.79	9.16	11.03
Maximum value	0.492	0.473	0.407	6.805	6.731	6.987	17.64	16.19	17.81
Coefficient of variation (%)	10.76	11.78	10.69	9.01	8.89	8.59	17.49	16.10	12.91
95% Confidence Interval	0.370-0.379	0.359-0.368	0.330-0.352	5.206-5.697	5.011-5.543	4.908-5.611	12.623-15.916	12.729-15.082	14.271-16.001
p value (*Statistically significant)	Cases 1 *vs.* Controls $p=0.001$** Cases 2 *vs.* Controls $p=0.045$* Cases 1 *vs.* Cases 2 $p=0.059$			Cases 1 *vs.* Controls $p=0.15$ Cases 2 *vs.* Controls $p=0.11$ Cases 1 *vs.* Cases 2 $p=0.10$			Cases 1 *vs.* Controls $p=0.017$* Cases 2 *vs.* Controls $p=0.012$* Cases 1 *vs.* Cases 2 $p=0.11$		

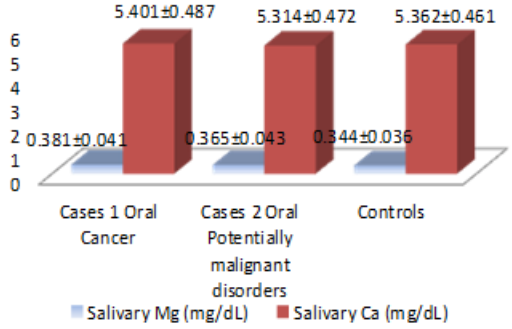

Fig. 2. Mean values of Salivary biochemical levels for magnesium and calcium

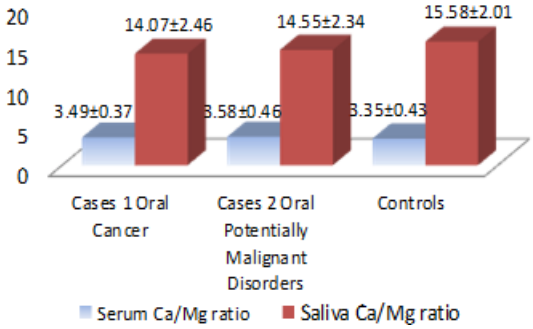

Fig. 3. Calcium-magnesium balance

Study of the calcium-magnesium balance in serum and saliva

The ideal ratio of calcium to magnesium (4-13.5) is very difficult to be settled in patients with oral cancer [11] on the one hand due to the main disease, and on the other hand due to the difficulties to eat properly, as is the case of these patients [16].

In order to be able to evaluate the balance between Ca and Mg, there have been calculated the mean values for Ca/Mg ratio in serum and saliva, the results for the two studied groups being presented by comparison to the control group (fig. 3).

Calcium and magnesium have a complicated relationship that is not fully understood.

The human body needs adequate levels of Mg in order to properly use Ca, and Mg affects Ca homeostasis and alters levels of certain hormones that regulate Ca in the body. The two minerals may also compete with other and interfere with the other's function [17, 20].

In these kind of patients it was established a prosthetic treatment in 48.57% of cases. Patients received treatment by fixed denture means, conjunct in 22.86% of the cases and the remaining mixed treatments, fixed and mobile in 14.28, 11.43% only removable dentures, 11 % of patients were treated with oclusal splints, the category with SDSS , thus being rebuilt the morphological and functional dental arches thereby restoring the homeostasis of the stomatognathic system [21-25].

Conclusions

The following conclusions were emphasized from the study results:

Oral malignant disorders induces Mg disturbances; in the study groups there were highlighted statistically significant increases of serum total Mg for patients with oral cancer (of +11.53%), as well in patients with oral potentially malignant disorders (of +11.48%), by comparison to the control group. There are no statistically significant differences concerning serum variations of total Ca in the studied groups, by comparison to the control group.

The results of the study reveal a statistical significant increase regarding salivary biochemical level of Mg in patients with oral cancer (of +10.75%), as well as in patients suffering from potentially malignant disorders (of +6.10%) by comparison to the controls. The Mg salivary excretion does not statistically correlate at a significant level with the serum Mg level for neither of the groups taken into consideration for study. There are no statistically significant differences as regarding variations of salivary Ca in studied groups by comparison to the control group.

Statistically significant increases of serum Ca/Mg *ratio* in patients with oral cancer (of +4.18%), as well as in patients with oral potentially malignant disorders (of +6.43%) by comparison to the controls.

A better knowledge and understanding of this pathology offers to the public health dentistry the necessary modern methods for a more efficient prevention of diseases at a population level.

References

1. FERLAY, J., STELIAROVA-FOUCHER, E., LORTET-TIEULENT, J., ROSSO, S., COEBERGH, J.W.W., COMBER, H, FORMAN, D, BRAY, F. European Journal of Cancer, 2013, **49**, p.1374– 403.
2. WILLIAMS, D.M. J Dent Res, 2011, **90**, nr. 5, p.549-51.
3. POPOVSKA, M., RADOJKOVA-NIKOLOVSKA, V., MINOVSKA, A., AGOP FORNA, D., MURATOVSKA, I., FORNA, N.C,. Rev. Chim. (Bucharest), **66**, no. 11, 2015, p.1786
4. CHEVALLIER, L. Nutrition:principes et conseils, Ed. Masson, Paris, 2005.
5. GROBER, U., SCHMIDT, J., KISTERS, K. Nutrients, 2015, **7**, p. 8199-226.
6. WOLF, F.I., MAIER, J., NASULEWICZ, A., et al. Arch Biochem Biophys, 2007, 458, p.24–32.
7. WOLF, F., TRAPANI, V. Clinical Science, 2012, 123, p.417–27.
8. COLOTTA, F., ALLAVENA, P., SICA, A., GARLANDA, C., MANTOVANI, A. Carcinogenesis, 2009, **30**, p.1073–81.
9. SUNALI, K. Int J Environ Res Public Health, 2008, **5**, nr.5, p.418-22.
10. LEONE, N., COURBON, D., DUCIMETIERE, P., ZUREIK, M. Journal of Epidemiology, 2006, **17**, nr.3, p.308-14.
11. DAI, Q., SHU, X.O., DENG, X., et al. BMJ Open, 2013, **3**, e002111.
12. PASTERNAK, K., KOCOT, J., HORECKA, A. J Elementol, 2010, **15**, nr.3, p.601–16.
13. SCHULTZ, K.F. Lancet, 2002, 359, p.431-4.
14. EFTIMIE, TOTU, E., MANUC, D., Rev. Chim. (Bucharest), no. 9, 2008, p. 947
15. RATHNAYAKE, N., AKERMAN, S., KLINGE, B., LUNDEGREN, N., JANSSON, H., TRYSELIUS, Y. J Clin Periodontol, 2013, **40**, nr.2, p.140–7.
16. CHECHERITA, L.E., FORNA, N.C., STAMATIN, O., COBZARU, R., LEON, M.M., CIOLOCA, D., Rev. Chim. (Bucharest), **64**, no.10, 2013, p.1172
17. SWAIN, N., RAY, J.G. Int J Oral Maxillofac Pathol, 2011, **2**, p.2-6.
18. ALBU, A., MORARU, C., HODORCA, R. Procedia Social and Behavioral Sciences, 2015, 197, p.1947-51.
19. CHECHERITA, L.E., FORNA, N.C., SURDU, MACOVEI, A., RACOVITA, S., FILIP, F., CHIRIAC, A.,.Rev. Chim. (Bucharest), **64**, no.11, 2013, p.1312
20. CHECHERITA L.E., BELDIMAN, M.A., STAMATIN, O. Rev. Chim. (Bucharest), 2013, **64**, no.8, p.864
21.CHECHERITA,L.E., TRANDAFIR, D., STAMATIN,O., CARAUSU,E.M., Rev. Chim. (Bucharest), **67**, no.8, 2016, p. 1628
22.CHECHERITA,L.E., TRANDAFIR, V., STAMATIN,O., CARAUSU,E.M., Rev. Chim. (Bucharest), **67**, 2017, no.7, p.1415
23. CARAUSU,E.M ,CHECHERITA,L.E., STAMATIN,O., ALBU,A., Rev. Chim. (Bucharest), **67**, 2016, no.9, p.1832
24. *** www.labcorp.com.2010. Laboratory Corporation of America-Directory of Services and Interpretive Guide

Manuscript received: 14.02.2016