Study of Biochemical Levels of Magnesium in Serum and Saliva in Patients with Stomatognathic System Dysfunctional Syndrome Determined by Compromised Bone Integrity and Prosthetic Treatment

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Evaluation of biochemical levels of magnesium (Mg) in serum and saliva provides useful information on bone metabolic processes and bone healing process.In the present study we are concerned if in patients with Dysfunctional Syndrome of Stomatognat System (SSDS) due to mandibular fractures or zygomatic-maxillary complex, are there any statistical significant differences regarding biochemical levels of Mg in serum and saliva, compared with a control group.We measured serum and saliva levels of Mg in 59 patients (cases 1) with bone injury and SSDS and we used 43 healthy subjects as controls (group 2).Serum levels (cases 1 vs. controls 2; mean ± s.d., in mg/dL), were as it follows: Mg, 23.73±1.91 vs. 23.81±2.47; salivary levels (cases 1 vs. controls 2; mean ± s.d., in mg/dL), were as it follows: Mg, 3.62±0.37 vs. 3.57±0.41. In patients with mandibular fractures or zygomatic-maxillary complex and SDSS there are differences of the serum Mg (+0.80%), as well of the salivary Mg (-0.88%), compared to the controls, but these differences did not prove to be statistically significant (p>0.05).

Key words: Stomatognat System Dysfunctional Syndrome–SSDS, magnesium, serum, saliva, public health dentistry,prosthetical treatment.

The divalent cations considered in the study exercise important roles in the human body. From a clinical perspective, mineral homeostasis is reflected in the maintenance of circulating concentrations of magnesium (Mg) and calcium (Ca) in the normal range and integrity of the skeleton. Bone tissue is a complex mineralized structure that consists of a mineral phase intimately embedded into an organic matrix [1]. Bone is a nano-composite with unique properties due to its physico-chemical composition. The dry matter in bone tissue is composed of about 70% anorganic phase and 30% matrix or organic phase.

In the dental-maxillary apparatus, Mg and Ca are important structural elements [2].

Magnesium is the most important cation after Ca [3]. Magnesium is present in a small concentration in all the cells and it is necessary for cellular metabolism. This trace element is also present in bones, along with Ca [4].

Magnesium is 0.42 % of the elemental composition of dental enamel, 0.82% of the dentine and, respectively, 0.26% of the fundamental composition of the maxillary bones [5].

Calcium is an abundant mineral inside the human body, stored in the skeleton and teeth [6]. Calcium represents 37.9% of the composition of the dental email, respectively 25.9% din of the elemental composition of the dentine [7], while the maxillary bones contain 22.5% Ca.

Both the salivary secretion, as well the saliva's composition is influenced, together with other different factors, also by the biochemical levels of Mg and Ca [8].

Together with the structural role [9], these minerals serve numerous functional roles, such as serum Mg and serum Ca influences the profile of the salivary secretion [10]. The influence is exercised both directly, through the parenchyma of the salivary gland, as well as indirectly, through the vegetative innervation of these glands [11]; high biochemical levels of Mg reduces the action of acetylcholine to stimulate the amylase in the salivary gland [12].

In the human body, there are important enzymatic systems which depend on the Mg.

An activator of over 325 different enzymes, Mg participates in many metabolic processes, including transformation of proteins, lipids, carbohydrates and nucleic acids, as well as electrolyte transport across cells membranes. Magnesium is considered as antagonist of Ca as it often functions synergistically with Ca, yet competes with Ca in the gut and kidney for transport and other metabolic pathways [13].

Changes of the intracellular and extracellular homeostasis of Mg and Ca are involved in the pathophysiological mechanisms of numerous affections; changes of the homeostasis are reflected in the changes of the salivary levels [14]. In this context, it has been shown that at elderly patients, the salivary level of Ca is significantly increased, and the salivary levels of Mg are significantly increased compared to the control group made of healthy subjects [15], structured on comparable age groups and genders.

In literature there are studies which suggest the fact that an increased salivary level of the Mg is associated with an increase in the degradation of the carbohydrates and, consequently, would be involved in the determinism of dental caries.

Not only the concentrations of Mg and Ca in serum or saliva are important for the development of some

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pathological conditions, but also the balance between them [16]. The actions of Mg and Ca are closely related, the deficit of one of these elements significantly influencing the homeostasis of the other.

Increased understanding of the homeostasis and metabolism of Mg and Ca allows a better understanding of the pathophysiology of the resultant clinical disorders [17].

In the last decade, Dysfunctional Syndrome of Stomatognathic System (SDSS) became an important problem of public health in the domain of Dental Medicine due to the increasing trend of this pathology of prevailing once with aging (from 10% in the adults to almost 40% in people of 65 years old and over) [18]. Thus, SDSS represents a current and a much debated problem, with large perspectives in the research regarding incidence and prevalence, etiologic factors, pathophysiology, risk factors and, not the least important, symptomatic and etiologic treatment.

Triggering SDSS is the result of cumulative intra- and over-systemic factors. Although, the onset can be done on one element of the stomatognatic system [19], further homeostasis disorders trigger the other elements. The factors that generate SDSS are, among others, injuries within the facial part of the skull, including fractures of mandible and/or zygomatic-maxillary complex.

At this moment, there are very few direct studies, and the attitude of the clinicians is based on pathophysiology and on the extrapolation of some observation data from the experimental medicine.

Thus, the realization of the present study has been motivated by the need of finding proofs which attest the significant modification of the Mg homeostasis and medically justify the administration of mineral supplements at patients with SSDS, due to mandibular fractures or zygomatic-maxillary complex, even in the first 48 hours from the fracture.

From this perspective, the aim of the study was to underline the changes of the serum and salivary biochemical levels of the Mg and, implicitly of the Ca in patients from the study group compared to a control group formed of healthy subjects and the revealing of the eventual correlations between the analyzed biochemical parameters and the studied demographic characteristics (age and gender).

In order to achieve the proposed aim, the present study has fixed the following *objectives:*

-the determination of the serum Mg, serum Ca and Ca/Mg *ratio* in the considered groups;

-the determination of the salivary levels of the Mg, Ca and the Ca/Mg *ratio*;

-testing the statistical significance of the differences observed regarding the variation of the biochemical parameters analyzed and the main demographic characteristics (age and gender) studied;

-evaluation of the correlations highlighted between the biochemical parameters analyzed and the main demographic variables by calculating the Pearson „r" correlation coefficients.

Experimental part

Material and method

Our study respected the methodology of the case-control studies.

Out of 615 cases of mandible fractures and 173 cases of zygomatic-maxillary complex fractures reported in 2015 for the area of Moldavia, there was chosen a representative group.

In the study group (cases 1) were included patients with SDSS produced as a result of the damage of the bone integrity of the stomatognat system by mandible fractures or of the zygomatic-maxillary complex, admitted in the Oro-Maxillo-Facial Clinic of the "St. Spiridon" University Hospital of Iasi.

In the cases there were included ill people who had the following criteria simultaneously met:

- minimum age of 18 years;
- existence of the written informed consent regarding participation in the study;
- patients in which the collection of biological samples (blood and saliva) could be achieved prior to any therapeutic intervention (after at least 6 and maximum 48 hours from the producing of the fracture).

There were not included in the study group (cases):

-patients who were administered, by any route, drugs containing Mg and/or Ca, 4 weeks prior to entering the study;

-patients who received diuretic medication 4 weeks before entering the study;

-patients who were administered drugs/substances (eg., patients treated with EDTA or who have received radiological contrast substances) that can significantly alter Mg and Ca homeostasis, 4 weeks before entering the study;

- subjects with other conditions (acute or chronic) that can significantly alter the homeostasis of Mg and/or Ca.

For each case, the initial diagnosis has been established based on the history and physical examination, the correct diagnosis being confirmed by the radiological findings. In all the cases, the diagnosis was made according to clinical, radiological and laboratory criteria.

In this study there were respected the requirements of Good Clinical Practice (GCP) [20].

After fulfilling the inclusion criteria, the study group consisted of 69 subjects, including 22 females and 47 males.

The control group was formed of volunteer adults who expressed their informed and freely consent regarding their participation in the study. In the control group there were included 52 healthy subjects of which 28 females and 24 males.

The proposal for the participation in the study and the collection of biological samples was made to persons who addressed, in 2015, to the individual dental office.

In order to emphasize the comparison between the studied groups, there were calculate the main statistical indicators (medium value, standard deviation and the confidence interval 95%). Their values (table 1) have shown a good correspondence between the structures of the studied groups in the main demographic characteristics analyzed [21].

In table 2 it is shown the location of the bone fracture in the study group. Of the total cases studied it can be noticed that the most common location of the fracture is the mandibular one, followed by the zygomatic-maxillary complex fracture.

At the subjects from the study group, the saliva was harvested by the Holmes method which involves aspiration (for 5 minutes).

At the subjects in the control group, the collection of biological material (blood and saliva) was performed before any therapeutic interventions addressed to the underlying disease, before any other method was used (12 hours after the last meal, between 7-8 ante-meridian, α-jeun) [21], and for saliva it was used the atomic absorption spectrophotometry (AAS). Serum determinations were performed in an accredited laboratory for medical tests.

The database was created using Microsoft Excel 2010 for Windows and computerized statistical processing was

Table 1
STRUCTURE OF THE GROUPS CONSIDERED IN THE STUDY

Age (years)	Female gender		Male gender		Total	
	Study group	Control group	Study group	Control group	Study group	Control group
Absolute no. (n)	22	28	47	24	69	52
Medium age	46.41	45.85	47.72	54.78	47.03	50.18
Standard deviation (σ)	±16.13	±16.75	±15.12	±15.71	±15.79	±16.13
Minimum value	19	18	19	20	19	18
Maximum value	79	78	76	79	79	79
Variation coefficient (%)	34.01	35.70	34.29	34.61	34.11	35.13
Interval of confidence (CI 95%)	37.59-55.84	40.96-56.15	42.76-57.69	49.32-62.15	39.19-57.74	41.02-60.23
p value (cases *vs.* controls)	*p* = 0.06 - NS		*p* = 0.11 – NS		*p* = 0.10 - NS	

Table 2
LOCALIZATION OF THE FRACTURE

Localization of the fracture	Female gender		Male gender		Total	
	Absolute Number	(%)	Absolute number	(%)	Absolute number	(%)
Mandible fractures	17	77.27	35	74.45	52	75.36
Zygomatic-maxillary complex fractures	5	22.73	12	25.55	17	24.64

Table 3
SERUM LEVELS OF THE MAGNESIUM, CALCIUM AND THE Ca / Mg RATIO

Indicators	Mg (in mg/dl)		Ca (in mg/dl)		Ca/Mg ratio	
	Cases group	Control group	Cases group	Control group	Cases group	Control group
Absolute no. (n)	69	52	69	52	69	52
Medium value	2.381	2.297	8.197	7.917	3.44	3.45
Standard deviation (σ)	±0.204	±0.247	±0.781	±0.647	±1.42	±0.92
Minimum value	1.807	1.791	7.179	7.104	2.77	2.53
Maximum value	2.732	2.804	9.507	9.427	4.42	4.75
Variability coefficient (%)	9.07	9.87	8.71	8.23	12.08	14.30
Confidence interval 95% (CI 95%)	2.293-2.487	2.301-2.515	7.995-8.519	7.528-8.209	3.36-3.60	3.07-3.82
p value (cases *vs.* control)	*p* = 0.27 - NS		*p* = 0.07 – NS		*p* = 0.11- NS	

Conversion factor serum *Ca*: nmol/l x 4 = mg/dl; mg/dl x 0.25 = mmol/l. *Reference values:* 8.6-10 mg/dl (18-60 years). 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 values:* 1.7-2.2 mg/dl (18-20 years); 1.6-2.6 mg/dl (21-60 years); 1.6-2.4 mg/dl (60 and above).

realized with SPSS 18.0 for Windows. It has been used the descriptive statistics module which permitted the calculation of the main statistical indicators (medium value, standard deviation and confidence interval CI 95%). In order to check the statistical significance of the differences found, there were applied tests of statistical significance (test "t" Student). In order to assess the correlations between serum and salivary concentrations of the divalent cations considered in the study and other demographic variables investigated, it was calculated the Pearson "r" correlation coefficient.

We mention that the interpretation of the values of the"r" correlation coefficient was realized according to the classic grid [22].

Table 4

BIOCHEMICAL LEVELS IN SALIVA OF THE MAGNESIUM, CALCIUM AND THE Ca/Mg RATIO

Indicators	**Mg** (in mg/dl)		**Ca** (in mg/dl)		**Ca/Mg** *ratio*	
	Study group	Control group	Study group	Control group	Study group	Control group
Absolute number(n)	69	52	69	52	69	52
Medium value	0.373	0.341	5.294	5.426	14.19	15.91
Standard deviation (σ)	±0.071	±0.063	±0.537	±0.481	±1.87	±2.58
Minimum value	0.341	0.309	4.198	4.617	8.47	10.38
Maximum value	0.493	0.482	6.772	7.118	19.34	22.27
Variability coefficient (%)	10.67	11.35	10.54	8.96	13.07	15.58
Confidence interval 95% (CI 95%)	0.314-0.397	0.305-0.372	5.107-5.816	5.218-5.789	14.03-15.76	14.31-16.79
p value (cases *vs.* controls)	*p* = 0.15 - NS		*p* = 0.11 - NS		*p* = 0.06 - NS	

Results and discussions

Evaluation of biochemical levels of M and Ca in serum and saliva provides useful information on bone metabolic processes and bone healing process.

1. Serum concentrations of the total magnesium, total calcium and the Ca/Mg ratio

Results of the calculation of the main statistical indicators for the biochemical levels of the serum Mg and serum Ca in the two study groups considered are presented comparatively in the table 3.

The results presented in table 3 reveal the following aspects:

-even though in case of SDSS due to mandible fractures or of zygomatic-maxillary complex there were noticed ifferences regarding the biochemical levels in serum of +0.80% for Mg and of +0.48% for Ca compared to the control group (but also differences towards the reference values of the laboratory (of +0.082 mg/dl for Mg and of +0.082 mg/dl for Ca), these differences did not prove to be statistically significant ($p>0.05$);

-also, there were not noticed significant statistical ifferences regarding the ratio between biochemical levels in serum of the Ca and Mg in cases of SDSS determined by andible fractures or by zygomatic-maxillary complex *vs.* the control group.

2. Salivary concentrations of the magnesium, calcium and the Ca /Mg ratio.

There were calculated the main statistical indicators for the biochemical levels in the saliva of the Mg and Ca in the wo groups considered, the results being comparatively presented in table 4.

When analyzing the results presented in table 4, it can be noticed that:

-even though in cases when SSDS is determined by mandible fractures or of zygomatic-maxillary complex, there are differences regarding the biochemical levels in the aliva of -0.68% for Mg and of +1,63% for Ca, compared to the control group, these differences hadn't proven yet to be statistically significant ($p>0.01$).

3. Analysis of the correlations

In order to measure the interdependence between the biochemical levels in serum and saliva of the Mg and Ca and te demographic values considered in the study it was calculated the Pearson "r" correlation coefficient. Regarding the eventual correlations between the tested parameters and age, we may notice significant ($p<0.05$) medium negative correlations between the total serum Ca and salivary Mg („r" = 0.32) and between the serum total Mg and salivary Mg („r"= 0.24) in patients from the study group.

From the results of the feminine gender there may be noticed medium negative correlations, statistically significant ($p<0.05$), between age and the biochemical levels in saliva of the Mg („r" correlation coefficient of -0.502) and respectively Ca („r" correlation coefficient of -0.407), in patients with SDSS determined by mandible fractures or by zygomatic-maxillary complex, compared to the control group made of healthy patients.

Bone is a dynamic tissue possessing the ability to consequent remodel throughout life. Bone formation and resorption are important processes which are intimately coupled under normal circumstances. Optimum balance between bone formation and resorption is required to maintain the biochemical competence of the skeleton, its structural organization and function [23].

Our study was focused on the SDSS following mandible fractures or those of zygomatic-maxillary. The results obtained have shown that in patients considered there are no significant statistical differences regarding the biochemical levels in serum and saliva of Mg and Ca, compared to the control group formed of healthy persons.

If in some previous studies [24], the possible variations in serum and saliva of the Mg and Ca compared to the controls were seen as a predisposing factor, not as a consequence of the pathological condition, in the investigated cases, in the present study the eventual variations of the biochemical levels of the serum and saliva of the Mg and Ca were seen as a result of the fracture.

We mention that the studies which followed the detection of possible variations of the biochemical levels of Mg and Ca targeted patients in which the traumatic context of the fractures was less severe and there were targeted possible correlations between the phospho-calcic and phosphor-magnezic balance, eventually the bone fragility [25].

By comparison, our study took into consideration exclusively cases of medium severity in which the traumatic context of the fracture was clearly revealed and the bone fragility at the mandibular bone, respectively at the zygomatic complex did not represent a predisposing factor of the fracture.

In literature there are relatively only a few studies referring to the evolution of the serum concentration of Mg

and Ca in the first post fracture hours/days [26]. A recent study has demonstrated a decrease in the concentration of the ionized Ca in the first 24 hours, but in patients who were in critical condition [27]. It has also been demonstrated the fact that the normalization of biochemical level in serum Ca is produced in the calcification period of the callus, and the concentration of the parathormone, as a response to the evolution of the serum total Ca to the same patients, increases in first post-facture days (the fractures being treated traditionally) and returns to normal within 6 weeks.

A recent study evaluate the evolution of serum Mg in patients with multiple injuries and with severe traumatic lesions to the skull *vs.* patients with single or double bone fractures, but without any severe injuries to the skull [28]. The data showed a tendency to hypomagnesaemia in patients with multiple injury (embodied in magnesaemia values lower than 16.8 mg/l in patients with head injury compared to patients with trauma and bone fractures, but without any important lesions to the skull), but there were not detected any significant changes regarding the serum total Ca among the studied groups. We consider the data as being in accordance with our results, taking into consideration the inclusion in the current research of only the patients without any major cranial lesions, but with fractures [28].

A study conducted by Hitz F Mette and col., has tried to correlate the calcemia with the degree of restraint in hip, shoulder fracture [29]. Increased concentration of the serum Ca was evidenced in patients with fractures and by Castilioni et al., but since the 7 day post-fracture, while maintaining a minimum of 6 months, in accordance with the degree of restraint and in accordance with osteogenic response [30]. The existence of hypercalcemia (increased serum concentration of the total and ionized calcium) post-fracture has been demonstrated in 50% of the children with fracture (single) at members. Hypercalcemia is associated with increased urinary excretion of Ca, as evidenced by increased calcium/creatinine *ratio* in urine. Also, some authors support the immobilization as the cause of the growth of serum Ca and, consequently, of the urinary elimination of Ca.

Our study did not follow in evolution the biochemical levels in serum and saliva of the Mg and Ca but only prior to any kind of therapeutic method; we did not follow the evolution in dynamic of the Mg and Ca on long periods of time.

There are also studies which argue that the changes in the biochemical parameters related to the phosphor-calcic balance in children with accidental factures (post-fracture) are statistically insignificant. Thus, Allgrove, demonstrated statistically insignificant changes of the serum levels of the Mg and Ca towards the control group formed of healthy subjects [5]. However, low bone mineral density occurs in the majority of the children who had a Ca intake below 60% of the daily recommended dose, a situation not found in any of the subjects in the control group.

The reparative process after a fracture is complex and takes several steps of the modeling tissue. Initially, the answer is inflammatory, during which penetrates the outbreak mesenchymal precursors of osteoclasts, osteoblasts and their neo-formation vessels. The reparative process takes place in three stages: enchondral ossification, production of immature bone (woven bone) and its remodeling in mature bone (lamellar).

The healing process begins with the anabolic action of the osteoblasts, while remodeling involves osteoclasts function. In all this, Mg and Ca play an important role. The healing of the fracture is considered complete when the radiological fracture line disappears, the normal architecture restores to the callus and the bone becomes mechanically competent, this last aspect is crucial for the homeostasis of Mg and Ca in patients with fractures or trauma it is not known to us. Their tracking was, however, considered important by our study, especially under the immediate post-fracture conditions in which the ratio between parasympathyetic and sympathetic innervation of the salivary glands and, implicitly the salivary volume changes (consequence of the stress and inclusively of the dehydration and the more reduced parasympathetic stimulation).

In this type of cases a prosthetic treatment was established at 75.36% of the cases. The patients received treatment by fixed prosthetic means, conjunct in 34.78% of cases and the remaining received mixed treatments, fixed and mobile at the rate of 42.02%, the rest of removable dentures 24.64%, thus restoring the morphological and functional the dental arches, thereby restoring the homeostasis of the stomatognathic system.

Conclusions

Even though in cases with dysfunctional syndrome of the stomatognathic system due to mandible fractures or zygomatic-maxillary complex fractures there are differences of the biochemical levels in serum (of +0.80% for Mg and +0.48% for Ca) as well as in the saliva (of -0.88% for Mg and of +1.63% for Ca) compared to a control group formed of healthy subjects, these differences did not prove to be statistically significant ($p>0.05$); therefore, we consider that it is not medically justified the decision of administrating mineral supplements (with Mg and Ca) immediately post-fracture in SSDS cases of medium severity take into consideration in the study.

Contribution of the authors:

Associate professor Carausu Elena Mihaela, MD, PhD- study design, literature search, statistical processing of the data and manuscript preparation;

University assistant Checherita Laura Elisabeta, MD, PhD- collection of the data from the control group and the prosthetic treatment;

University assistant Trandafir Violeta, MD, PhD- collection of the data from the study group, clinical diagnosis and clinical interpretation of the results, generation and database administration.

The authors have declared that they are not in any conflict of interest.

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