

**ORIGINAL PAPERS**

**DIAGNOSTIC VALUES OF CALCIUM  
AND MAGNESIUM FORMS DETERMINED  
IN HUMAN SERUM AND SALIVA**

**Iryna N. Andrusishina**

**Institute for Occupational Health of AMS of Ukraine**

Abstract

Calcium and magnesium are known to be necessary for the normal function of various systems in animal and human organisms. There are many diseases caused by abnormal concentration of electrolytes, e.g. arterial hypertension or nervous system diseases such as multiple sclerosis, Mb. Alzheimer or Mb. Parkinson. The mechanisms of homeostasis indicate only the ionized forms of these elements. It is known that ionized calcium serves as an endocellular intermediary in action of enzymes and hormones in cells.

Therefore, it is very important to define levels of total and ionized forms of  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  in blood serum and saliva by the method of atomic absorption spectrometry and to show their diagnostic value for various pathological conditions of a human body. The 39 persons, aged 21 to 47 years take part in these investigations.

The results of determinations of calcium and magnesium forms present in human serum and saliva, representing physiological states are presented. The age and daily fluctuations of  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  content in serum and saliva were studied by atomic absorption spectrometry. The levels of non albumin forms of these elements were found by FAAS. The significance of determination of calcium and magnesium levels in serum and saliva under various pathological conditions (arterial hypertension and osteoporosis) was shown.

**Key words:** calcium, magnesium, serum, saliva, forms of elements, atomic absorption spectrometry.

## WARTOŚĆ DIAGNOSTYCZNA FORM WAPNIA I MAGNEZU OZNACZONYCH W LUDZKIEJ SUROWICY KRWI I ŚLINIE

### Abstrakt

Wapń i magnez to pierwiastki niezbędne do prawidłowego funkcjonowania organizmów zwierzęcych i ludzkich. Istnieje wiele chorób wywołanych przez nieprawidłowe stężenia elektrolitów, np. nadciśnienie tętnicze lub choroby układu nerwowego, takie jak SM, choroba Alzheimera lub Parkinsona. Mechanizmy homeostazy wskazują jedynie na zjonizowane formy tych pierwiastków. Wiadomo, że zjonizowana forma wapnia służy jako międzykomórkowy pośrednik między enzymami i hormonami w komórkach. Dlatego tak ważne jest określenie zawartości całkowitych tych pierwiastków oraz jonów  $\text{Ca}^{2+}$  i  $\text{Mg}^{2+}$  w surowicy krwi oraz w ślinie za pomocą metody absorpcyjnej spektrometrii atomowej oraz wykazanie ich roli w diagnostyce różnych stanów patologicznych w organizmie człowieka. Badaniami objęto 39 osób w wieku od 21 do 47 lat.

W pracy przedstawiono wyniki oznaczeń form wapnia i magnezu występujących w ludzkiej surowicy krwi oraz ślinie odpowiadające stanom fizjologicznym. Wahania w zawartości  $\text{Ca}^{2+}$  i  $\text{Mg}^{2+}$  w surowicy i ślinie, w zależności od wieku i pory dnia, badano za pomocą absorpcyjnej spektrofotometrii atomowej. Zawartości niealbuminowych form tych pierwiastków określono za pomocą FAAS. Wykazano istotność oznaczeń zawartości wapnia i magnezu dla różnych stanów chorobowych (nadciśnienie tętnicze, osteoporoza).

Słowa kluczowe: wapń, magnez, surowica, ślina, formy pierwiastków, absorpcyjna spektrofotometria atomowa.

## INTRODUCTION

The biological role of calcium and magnesium ions in vital activities of various organisms and their influence on development of pathological processes are well known (PARFENOV 1977, BAZARNOVA 1990, KONOVALOVA 2002). Calcium and magnesium participate in many processes, which sustain vital activities of a living organism. Calcium takes part in reactions of neuromuscular transmission of impulses, renders positive inotropic effect on the cardiac muscle activity, provides the control and activation of hormones and neurotransmitters, participates in blood coagulation, in the metabolism of osseous tissues. Magnesium is a physiological antagonist of calcium. Magnesium ions is an important link of neuromuscular conductivity, make depressive impact on the central nervous system, participate in heart beat and have a vasodilatation effect as a fibrinolysis promoter. Magnesium plays an important role in establishing immunity (TRAKHTENBERG 2006, KUDRIN 2006).

Mechanisms of homeostasis indicate only the ionized forms of these elements. It is known that ionized calcium serves as an endocellular intermediary of action of enzymes and hormones in cells. Ionized calcium with calmodulin influence functions of many structural elements in the cell. The role of ionized calcium as a mediator of actions of antidiuretic, adrenocorticotropic and other hormones is also important. The behaviour of ionized

forms of this macroelement in blood plasma (serum) and saliva is of interest to researchers, who study many physiological and pathological states (JARMAGOMEDOV et al. 1978, RADISHEVSKA 1998, ZABOLOTSKI, KULEV 2007). In clinical diagnostics, determination of ionized calcium in blood plasma is useful for surgical interventions in the cardiovascular system, in hypertension, anomalies of pregnancy and diseases of the inflammatory genesis (LOSKUTOVA 2004, KISTERS et al. 2005, VERESCHAGIN et al. 2006, TYPIAKOVA 2006, MAKARA-STUDZINSKA et al. 2008).

Traditionally, general behaviour of calcium and magnesium in blood serum and saliva is analyzed by photometric, potentiometric and atomic-absorption spectrometry (AAS) methods (SIGNIFOLI et al. 1989, KONOVALOVA 2002, KUDRIN, GROMOVA 2006, KOVALCHUK et al. 2007). Concentration of ionized forms of calcium and magnesium is most often determined via potentiometry or using ion-selective electrodes (JARMAGOMEDOV et al. 1978, TITOV 1995, TYPIAKOVA 2006, MELNICHENKO 2008). A drawback of the latter method is the need to secure an acid-alkaline balance test, which requires a large volume of a sample (5 ml of serum for testing). Also, ion-selective membranes of electrodes are not resistant to microflora action or specific sedimentation of proteins and there are other technical defects.

In view of the above, it has been decided to assess the usefulness of the AAS method for determination of calcium and magnesium forms in blood serum and magnesium. Therefore, the aim of this study was to determine levels of total and ionized forms of  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  in serum and saliva by the method of atomic absorption spectrometry, and to show the diagnostic importance of such determination for various pathological states of the human body.

## MATERIAL AND METHODS

This investigation included 39 persons, aged 21 to 47 years. The control group covered healthy volunteers (12 patients). Experimental groups consisted of patients of the Institute Clinic, who had been diagnosed to suffer from arterial hypertension (14 patients) and osteoporosis (13 patients).

Analyses of serum and saliva were performed using the standard method of selection for a given fluid (SIGNIFOLI et al. 1989, BAZARNOVA 1990, RADISHEVSKA 1998). The content of  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  forms in serum and saliva were determined by the flame atomic absorption spectrometry (FAAS). The total content of calcium and magnesium in the investigated substrates was determined by a generally accepted method for preparation of samples (SIGNIFOLI et al. 1989, TITOV 1995). The determination of non-albumin forms of metals was conducted after stabilization of proteins in serum and saliva with 5% isotonic glutaraldehyde solution (pH 7.4) (VOLKOVA 1987). Then, the proteins were mixed with 10% nitric acid solution and the samples were

cultured with 0.1 % solution lanthanum chloride (1:10 and 1:5, respectively) (ANDRUSISHINA 2007). The results were processed statistically, using the software program Statistica ver.6. The statistical importance of intergroup differences was estimated by the Student's-test (ANTOMONOV 2006).

## RESULTS AND DISCUSSION

Content of the total and ionized forms of  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  in serum and saliva of healthy persons (the control) is presented in Table 1.

Table 1

Content of the total and ionized forms of calcium and magnesium in serum and saliva of the control group

Biological material	Forms of chemical elements	Ranges of concentration of calcium ( $\text{mmol dm}^{-3}$ )	Ranges of concentration of magnesium ( $\text{mmol dm}^{-3}$ )
Blood serum	total	1.95-2.25	0.85-1.02
	ionized	0.96-1.20	0.43-0.55
Saliva	total	0.67-1.33	0.70-1.67
	ionized	0.48-0.62	0.45-0.71

The fluctuations in the total content of macroelements did not differ from the published data (PARFENOV 1977, BAZARNOVA 1990, KUDRIN, GROMOVA 2006). The non-significant differences between the behaviour of the total forms of electrolytes in blood serum and saliva were due to the active function of salivary glands and albumin structure (ZAVIALOVA 1998, KOWALCZUK et al. 2007, NACHAROV et al. 2007). Identical percentages of the ionized forms of metals in the investigated substrates are found (50% for ionized calcium and 70% for ionized magnesium), which is in agreement with the data published in literature (JARMAGOMEDOV et al. 1998, LOSKUTOVA 2004, KUDRIN, GROMOVA 2006, TYPIAKOVA 2006). That confirms the suitability of the technique, used for preparation of samples for the FAAS determinations of the ionized forms of electrolytes.

It is known that the content of elements in serum and saliva fluctuates, depending on the time of the day, the subject's age, pH value, functional activity of the vegetative nervous system, as well as on individual daily rhythm („owls” and „early birds”).

Therefore, determination of electrolytes in serum and saliva depending on the functional state of the body is important for recognition of their daily dynamics. The results are presented in Figures 1 and 2. The highest level

of  $\text{Ca}^{2+}$  in serum registered at 9.00 p.m. and in saliva – at 3.00 p.m. The lowest calcium level in serum was at 7.00 a.m. and in saliva at 11.00 p.m. At the same time, the maximum  $\text{Mg}^{2+}$  differed from  $\text{Ca}^{2+}$ , both in serum and in saliva. The highest level of  $\text{Mg}^{2+}$  in blood serum appeared at 6.00 p.m. and in saliva – at 9.00 p.m. The lowest content of that ion in serum and saliva was at 7.00 a.m. Thus, the daily changes of electrolytes, as revealed in this study, can be compared to the data reported by other authors (PARFENOV 1977, MELNICHENKO 2008), who found that release of electrolytes in the afternoon was higher than at night. This regularity is attributable to the activity of the sympathetic and parasympathetic vegetative nervous systems.

It is important that the release of  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  at 7:00 in the morning into biosubstrates is minimal, the fact that has to be remembered about during daily monitoring of electrolytes in human fluids. Nevertheless, the use of saliva for estimation of the body functional state has certain advantages over blood serum in that it is an adequate, noninvasive substratum.

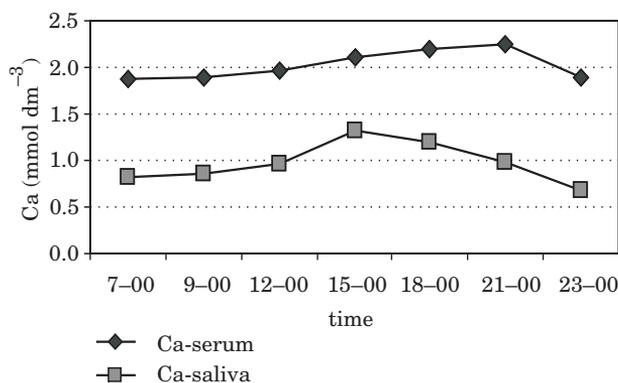


Fig. 1. Dynamics of daily fluctuations of the total calcium in human biosubstrates

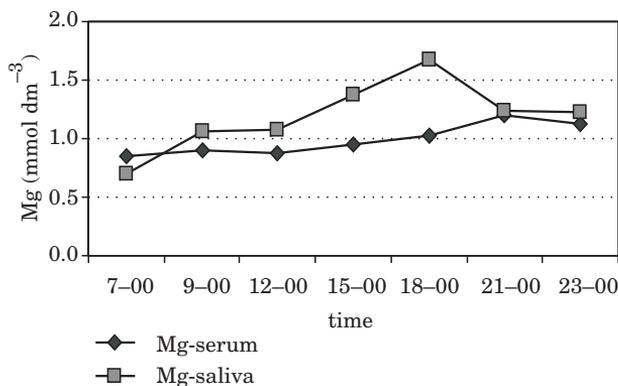


Fig. 2. Dynamics of daily fluctuations of the total magnesium in human biosubstrates

It was of interest to show the informative importance of the method of sample preparation for AAS determinations of ionized forms of  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ , described in this paper, for diagnosing some pathological states.

It has been demonstrated that high arterial pressure caused the decreased concentration of  $\text{Mg}^{2+}$  in cardiocytes and blood serum, whereas  $\text{Ca}^{2+}$  under such conditions either increases or remains unchanged (KISTERS et al. 2005, VERESCHAGIN et al. 2006). Normal levels of  $\text{Ca}^{2+}$  in serum are often maintained by Ca, liberated from the depot (bones), thus preventing its decrease in blood serum (BAZARNOVA 1990, ZABOLOTSKI 2007). The amount of  $\text{Mg}^{2+}$  in the heart makes up 1/5 of all magnesium in the human body. It is one of the active regulators of vascular tone; it tones the heart and causes vasodilatation. Researchers have demonstrated that 90 % of patients with myocardium heart attack have  $\text{Mg}^{2+}$  deficiency (TITOV 1995, HUNTER 2005). Therefore, it is important to determine levels of the total and ionized  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  in serum and saliva of patients with arterial hypertension (AH) by the AAS method. The results are presented in Table 2.

Table 2

Content of total and ionized forms of calcium and magnesium in serum and saliva of patients with arterial hypertension

Biological material	Treatment group	Forms of chemical elements	Calcium (mmol dm <sup>-3</sup> )	% of total content	Magnesium (mmol dm <sup>-3</sup> )	% of total content
Serum blood	control	total	1.95 ±0.07		1.02±0.02	
		ionized	0.98 ±0.11	50.26	0.77±0.07	75.49
	experiment	total	2.26±0.06*		0.74±0.02*	
		ionized	1.26±0.06*	55.75	0.50±0.02*	67.57
Saliwa	control	total	1.03 ±0.13		1.20± 0.17	
		ionized	0.51± 0.07	49.51	0.90 ±0.13	75
	experiment	total	1.30± 0.21*		0.60±0.02*	
		ionized	0.60± 0.06	46.15	0.34 ±0.13*	56.67

The growth of the total  $\text{Ca}^{2+}$  concentration in serum of patients with AH was revealed in 14.15% of cases. Thus, the ionized form of the metal did not change its level. The total  $\text{Mg}^{2+}$  in serum of patients with AH decreased by 31.49% as compared to the control group. The ionized  $\text{Mg}^{2+}$  level in serum increased by 14.51%. The imbalance between the total and ionized  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  in serum of patients with AH indicates the physiological antagonism between these macroelements. The increase in the ionized  $\text{Mg}^{2+}$  could be some evidence of genetic predisposition to this disease (KISTERS et al. 2005, KUDRIN, GROMOVA 2006, VERESCHAGIN et al. 2007).

At the same time, levels of the total and ionized forms of  $Mg^{2+}$  decreases by 47.86% and 37.93%, respectively. The difference between fluctuations in the forms  $Ca^{2+}$  and  $Mg^{2+}$  are the evidence that disorders of the vegetative nervous system can be accompanied by disturbances in the power exchange as well as by changes in the transmembrane ionic transport, mostly of magnesium (KISTERS et al. 2005, HUNTER 2005).

Osteoporosis is a disease which is characterised by severe destruction of organic and mineral parts of the bone tissue and by calcium loss. Osteoporosis is primary depends on the patient's age and depressed production of sex hormones. In the second, osteoporosis is caused by thyroid and parathyroid gland dysfunction (calcitonin and parathyroid hormones), xenobiotics and other factors (TYPIAKOVA 2006, NACHAROV et al. 2007). Thus, in practice determination of the total  $Ca^{2+}$  and  $Mg^{2+}$  is not always effective in clinical diagnostics of the disease (KISTERS et al. 2005, MELNICHENKO 2008). Therefore, it is often necessary to monitor ionized  $Ca^{2+}$  in serum, which makes the diagnosis more complicated. Taking into account that the ratios of  $Ca^{2+}$  and  $Mg^{2+}$  in serum and saliva are identical, determination of the ionized forms of these elements in saliva can be acceptable.

It was of interest to study the suitability of the sample preparation method, discussed in this paper, for the assessment of the behaviour of the total and ionized  $Ca^{2+}$  and  $Mg^{2+}$  in patients with osteoporosis. The results are presented in Table 3. The increased total  $Ca^{2+}$  in serum appeared in 24.10% of cases; the level of ionized  $Ca^{2+}$  did not change in comparison with the control, but its share decreased by 6.46 %. In saliva of patients with osteoporosis the level of the total  $Ca^{2+}$  increased by 41.75% in comparison with the control. Thus, the level of ionized  $Ca^{2+}$  did not change considerably, although its share fell by 7.78%. The behaviour of the total  $Mg^{2+}$  changed

Table 3

Content of the total and ionized forms of calcium and magnesium in serum and saliva of patients with osteoporosis

Biological material	Treatment group	Forms of chemical elements	Calcium (mmol dm <sup>-3</sup> )	% of total content	Magnesium (mmol dm <sup>-3</sup> )	% of total content
Blood serum	control	total	1.95±0.07		1.02±0.02	
		ionized	0.98±0.11	50.26	0.77±0.07	75.49
	experiment	total	2.42±0.06*		0.74±0.02*	
		ionized	1.06±0.06	43.80	0.60±0.02*	81.08
Saliva	control	total	1.03±0.13		1.20±0.17	
		ionized	0.51±0.07	49.51	0.90±0.13	75
	experiment	total	1.46±0.20*		1.31±0.02	
		ionized	0.61±0.06	41.78	0.75±0.02*	57.25

only in blood serum, where its level decreased by 27.45%, relative to the control. Levels of ionized  $Mg^{2+}$  in serum and saliva changed differently. In blood serum, the level of ionized  $Mg^{2+}$  decreased by 22.08 %, and in saliva it increased by 16.67%. Thus, the percentages of the metals in both environments also decreased by 5.59% (in serum) and increased by 17.75% (in saliva). The results evidence the surplus of the total form of calcium and shortage of its ionized form, which can intensify physiological action of calcitonins, causing destruction of bone tissues (ZAVIALOV, KAXRI 1998, KUDRIN, GROMOVA 2006, NACHAROV et al. 2007). Different changes in  $Mg^{2+}$  forms confirm the general deficiency of magnesium in patients with osteoporosis.

The results prove that the determined concentration of the total and ionized forms of  $Ca^{2+}$  and  $Mg^{2+}$  in the analyzed biological environments are in accordance with the "conditioned norm," in literature (KONOVALOVA 2002, TRAKHTENBERG 2006). The method of preparation of blood serum and saliva samples for AAS determination of ionized  $Ca^{2+}$  and  $Mg^{2+}$  can be used as an alternative to the existing methods, used for diagnosing some diseases which are characterized by changes electrolytes, found in blood serum and saliva. Also, determination of  $Ca^{2+}$  and  $Mg^{2+}$  in saliva has certain advantages in clinical and epidemiological practice when it is necessary to sample a high volume of biomaterial and to monitor patient's health.

## CONCLUSIONS

1. The deviations from the optimum level of the macroelement forms in serum and saliva in the control group testify that the method for sample preparation for the AAS determination of calcium and magnesium fractions gives results, which agree with the relevant literature data.

2. The differences in the concentrations of  $Ca^{2+}$  and  $Mg^{2+}$  fractions in serum of patients with arterial hypertension and osteoporosis, found in this study, confirm their diagnostic importance for evaluation of these diseases.

3. Determination of calcium and magnesium fractions in saliva gives certain advantages in clinical and epidemiological practice when it is necessary to obtain a higher volume of biomaterial and to carry out a long-term monitoring of the health status of patients.

## REFERENCES

- ANDRUSISHINA I.M. 2007. *Method for determination of ionized form of calcium in serum*. Inf. Letter , 047-07, Kyiv.
- ANTOMONOV M.J. 2006. *Mathematical processing and the analysis of medical and biological data*. Kyiv.
- BAZARNOVA M. A. 1990. *Management of clinical laboratory diagnostics*. P.3. Clinical Biochemistry. Vicsha shkola, Moscow.

- JARMAGOMEDOV A.A., MATEROVA E.A., KOLOSOV I.V. 1978. *Express method for determination of ionized calcium in serum and blood*. Lab. Delo, 9: 552-556.
- KONOVALOVA S.O. 2002. *Comparative analyses of information derived from studying various biosubstrates for monitoring mineral exchange*. Ukr. Biochem. J., 4 (4): 145-146.
- KOWALCZUK K., STRYJECKA-ZIMMER M., BACHANEK T., NAKONIECZNA-RUNICKA M. 2007. *The level of magnesium and calcium in saliva of patients with dental caries*. Pol. J. Environ. Stud., 16 (3): 147-150.
- KUDRIN A.V., GROMOVA O.A. 2006. *Microelements in neurology*. GEOTAR-media, Moscow.
- MAKARA-STUDZINSKA M., MOROLOWSKA J., BUCZYJAN A. 2008. *The importance of magnesium in the pathogenesis and treatment of depression – literature review*. Pol. J. Environ. Stud., 17 (1): 98-100.
- NACHAROV J.V., MELNIKOV V.A., MELNIKOV V.I. 2007. *Dynamics of parameters of the immunoflammatory process in the oral cavity of women on the background of dental implantations*. Stomatology, 7: 551-557.
- PARFENOV M.F. 1977 *The man. Medical and biological data*. The report of a working group of the II ICWG committee on condition of humans. Medicine, Moscow.
- PRAIS. V. 1976. *Analytical atomic absorption spectrometry*. Peac., Moscow.
- SIGNIFOLI G.P., GORDONI C., BONORLI O. 1989 *Comprehensive determination of trace elements in human saliva by ETA-AAS*. Microchim. Acta, 1: 171-179.
- TITOV V.N. 1995. *Diagnostic value of determinations of magnesium in serum*. Clin. Lab. Diagn., 2: 3-7.
- VERESCHAGIN G.S., KASATOVA T.B., MALYSHEVA N.V., POSTNIKOVA S.L. 2006. *Magnesium and cardiovascular diseases*. Reanimatology, 2: 36-42.
- VOLKOVA O.V. 1987. *The atlas of scanning microscopy*. Medicine, Moscow.
- WIELEBA E., PASTERNAK K. 2001 *Changes in serum and erythrocytic magnesium concentrations in sportsmen after physical exercise*. Biul. Magnezol., 3(6): 396-404.
- ZAVIALOVA G.V., KAXRI .S.H. 1998. *Changes in electrolytes in saliva structure as an indicator of the influence of ecological factors on organisms of schoolboys*. Povolzh. Ekol. Vestn., 5: 319-322.

