Polish J. Chem., **78**, 1619–1625 (2004)

The Application of Magnesium Ion Selective Electrode in Clinical Analysis*

by A. Malon[1], Ch. Brockmann[2], J. Fijalkowska-Morawska[3], P. Rob[4] and M. Maj-Zurawska[1]**

[1]*Warsaw University, Faculty of Chemistry, Pasteura 1, 02093 Warsaw, Poland*
[2]*Medical University Luebeck, Department of Anesthesiology, 23538 Luebeck, Germany*
[3]*Medical Academy, Department of Nephrology, 90153 Lodz, Poland*
[4]*Medical University Luebeck, Department I of Internal Medicine, 23538 Luebeck, Germany*

(Received March 1st, 2004; revised manuscript April 5th, 2004)

The role of magnesium is primarily that of a cofactor in intracellular biochemical reactions, therefore, the concentration of intracellular ionized magnesium is much more physiologically relevant. The determination of the ionized magnesium (iMg_e) in erythrocytes by ion-selective electrode for routine clinical measurements was first time investigated. Intracellular and extracellular magnesium concentration in critically ill postoperative patients and in dialyzed patients was compared with healthy individuals. Of the investigated parameters, iMg_e seems to be the best magnesium parameter to observe hypo- or hypermagnesemia for both groups of patients. The correlation that was found between extracellular and intracellular magnesium concentrations can be also used to evaluate the magnesium status.

Key words: magnesium ion selective electrode, erythrocyte

Magnesium is one of the most fundamental ions in the human body with a well-documented physiological and clinical role [1,2]. In modern clinical laboratories magnesium is measured mainly as total substance concentration. Although less than 1% of the total body magnesium is present in blood, the determination of this parameter is mainly done for blood serum or plasma in routine clinical analysis. Several methods for the measurement of total magnesium content in serum (tMg_s) have been described. To obtain more reliable information about the functional magnesium status of a patient, a method that measures the biologically active Mg fraction was necessary. A relevant method for measurement of ionized magnesium in serum (iMg_s) was developed in the beginning of the nineties and is now gaining the status of a routine method in clinical analysis [3–18].

Because the role of magnesium is primarily that of a cofactor in intracellular biochemical reactions, and almost 99% of the total body magnesium can be found intracellularly, the benefit of the magnesium measurement in blood serum alone has been questioned. Therefore, several methods of the measurement of total cellular

* Dedicated to Prof. Dr. Z. Galus on the occasion of his 70th birthday.

** Corresponding author; Fax: +48228225996, phone: +48228220211 ext.334, email: mmajzur@chem.uw.edu.pl

magnesium, also in erythrocytes (tMg_e), have been described. However, the concentration of intracellular ionized magnesium is much more physiologically relevant and therefore of special interest. The knowledge of a relation between the ionized and the total magnesium concentration in serum and erythrocytes together with the knowledge of exchange of erythrocyte-serum gives a fuller picture of biochemical transformations occurring in the organism.

Ionized magnesium in erythrocytes (iMg_e) is a new parameter that can help to better establish more reliable information on the functional magnesium status. Nowadays ionized magnesium concentration in erythrocytes is determined by comparatively expensive methods requiring specialized staff. These methods are the ^{31}P nuclear magnetic resonance spectroscopy (^{31}P NMR), the zero-point titration using the atomic absorption spectrometry (AAS), the fluorimetry using video-microscope or ion-selective microelectrode for intracellular measurements. That is why accurate, reproducible and also cheap methods for the measurement of cytosolic free magnesium ion concentration are required in order to assess its physiologic role. Recently a simple, rapid and accurate method of determining ionized magnesium in erythrocytes using a potentiometric clinical analyzer Microlyte 6 (Kone, Finland) for routine clinical use was investigated [19,20]. The present paper reports results of application this new method for clinical measurements.

EXPERIMENTAL

Freshly drawn heparinized blood (6 U.I. of sodium heparin pro 1 mL of blood) was centrifuged (Centrifuge type MPW–340, Poland, G = 480 g, 20 min, 4000 rev./min). The serum was separated from the erythrocytes and the coating of lymphocytes was removed. Ionized magnesium concentration in serum was measured in fresh samples by a magnesium ion-selective electrode, which was placed in an automatic potentiometric clinical analyzer Microlyte 6 (KONE Instruments, Espoo, Finland) [3,6–8]. Total magnesium content in serum was measured by flame atomic absorption spectroscopy (AAnalyst 300, Perkin Elmer, Ueberlingen, Germany).

Isolated erythrocytes were washed three times with 0.16 mol/L NaCl (1+1 by volume). The sample was centrifuged after every washing and the washing solution was removed (Centrifuge type MPW–340, Poland, G = 480 g, 20 min, 4000 rev./min). The distilled water and TRIS/TES buffer of pH 7.2 (1+1+1, sample + water + buffer) were added to the erythrocytes. Then the sample was lysed in ultrasonic bath (20 min) (ultrasonic cleaner "Polsonic" sonic-0.5, Poland). Ionized magnesium concentration in erythrocytes was measured in the samples by a magnesium ion-selective electrode, which was placed in an automatic potentiometric clinical analyzer Microlyte 6 (KONE Instruments, Espoo, Finland) [19,20]. Total magnesium content in erythrocytes was measured by atomic absorption spectrometry (AAnalyst 300, Perkin Elmer, Ueberlingen, Germany); erythrocytes after lysis and centrifugation of cell membranes were diluted 100 times with deionized water.

All reagents were of analytical grade. Doubly distilled and deionized water (resistance 18.2 MΩcm, Milli-Qplus, Millipore, Austria) was used.

Experimental potentiometric data were corrected for changes in ionic strength and the liquid junction potential using the Debye-Hückel and Henderson formalism [6,7], respectively. All potentiometric measurements were made at 37°C with a Microlyte clinical analyzer (Kone, Finland) connected online to a computer.

The fraction of iMg_s ($friMg_s$) and iMg_e ($friMg_e$) were calculated as $iMg_s/tMg_s \times 100\%$ and $iMg_e/tMg_e \times 100\%$, respectively.

Reference ranges for all parameters were defined as mean ± 2 SD. Comparison of magnesium values measured in erythrocytes in dialyzed patients and in critically ill postoperative patients with the reference values was done using the Student *t* test. Correlations between the magnesium parameters and hematocrit were calculated with the use of the Kendall rank correlation.

RESULTS AND DISCUSSION

Critically ill postoperative patients. Hypomagnesemia is very common in critically ill postoperative patients. Compared to 11% of hospitalized patients [21,22], 65–70% of critically ill postoperative adults [23–25] and 30% children [26] have lower level of magnesium. Accordingly, the present part of this study was undertaken to investigate the relation between the levels of iMg_s, tMg_s and iMg_e of critically ill patients. We compared intracellular and extracellular magnesium concentration in critically ill postoperative patients with healthy subjects and examined whether or not there is a significant correlation between magnesium parameters and level of hematocrit.

In Table 1, an overview of the mean intracellular and extracellular magnesium parameters and level of hematocrit measured in patients and representatives of the healthy group are given. The mean of hematocrit concentration of critically ill postoperative patients was statistically significantly decreased, whereas the mean of iMgs, tMgs and iMge concentration did not differ (Student *t* test, $\alpha = 0.01$).

Table 1. Comparison of blood parameters of two groups of patients and healthy population.

	Critically ill postoperative patients n = 69	Dialyzed patients n = 15	Healthy subjects n = 70
tMg_s [mmol/L] (SD)	0.97 (0.35)	–	0.90 (0.20)
iMg_s [mmol/L] (SD)	0.57 (0.17)	0.51 (0.09)	0.58 (0.13)
tMg_e [mmol/L] (SD)	–	2.29 (0.37)	2.42 (0.34)
iMg_e [mmol/L] (SD)	0.72 (0.19)	1.26 (0.10)	0.73 (0.15)
Hematocrit [%] (SD)	31.0 (4.7)	31.6 (5.2)	40.0 (5.0)

The significant correlation between the two serum markers, iMg_s and tMg_s (Kendall rank correlation coefficient $\tau = 0.611$; $P < 0.001$), and between iMg_e and $friMg_s$ ($\tau = 0.235$, $P < 0.001$) for the 69 patients was made. We also studied the relationship between both serum and erythrocytes magnesium concentration and hematocrit content. We found a significant correlation between tMg_s and hematocrit ($\tau = 0.225$, $P < 0.01$).

The frequencies of hypomagnesemia calculated for the three measured magnesium parameters were also checked. Based on tMg_s measurements, 15.87% of the patients admitted to the intensive care unit are hypomagnesemic. A decreased iMg_s and iMg_e was measured in 22.22% and 36.51% of the patients, respectively.

The prevalence of hypomagnesemia based on iMg_s (0.45 mmol/L) was 22.22%, a value that differs from the frequency reported in other studies on iMg_s in critically ill patients: 4.30% of the 91 critically ill postoperative patients with hypoalbuminemia [17] or 15.00% of the 34 critically ill postoperative patients [27] were found with a level of iMg_s of less than 0.44 mmol/l. Nevertheless, it is still a very small percentage of the population to be treated as a marker of hypomagnesemia.

The measurement of tMg_s also resulted in a very low number of hypomagnesemic patients (15.87%). The results reported in previous studies varied from 9.40% in critically ill patients with chronic obstructive pulmonary disease [28] or 11.00% in general hospital inpatients [21], through 30.00% in neonatal intensive care patients [26] or 32.60% in critically ill postoperative patients with hypoalbuminemia [17] to 61.00% [29] in critically ill postoperative patients or 65.00% in adult intensive care patients [23], depending on the population studied and whose tMg_s threshold value was chosen. The reasons for these rates of magnesium deficiency are multifactor and include: decreased absorption caused by impaired gastrointestinal activity; malnutrition; renal wasting of various drugs; diabetes mellitus; hypokalaemia; and hypocalcaemia [1,30].

The major finding of this study is that the frequency of hypomagnesemia as measured by intracellular ionized levels is over 36% in critically ill patients. This is in contrast to the 16% found when only total serum magnesium levels were measured. The new magnesium marker that should be a rate of hypomagnesemia iMg_e looks promising. This new parameter used to routine, clinical measurements can help to better establish more reliable information on the functional magnesium status and that knowledge gives a fuller picture of the magnesium status of a patient.

Dialyzed patients. Because the kidneys play a major role in magnesium homeostasis, chronic dialysis patients are often hypermagnesemic [31], the levels of iMg_s, iMg_e and tMg_e of dialyzed patients were investigated. In the present study the levels of hematocrit, iMg_s, iMg_e, tMg_e were compared with the values measured in blood from healthy subjects. Moreover, we examined whether or not there is a significant correlation between magnesium parameters and level of hematocrit.

Table 1 presents the mean of intracellular and extracellular magnesium markers and level of hematocrit in 15 dialyzed patients. When comparing the dialyzed group with the healthy one, the mean of hematocrit concentration in patients decreased significantly, while there was no significant difference in iMg_s or tMg_e concentration (Student t test, $\alpha = 0.01$). Nevertheless, iMg_e was significantly higher in the dialyzed patients, namely, 1.7 times the mean value for the healthy group (Student t test, $\alpha = 0.01$).

There was a negative correlation between iMg_s and tMg_e ($\tau = -0.485$, $P < 0.05$), but also between $friMg_e$ and tMg_e ($\tau = -0.727$, $P < 0.001$). No correlation was found between iMg_s and $friMg_e$, iMg_e and $friMg_e$, iMg_e and tMg_e or iMg_e and iMg_s.

Hypo- (< 1.66 mmol/L) or hypermagnesemia (> 3.06 mmol/L) based on tMg_e was not observed in this dialyzed group. However, 5 of the 15 patients (33.30%) had a low level of ionized magnesium in serum and in all the patients there was increased ionized magnesium in erythrocytes (statistically significant, Student *t* test, $\alpha = 0.01$).

Our values for ionized magnesium in erythrocytes were similar to the results obtained by Markell and co-workers [32]. However, in another study [31] it was shown that iMg_s and tMg_e concentration in the hemodialysis patients were greater than in the healthy volunteers. Huijgen *et al.* [31] explained that in uremic patients erythrocytes have a decreased life span, which results in increased erythropoiesis. Because young erythrocytes contain much more magnesium than older cells, hemodialysis patients can be expected to have an increased tMg_e [33–35]. Moreover, the increased serum magnesium concentration during erythropoiesis can enhance this effect. In their work, tMg_e correlated with iMg_s and tMg_s. Similar results are obtained in our work for iMg_s and tMg_e. However, in our study these magnesium parameters are in the same range as the magnesium values measured in the healthy population. On the other hand, an increased rate of Na/Mg antiport in hemodialysis patients, which leads only to a magnesium efflux out of erythrocytes, partially compensates for the intracellular increase [36]. In their opinion, all these influences combine to make erythrocytes magnesium an unsuitable measure of magnesium overload in hemodialysis patients [31]. In our work, we observe that only iMg_e concentration significantly increased, in opposite to tMg_e that was at the same level in comparison with the healthy persons. Because the total magnesium concentration in erythrocytes is much higher in reticulocytes than in ordinary red cells [35], it is possible that reticulocytes, during the process of entering the blood circulation, when they lost intracellular organelles and ability of protein synthesis, were holding much more ionized magnesium than ordinary erythrocytes. This might result in an increase in the population of younger erythrocytes with higher ionized magnesium concentration and thus explains our results.

CONCLUSIONS

In spite of earlier studies, which suggested that hypomagnesemia estimated on the level of tMg_s or iMg_s was common in these specific patients, our data showed hypomagnesemia only in a small part of the population. However, the prevalence of hypomagnesemia based on iMg_e was almost twice as high as that on tMg_s or iMg_s. This parameter, used in routine, clinical measurements can give a fuller picture of the magnesium status of a patient. Because the function of magnesium is mainly intracellular the correlation that was found between extracellular and intracellular magnesium concentration in critically ill postoperative patients can be also used to evaluate

the magnesium status. Moreover, a statistically significant increase iMg_e in dialyzed patients by the simultaneous normal levels of other magnesium parameters once more emphasized that the knowledge of concentration of ionized intracellular magnesium (the biologically active magnesium fraction) can help to better establish more reliable information about the functional magnesium status.

Acknowledgments

The authors thank the Polish State Committee for Scientific Research (KBN) for supporting part of this research (Grant 3 P05B 096 24).

REFERENCES

1. Fawcett W.J., Haxby E.J. and Male D.A., *Br. J. Anaesth.,* **83**, 302 (1999).
2. Saris N-L.L., Mervaala E., Karppanen H., Khawaja J.A. and Lewenstam A., *Clin. Chem. Acta,* **294**, 1 (2000).
3. Maj-Zurawska M. and Lewenstam A., *Anal. Chim. Acta,* **36**, 331 (1990).
4. Altura B.T. and Altura B.M., *Magnes. Trace Elem.,* **10**, 90 (1991–92).
5. Altura B.T., Shirey T.L., Young C.C., Hiti J., Dell'Orfano K., Handwerker S.M. and Altura B.M., *Methods Find Exp. Clin. Pharmacol.,* **14**, 297 (1992).
6. Lewenstam A., Maj-Zurawska M., Blomqvist N. and Öst J., *Clin. Chem. Enzym. Comms.,* **5**, 95 (1993).
7. Maj-Zurawska M., Hulanicki A., Drygieniec D., Pertkiewicz M., Krokowski M., Zebrowski A. and Lewenstam A., *Electroanalysis,* **5**, 713 (1993).
8. Maj-Zurawska M., *Scand. J. Clin. Lab. Invest.,* **54/217**, 69 (1994).
9. Altura B.T., Shirey T.L., Young C.C., Dell'Orfano K., Hiti J., Welsh R., Yeh Q., Barbour R.L. and Altura B.M., *Scand. J. Clin. Lab. Invest.,* **54/217**, 21 (1994).
10. Marsoner H.J., Spichiger U.E., Ritter C., Ghahramani M., Offenbacher H., Kroneis H., Kindermans C. and Dechaux M., *Scand. J. Clin. Lab. Invest.,* **54/217**, 45 (1994).
11. van Ingen H.E., Huijgen H.J., Kok W.T. and Sanders T.B., *Clin. Chem.,* **40**, 52 (1994).
12. Altura B.T., Bertschat F., Jeremias A., Ising H. and Altura B.M., *Scand. J. Clin. Lab. Invest.,* **54/217**, 77 (1994).
13. Hristova E.N., Cecco S., Niemela J.L., Rehak N.N. and Elin R.J., *Clin. Chem.,* **41**, 1649 (1995).
14. Ising H., Bertschat F., Günther T., Jeremias E. and Jeremias A., *Eur. J. Clin. Chem. Biochem.,* **33**, 365 (1995).
15. Huijgen H.J., Sanders R., Cecco S., Rehak N.N., Sanders G.T.B. and Elin R.J., *Clin. Chem. Lab. Med.,* **37**, 465 (1999).
16. Dewitte K., Stöckl D. and Thienpont L.M.R., Advances in magnesium research: nutrition and health, Ed. Y. Rayssiguier, A. Mazur, J. Durlach, John Libbey & Company Ltd, 2001, p. 241.
17. Brockmann C., Meier T., Maj-Zurawska M., Schmucker P. and Rob P., Advances in magnesium research: nutrition and health, Ed. Y. Rayssiguier, A. Mazur, J. Durlach, John Libbey & Company Ltd, 2001, p. 263.
18. Brockmann C., Meier T., Maj-Zurawska M., Schmucker P. and Rob P., *Intens. Care Med.,* **27**, 469 (2001).
19. Malon A. and Maj-Zurawska M., *Anal. Chim. Acta,* **448**, 251 (2001).
20. Malon A., Wagner B., Bulska E. and Maj-Zurawska M., *Anal. Biochem.,* **302**, 220 (2002).
21. Woods K., *Br. J. Clin. Pharmacol*., **32**, 3 (1991).
22. Wong E.T., Rude R.K., Singer F.R. and Shaw S.T., *Am. J. Clin. Pathol.,* **79**, 348 (1983).
23. Ryzen E., Wagers P.W., Singer F.R. and Rude R.K., *Crit. Care Med.,* **13**, 19 (1985).
24. Reinhart R.A. and Desbiens N.A., *Crit. Care Med.,* **13**, 506 (1985).
25. Huijgen H.J., Soesan M., Sanders R., Mairuhu W.M., Kesecioglu J. and Sanders G.T.B., *Am. J. Clin. Pathol*., **114**, 688 (2000).

26. Munoz R., Khilnani P., Ziegler J., Salem M., Catlin E.A. and Nussbaum S., *Crit. Care Med.,* **22**, 815 (1994).
27. Hebert P., Mehta N., Wang J., Hindmarsh T., Jones G. and Cardinal P., *Crit. Care Med.,* **25**, 749 (1997).
28. Fiaccadori E., Del Canale S., Coffrini E., Melej R., Vitali P. and Guariglia A., *Crit. Care Med.,* **16**, 751 (1988).
29. Chernow B., Bamberger S., Stoiko M., Vadnais M., Mills S. and Hoellerich V., *Chest,* **95**, 391 (1989).
30. McLean R.M., *Am. J. Med.,* **95**, 63 (1994).
31. Huijgen H.J., Sanders R., van Olden R.W., Klous M.G., Gaffar F.R. and Sanders G.T.B., *Clin. Chem.,* **4**, 639 (1998).
32. Markell M.S., Altura B.T., Sarn Y., Delano B.G., Ifudu O., Friedman E.A. and Altura B.M., *ASAIO Journal,* **39**, 801 (1993).
33. Walser M., *Rev. Physiol. Biochem. Exp. Pharmacol.,* **59**, 185 (1967).
34. Watson W.S., Lyon T.D.B. and Hilditth T.E., *Metabolism,* **29**, 397 (1980).
35. Elin R.J., Utter A., Tan H.K. and Corash L., *Am. J. Pathol.,* **100**, 765 (1980).
36. Vormann J., Günther T., Perras B. and Rob P.M., *Eur. J. Clin. Chem. Clin. Biochem.,* **32**, 901 (1994).