Clinical Biochemistry 133–134 (2024) 110815

Contents lists available at ScienceDirect

Clinical Biochemistry

journal homepage: www.elsevier.com/locate/clinbiochem

Short Communication

In vivo and in vitro relationship between ionized magnesium and ionized calcium

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ARTICLE INFO

Keywords:
Ionized magnesium
Ionized calcium
Calcium
pH
Albumin
Interference
Ion selective electrode

ABSTRACT

Objectives: The objective of this study was to determine the in vivo correlation of ionized magnesium (iMg) with ionized calcium (iCa), total calcium, albumin and pH. In addition, the analytical interference of iCa on iMg measurement on the Stat Profile Prime Plus (Nova Biomedical) and vice versa was defined.
Methods: In vivo correlation of iCa, iMg and pH was studied in 238 paired blood gas samples of 109 different patients admitted to the intensive care unit. Albumin and total magnesium (tMg) were measured in heparinized plasma samples. Measurement of iMg was performed with the ion selective magnesium electrode (ISE) of the Stat Profile Prime Plus (Nova Biomedical) and iCa and pH were measured with a Rapid Point 500 blood gas analyzer (Siemens). Albumin, total calcium and total magnesium were analyzed with a Siemens Atellica CH. Analytical interference of iCa with iMg and vice versa was investigated using unbuffered saline solutions.
Results: In the studied patient population, no significant correlations were observed between iMg and iCa, albumin, and pH. An inverse relationship was observed between iCa and Mg-ISE. For every 0.1 mmol/L change in iCa concentration, the iMg concentration deviated by 0.01 mmol/L at an iMg concentration of 0.5 mmol/L and by 0.013 mmol/L at an iMg concentration of 1.0 mmol/L. The measurement of iCa was not affected by iMg.
Conclusions: In vivo, no correlation was observed between iMg with iCa, albumin and pH. Interference of iCa on iMg measurement was noted, with a maximum deviation of ±0.02 mmol/L iMg across the reference range of iCa (1.15–1.32 mmol/L). Additionally, the iCa measurement was not affected by the iMg concentration.

1. Introduction

Magnesium (Mg) is an abundant cation and a crucial cofactor in several biological processes. Dysregulation of Mg is primarily linked to neuromuscular and cardiovascular dysfunctions and can lead to severe symptoms such as seizures and coma. In the blood, approximately 70 % of magnesium exists in the physiologically active ionized (iMg) form, while the remaining 30 % is mainly bound to albumin and plasma anions [1]. Plasma Mg concentration is tightly regulated within a narrow range (0,49–0,71 mmol/L) [2]. Plasma magnesium can be measured as total magnesium (tMg), which is currently the standard of clinical care, or in its ionized form. The concentration of iMg represents physiological functions and is thought to be affected by ionized calcium (iCa), albumin, pH, and medications. Therefore, it is hypothesized that iMg should be measured to detect patients with pathological magnesium levels [3,4]. Recently, the benefit of measuring iMg over tMg in patients receiving continuous renal replacement therapy with citrate as an anticoagulant was demonstrated [2]. iMg, and not tMg, was identified as a discriminating marker of hypomagnesemia in this patient population. To accurately measure iMg, the International Federation of Clinical Chemistry "guideline for sampling, measuring and reporting iMg in plasma" made several recommendations [5]. It is well established that iCa interferes with Mg ion-selective electrodes (ISE) and that the magnesium binding in plasma is pH-dependent. Therefore, the guideline recommends simultaneous measurement of iCa, pH, and iMg to correct iMg results for iCa and pH interference. Of note, experiments demonstrating these relationships were conducted in vitro without in vivo homeostatic mechanisms that closely regulate iMg concentrations [6,7].

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https://doi.org/10.1016/j.clinbiochem.2024.110815
Received 28 May 2024; Received in revised form 29 August 2024; Accepted 30 August 2024
Available online 4 September 2024

Thus, we aimed to study the in vivo relationships between iMg, iCa, albumin, and pH, as well as the interference of iCa on the magnesium-ISE and vice versa.

2. Materials and methods

iMg concentrations were analyzed on a Stat Profile Prime^{+}® Analyzer (Nova Biomedical, Waltham, USA) which was verified, including establishing the reference range, as previously described [2]. The analyzer automatically corrects the result of iMg concentration for iCa interference according to the known selectivity of iMg over iCa. The correction used by the manufacturer (Nova Biomedical) was not disclosed. In addition, the Stat Profile Prime^{+}® Analyzer does not correct iMg for pH. iCa and pH were measured on a Siemens RAPIDPoint 500 blood gas analyzer (Siemens Healthcare Diagnostics, Tarrytown, NY). Interference of iCa on iMg measurement and vice versa was investigated by adding increasing concentrations of calcium chloride ($CaCl_2$, Merck, Darmstadt, Germany) or magnesium chloride ($MgCl_2$, Merck, Darmstadt, Germany) to 0.9 % NaCl with fixed concentrations of iMg or iCa, respectively. All samples where measured in duplicate.

iMg, pH and iCa were measured in 238 arterial blood gas samples from 109 different adult intensive care unit patients and collected in balanced heparin syringes. All analyses were conducted within one hour after blood collection. At the same time point albumin and total magnesium were analyzed in lithium heparin plasma (gel vacutainer®; BD, Franklin Lakes, USA) with an Atellica CH930 Analyzer (Siemens, Erlangen, Germany) according to manufacturer's instructions. Correlation of albumin, iMg and iCa was studied in 136 paired samples from 74 different patients. Albumin and tMg were measured in 107 paired samples from 63 different patients.

Results were assessed using Passing–Bablok regression analysis. Data were analyzed with EP Evaluator (Data innovations LLC, V11.3.023), Graph Pad Prism (GraphPad Software, LLC, V8.3.0) and SPSS (IBM Statistics, V25). Samples and data were obtained in accordance with the Declaration of Helsinki and institutional regulations (METC 19-020/C and METC 07-125).

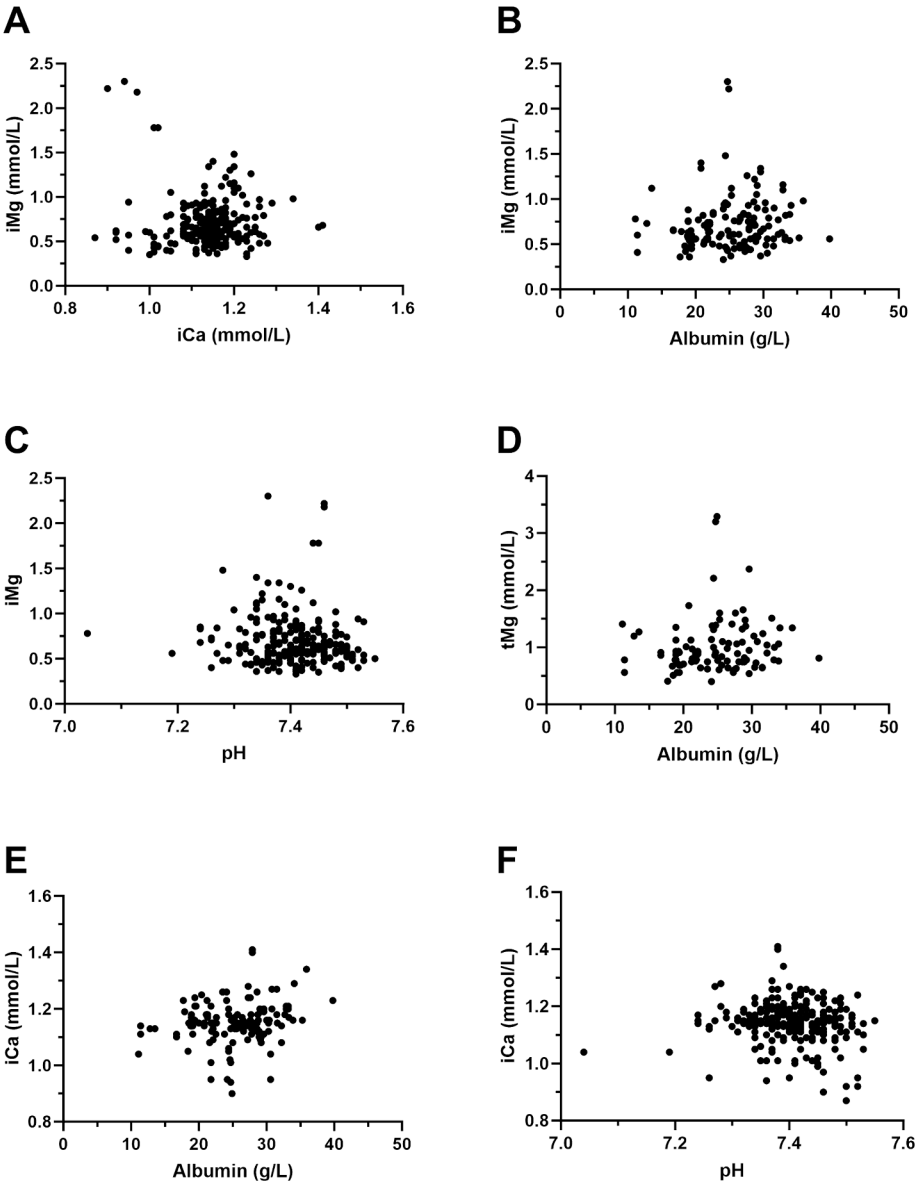

Fig. 1. In vivo correlation of ionized magnesium (iMg) with ionized calcium (iCa), albumin and pH. In vivo correlation of iMg concentration with iCa (n = 238, A), albumin (n = 136, B), pH (n = 238, C), tMg with albumin (n = 107, D) and iCa with albumin (n = 136, E), pH (n = 238, F).

3. Results

In vivo, no significant correlation was observed between iMg and iCa concentrations (Fig. 1A), with a Pearson coefficient of −0.095 and R^2 of 0.0090. Furthermore, no correlation was observed between iMg and albumin concentration (Pearson coefficient = 0.092 and R^2 = 0,0085) (Fig. 1B) or pH (Pearson coefficient = −0.1033 and R^2 = 0,0107) (Fig. 1C). No correlation between tMg and albumin was observed (Pearson coefficient 0,0883 and R^2 = 0,0078 (Fig. 1D). Moreover, no significant correlation was observed between iCa and albumin (Pearson's coefficient 0.0407 and R^2 = 0.0309) or pH (Pearson's coefficient 0.0735 and R^2 = 0.0135) (Fig. 1E and F), respectively. The correlation between iMg and tMg in this cohort has been previously published [2].

The interference of the iCa and iMg ISE by iMg and iCa was studied by increasing the concentrations of the interfering ions in aqueous solutions with a fixed concentration of the other cation.

At iMg concentrations of 0.5 and 1.0 mmol/L a significant effect on iMg measurement was demonstrated with varying iCa concentrations, ranging from 0.5 to 2.1 mmol/L. As shown in Fig. 2A, upon increasing the iCa concentration, there was an inverse correlation with the measured iMg concentration. At an iMg concentration of 0.5 mmol/L, the iCa value at the intersection of the regression curve was 0.84 mmol/L. For an iMg concentration of 1.0 mmol/L the iCa value at the point of intersection was 1.46 mmol/L. This shows that iMg at the studied concentrations was overestimated below and underestimated above the iCa cut-off points. Each 0.1 mmol/L change in iCa concentration caused a deviation of 0.01 mmol/L in iMg concentration at the tested iMg concentration of 0.5 mmol/L and of 0.013 mmol/L at the tested iMg concentration of 1.0 mmol/L. Based on the regression line, a maximum deviation of ±0.02 mmol/L iMg can be expected spanning the reference range of iCa (1.15–1.32 mmol/L). Fig. 2B shows that iCa measurements were unaffected at levels of 0.8, 1.2 and 1.6 mmol/L by iMg concentrations ranging from 0.2-1.3 mmol/L.

4. Discussion

In this study we demonstrated that no significant correlation was observed between iMg with iCa, albumin and pH. Additionally, we showed that iCa interferes with iMg measurement and a maximum deviation of merely ±0.02 mmol/L iMg could be expected spanning the reference range of iCa (1.15–1.32 mmol/L). Finally, iCa was not affected by the iMg concentration.

We did not find a significant correlation between albumin and iMg levels in vivo, as previously demonstrated [8]. Experiments on the effect of albumin on iMg merely studied the competition between iCa and iMg for binding albumin in vitro, in the same way that iCa interference on iMg was studied by spiking whole blood samples with $CaCl_2$.

Similarly, it has been demonstrated that albumin and pH inversely and proportionally influence the iMg concentration in vitro [6,7]. These studies showed that for Nova Prima Plus, iMg decreased by 0.005 mmol/L per 1 g/L albumin and 0.002 mmol/L per 0.1 pH unit. This effect of pH on iMg concentration was not observed by Hutten et al. [2], who measured iMg in whole blood samples stored for up to 8 h at room temperature, during which the average pH gradually decreased by 0.15. It is possible that the effect of pH on the iMg concentration was too small to be detected in the studied samples. Theoretically, changes in pH could alter the competition between protons and iMg, mainly for albumin-binding sites, increasing or decreasing the concentration of iMg with decreasing or increasing pH, respectively. However, in vitro studies have shown that the effect of the studied pH range on the iMg concentration for NOVA Prime Plus is not statistically significant and can be considered clinically negligible. This suggests that simultaneous pH measurements, as recommended to correct iMg for pH-interference, might not be necessary [5]. In addition, no effect of pH was demonstrated by Elin et al. on iMg measurements using an AVL system (Roche Diagnostics, Roswell, GA, USA) [9]. Moreover, the present study demonstrated no correlation between the pH and iMg concentration in vivo. However, in this study, no in vitro experiments have been performed to determine the effect of albumin on iMg measurements. In theory, changes in albumin and pH in vivo are counteracted by homeostatic mechanisms that maintain normal plasma iMg levels, thereby not influencing the correct measurement of iMg and thus reflecting the true iMg concentration.

It is well-established that iCa interferes with Mg ISEs, necessitating the simultaneous measurement of iCa and iMg to correct the iMg result for iCa interference. In contrast, the selectivity of calcium ISE is well known as been demonstrated previously [10]. For example, Hutten et al. [2] demonstrated a positive linear relationship between iMg and iCa in whole-blood samples spiked with $CaCl_2$. The positive linear relationship was most likely the result of the competitive effect of iCa and iMg on protein (predominantly albumin) binding sites. Thus, increasing the whole blood iCa concentration in vitro resulted in an increased iMg concentration. However, this experiment did not study the interference of iCa on the magnesium ISE but rather the competition between iCa and iMg for plasma proteins. This is supported by the present study, which showed the opposite result for the same analyzer, with an inversely proportional relationship between iMg and iCa in aqueous solutions. Rehak et al. [10] demonstrated in a similar experiment using NOVA CRT, a positive proportional relationship between the concentrations of iMg and iCa. This indicates that the formula that corrects the iMg measurement for iCa interference has been adjusted and improved for iCa interference on NOVA Prime Plus, although there is still room for further improvement. Our results strongly indicate that iCa in vivo can affect iMg measurement, as iCa has a direct effect on the measurement of Mg-ISE. Although the measurement of iCa on RapidPoint500 was not affected by the iMg concentration, the results might be different for ion-selective electrodes from other manufacturers.

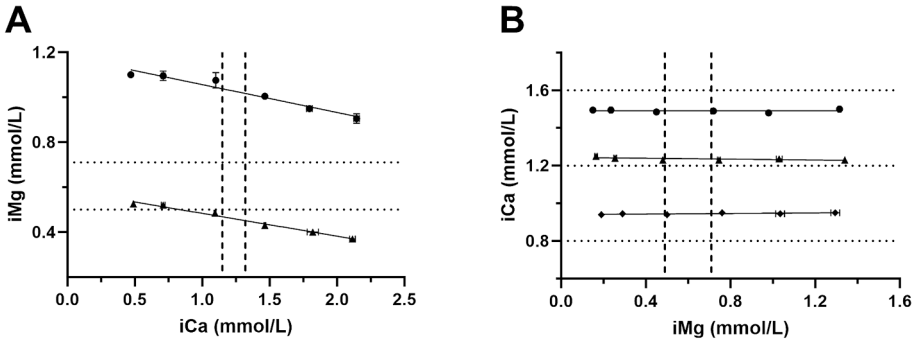

Fig. 2. In vitro interference on ionized magnesium (iMg) and ionized calcium (iCa) measurement. The effect of iCa interference on the iMg selective electrode was determined at two iMg concentration levels (low, triangle = ~0.5 mmol/L: Y = −0,1013*X + 0,5849; high, square ~1.0 mmol/L: Y = −0,1241*X + 1,181) with increasing concentrations of iCa (A). The effect of iMg interference on iCa selective electrode was determined at three different iCa concentrations (low, triangle = ~0.8 mmol/L; middle, square ~1.2 mmol/L; high, circle = ~1.60 mmol/L) with increasing concentrations of iMg. All data are presented as means ± SD (n = 2).

In conclusion, we demonstrated that in vivo, iMg does not correlate with iCa, albumin, or pH. Additionally, we quantified the degree of iCa interference in iMg measurements, providing insights for improving Mg-ISE accuracy.

Funding

This research was partly funded by Nova Biomedical.

CRediT authorship contribution statement

Wouter M. Tiel Groenestege: Writing – review & editing, Writing – original draft, Methodology, Investigation, Conceptualization. **Ron H. Stokwielder:** Writing – review & editing, Investigation, Formal analysis, Conceptualization. **Leosa R. Soels:** Writing – review & editing, Investigation. **Maaike A. Sikma:** Writing – review & editing, Validation, Methodology, Conceptualization. **Tim J.A. Hutten:** Writing – review & editing, Writing – original draft, Supervision, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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