

Study of the Total Serum Concentration of Serum Ionized Magnesium in Children and Adolescents from Sibiu Area

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Magnesium is an essential nutrient for the living organisms and plays an important part in the prevention and treatment of many diseases. It is an enzymatic cofactor for more than 300 reactions. Magnesium is essential for regulating blood pressure, muscle contraction, cardiac excitability, insulin metabolism, vasomotor tonus. Studying the way in which serum magnesium concentration varies in children and adolescents in the Sibiu area according to the reference intervals we especially set for this area. The study is a retrospective one, using approximately 4900 data from the archives of the Medical Analysis Laboratory within the Sibiu Pediatric Clinical Hospital. Serum magnesium was dosed using the Konelab Prime 30i analyser. The data from the literature was used to compare the results. The reference ranges obtained in the current study were similar to the literature studied. The percentage of patients with magnesium concentration outside the reference ranges was roughly equal for all age groups. The difference was between 1 month and 2 year-old children with very few deviations from the reference range. The results of our study reflect more accurately the real reference range for the population in the Sibiu area, helping clinicians to establish a diagnosis as quickly and accurate as possible. These results were not significantly different from the literature studied.

Keywords: serum, magnesium, children, adolescents

Magnesium is an essential nutrient for the living organisms [1]; in the human body, it is the fourth most abundant mineral, with an important role in the prevention and treatment of many diseases [2].

An adult body (70 Kg) has about 25 g of magnesium. Of the total magnesium in the body, 30-40% is found in muscles and soft tissues [3-5], 1% is found in the extracellular fluid and the rest is in the skeleton and (50-60)% is located in bones [3, 5, 6] where, it is believed to form a constituent of the surface of the mineral component, hydroxyapatite (calcium phosphate). A small amount of magnesium is found in the plasma (1%), 25% is protein bound and the rest is found under the form of Mg^{2+} . Magnesium along with the Na^+ , K^+ and Ca^{2+} ions regulate the mechanism of coagulation and neuromuscular excitability [3, 4]. Magnesium is a cofactor of many enzymes involved in energy metabolism, protein synthesis, synthesis of RNA and DNA [2], including synthesis of organic molecules, but also in glucose homeostasis and mineral metabolism [7].

Practically, Mg^{2+} is involved in all major metabolic and biochemical processes within the cell. Mg^{2+} is an essential component of RNA and DNA. In DNA, Mg^{2+} forms hydrogen bonds with the electronegative (O, N) elements to stabilize natural DNA conformation, called B-DNA. Also, by competition with monovalent ions, Mg^{2+} plays an important part in the secondary and tertiary DNA structure.

When Mg^{2+} concentration is low, DNA is more prone to oxidative stress due to the accessibility of free oxygen radicals.

Maintaining Mg^{2+} concentration within normal limits is essential for DNA stability. Mg^{2+} serves as cofactor for about 600 enzymes, for about 300 enzymes it can act as an activator [6, 8-10].

In normal doses, magnesium is not toxic. Magnesium daily recommended intake for adults is about 300 mg/day. Magnesium deficiency is much more common than the problems caused by toxicity. Magnesium deficiency causes fatigue, irritability, nervousness, stiffening of the muscles, and concentration deficiency, which makes magnesium one of the most important essential minerals in the human body [11, 12]. Low plasma concentrations of Mg have been associated with impaired insulin action, glucose homeostasis, increased blood pressure, peripheral blood flow defects and electrocardiogram abnormalities [13]. However, recent reports estimate that a large proportion of the world's population does not intake the amount of Mg^{2+} recommended daily. In recent years, the Mg^{2+} intake in fruits and vegetables has fallen by (20-30)%, because the soil used in agriculture is poorer in minerals, representing a part of this problem.

Also, during food processing (80-90)% of Mg^{2+} content is lost. Also, a significant number of people prefer and consume more processed foods such as fast food, resulting in Mg^{2+} deficiency [8, 9].

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In order to prevent magnesium deficiency, a balanced consumption of products containing magnesium is required, the recommended daily intake varying according to needs, making it difficult to accurately define the exact optimal intake [2]. Mg^{2+} is determined by measuring total serum concentrations. For a correct classification of the obtained results, it is necessary to establish correct reference ranges (lower and upper limits of analyze), for the population served, depending on the equipment and reagents used [14-18].

This is especially important for the pediatric population due to the changes that accompany the child's growth and development [19].

Specific recommendations regarding the statistical methods by which reference ranges can be calculated are given by the International Federation of Clinical Chemistry (IFCC) and the Clinical Laboratory and Standards Institute (CLSI) [20].

Experimental part

The study was conducted at the Pediatric Hospital in Sibiu. The study included the results for serum Mg concentrations collected for two years from the electronic records of the Medical Analysis Laboratory. The results come from admitted and ambulatory children and adolescents. Data from approximately 4900 patients aged between 1 month and 18 years and patients over 18 years of age were analyzed, divided into 4 categories, namely: one month-two-year-old children; 3 to 4 years old, 5 to 18 years old, and the 4th category included patients aged over 18 years.

Blood samples for magnesium determination were collected by venipuncture in vacuum collection systems without anticoagulant. The harvested blood was allowed to stand at room temperature for 30 min, then centrifuged for 10 min at 3000 rpm, then immediately analyzed at 510 nm wavelength on the Konelab Prime 30i analyser, checked with control serum (normal and pathological) and calibrator.

To establish the reference intervals, the indirect Hoffmann method is the easiest to use because patients' results from the database can be used. This method is widely described in the literature [13-17].

For the application of the Hoffmann method, first, there were eliminated the aberrant values [16]. After removing the values considered inappropriate, according to the Chauvenet criterion, the data was re-analyzed and the cumulative frequency was determined [15, 16]. The variation of the cumulative frequency was graphically represented according to the serum magnesium concentration values, and then the linearity of the straight line and the maximum deviation were determined by visual assessment. The equation of the regression line ($Y_i = A \cdot X_i + B$, by analyzing as few squares) was used, where A is the slope and B is the intercept of the line; By solving the equation of the straight line, the minimum and maximum values were determined given that the values must be within the percentile of 2.5 and 97.5 [16].

The reference ranges obtained in the present study were compared to other reference ranges in the literature.

Results and discussions

In the first stage of the study, the reference ranges for serum magnesium were established using the results of the laboratory's electronic registers.

Magnesium reference intervals in children under 1 month old could not be calculated as a result of insufficient data given that, according to the Hoffmann method, a minimum of 120 data is required.

The cumulative frequency according to the serum magnesium concentration values for different age groups is shown in figure 1.

The regression function and the calculated reference ranges for magnesium by the Hoffman computerized method, involving indirect estimation of the reference ranges, are shown in table 1.

By comparing our results with the reference interval declared by the Lothar Thomas - Clinical Laboratory

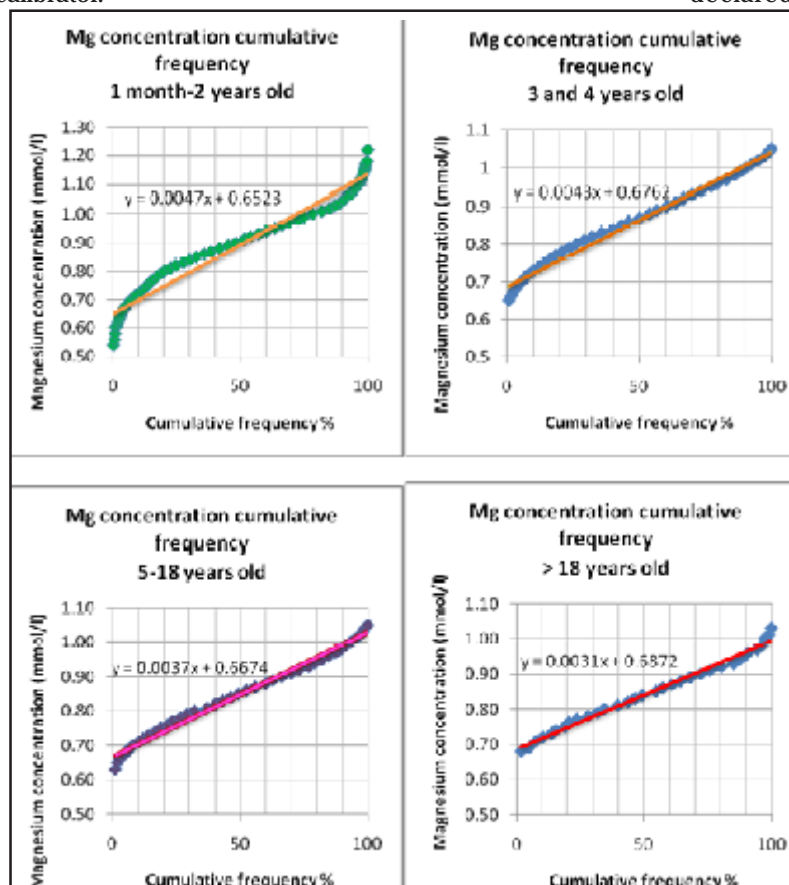


Fig.1. Cumulative frequency based on magnesium concentration values for different age groups

Age groups	Regression function	Reference range	
		mmol/l	mg/dl
1 month-2 years old	$0.0047x+0.6523$	0.66 – 1.11	1.60 – 2.70
3-4 years old	$0.0043x+0.6762$	0.68 – 1.09	1.65 – 2.65
5-18 years old	$0.0037x+0.6674$	0.68 – 1.03	1.65 – 2.50
>18 years old	$0.0031x+0.6872$	0.69 – 0.99	1.67 – 2.41

Table 1
REGRESSION FUNCTION AND REFERENCE RANGES
CALCULATED FOR MAGNESIUM BY HOFFMAN
COMPUTERIZED METHOD

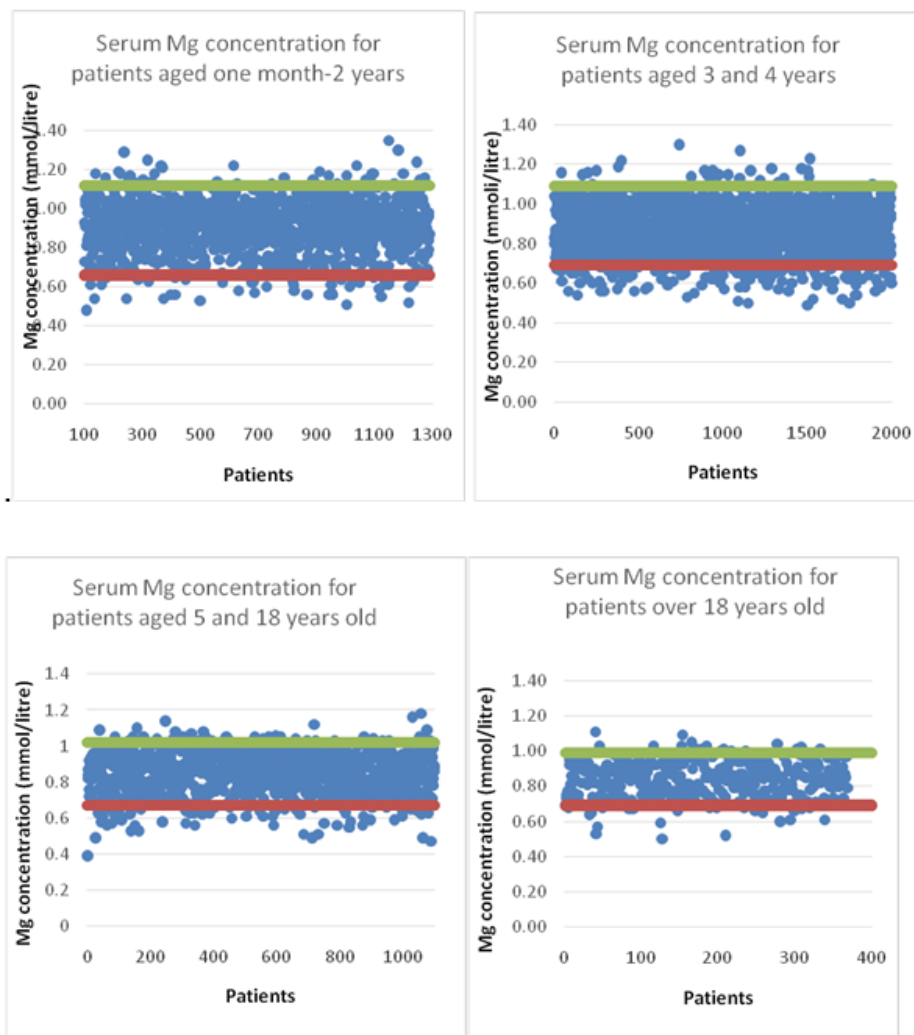


Fig. 2. Serum Mg concentration for different age groups

Diagnostics and the Roche Diagnostics Guidelines, it can be observed that the ranges obtained are very close to the reference ranges mentioned in the two publications, even if the age groups are divided differently. In the Lothar Thomas Guidelines - Clinical Laboratory Diagnostics, reference ranges are set for school-age children, the reference range being 0.60-0.95 mmol/L [21], and the Roche Diagnostics Guidelines publishes a reference range of 0.70-1.05 mmol/L for children and adults in general [22].

In the second part of the study, we will analyze the way in which serum magnesium concentration values obtained in the laboratory fall within the reference ranges.

By studying the percentage of patients with magnesium concentration outside the reference ranges, it is observed that they are roughly equal for all age groups.

There is a small difference in the patients aged 1 month to 2 years. Among these patients, there is a lower percentage of deviation from the reference ranges. This is explained by the fact that the baby takes Mg intake from the mother, by breastfeeding [3]. Also, if at birth, children

have a sufficient amount of Mg, the reserve is not consumed until the age of 2. Often, in this age category, dietary supplements are also recommended or these supplements are added to the milk powder.

We could not establish distinct reference intervals for girls and boys because there were no gender specifications in the database. However, a study conducted in the Hospital for Sick Children, Toronto, Canada and published in 2012, shows that these reference intervals do not vary according to gender [23].

It is important to monitor the level of magnesium in the child's body because once the deficiency become chronic, it can contribute to the development of conditions such as diabetes [24-26], insulin resistance, cardiovascular disease and ADHD [27].

The serum levels of Mg^{2+} reflect only 1% of its content in the body, most of Mg^{2+} is stored in muscles, bones and soft tissues. Although serum Mg^{2+} levels are within normal range, the body may be severely deficient in Mg.

Table 2
THE PERCENTAGE OF PATIENTS WITH MAGNESIUM CONCENTRATION OUTSIDE THE REFERENCE RANGES

Age	1 month–2 years		3-4 years old		5-18 years		≥18 years old	
	old				old			
No. of patients	1287		2204		1117		368	
No. of patients with values below the reference range	68	5.28%	174	7.89%	85	7.61%	23	6.25%
No. of patients with values above the reference range	41	3.19%	121	5.49%	46	4.12%	18	4.89%

Conclusions

To determine the diagnosis of hypo or hypermagnesemia, it is important to accurately measure the magnesium concentration. Therefore, the availability of reference materials and methods is essential for standardizing and validating the magnesium measurement process.

The Hoffmann method of establishing the reference ranges is easy to use when the medical analysis laboratory has a vast database, because patients' identification data is not required to apply this method, so it is not necessary to obtain their agreement.

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