Comparison of electrolytic status (Na^+, K^+, Ca^{2+}, Mg^{2+}) in preterm and term deliveries

A. Mitrovic-Jovanovic[1], S. Dragojevic-Dikic[1], M. Zamurovic[1], B. Nikolic[1], M. Gojnic[2], S. Rakic[1], T. Jovanovic[3]

[1]University Clinic of Gynecology and Obstetrics "Narodni Front", School of Medicine, Belgrade
[2]Institute of Gynecology and Obstetrics, Clinical Center of Serbia, University of Belgrade Medical Faculty
[3]Institute of Physiology, School of Medicine, Belgrade (Serbia)

Summary

Purpose of investigation: The objective of this study was to evaluate the electrolytic status of Na^+, K^+, Ca^{2+}, and Mg^{2+} in serum and red blood cells in idiopathic preterm and term deliveries. *Methods:* The study included 105 pregnant women diagnosed with idiopathic premature delivery (study group) and 36 pregnant women with physiologically term delivery (controls). Samples of mother's blood were collected and analyzed for the level of electrolytes in the serum/plasma and red blood cells. *Results:* Measured values of magnesium in red blood cells in the study group were far lower than physiological values, intracellular calcium levels were higher in the study group compared to levels measured in the controls. Sodium concentrations in cells were significantly lower in subjects with premature delivery. *Conclusion:* The magnesium intracellular level is the best representative value of magnesium in the body.

Introduction

Is the physiological mechanism of contraction and electrolytic status of the gravid uterus always the same? Electrolytic status is vitally important for biochemical processes in the cell. Recognition of intracellular processes, enzymatic activity, effects of neurohumoral factors, interacting effects and relation of electrolytes, particularly the correlation of intracellular deficit of magnesium, and altered concentrations of sodium, potassium, and calcium, allows for a more thorough and precise understanding of uterine contractions both in term and idiopathic preterm deliveries [1].

From the current aspect of electrophysiology in spite of numerous non-clarified facts, the mechanism of contractions of the gravid uterus is in direct relation with slow, voltage-gated channels and their interaction with intracellular processes. The change of electric potential in cell membranes controlling the cellular calcium level, as well as feedback on the cell membrane which is regulated by intracellular events, defines muscle cell tonus [2, 3]. Out of all electrolytes, magnesium is the most important regulator of cellular function and an essential activity factor of over 320 enzymes [4, 5]. Magnesium is an integral part of the intracellular processes such as oxidative phosphorylation, membrane transport, muscular contractions, and neural conduction, and it is involved in the synthesis of nucleic acids and proteins [6]. The most valuable discovery regarding the role of magnesium is the knowledge that the concentration of free magnesium ions in the cell regulates metabolic activity of the cell.

Smooth muscle contraction is closely related to cellular metabolism and electrophysiological characteristics of the cell. Smooth muscles are so-called oxidative tissue with more than 80% of ATP supplied from oxidative phosphorylation in mitochondria which requires magnesium ions to proceed. Until now, it is known that smooth muscle contraction is initiated by Ca^{2+} bonding to calmodulin, and such formed complex activates the kinase of the myosin light chain. It is considered that the presence of Mg^{2+} ion is necessary for modulation of the kinase of the myosin light chain as well as for identification of site and mode of action of the respective enzyme [7-11]. If there is any magnesium deficit in the cell, it brings about the impairment of physiological processes.

Magnesium is the most important bivalent cation in the cell. Less than 1% of total body magnesium is found in circulating blood, and therefore, the evaluation and analysis of serum magnesium concentration is not the true indicator of magnesium level [12-14]. The objective of our study was to evaluate the electrolytic status of Na^+, K^+, Ca^{2+}, and Mg^{2+} in extracellular (serum) and intracellular (red blood cell) compartments in idiopathic preterm and term deliveries.

Materials and Methods

A randomized clinical study based on fundamental physiological principles was carried out. The study included 105 pregnant women diagnosed with idiopathic premature delivery (study group) and 36 pregnant women with physiologically term delivery (controls). The study group consisted only of pregnant women at 28-36 gestational weeks with preterm delivery whose etiology could not be explained by etiological agents, i.e. the study excluded all premature births whose causes could have been due to maternal or fetal factors (e.g., multifetal pregnancy, hypertension, diabetes mellitus, amnionitis, PROM, urogenital tract infections, IUGR, macrosomia, polyhydramnios, oligoamnios, etc.). The controls involved women with physio-

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logic pregnancy and with term delivery between 38 and 42 gestational weeks.

Other than regular biochemical analyses, a sample of mother's blood was collected and analyzed for the level of electrolytes as follows: sodium, potassium, magnesium and calcium in serum/plasma (extracellular compartment) and red blood cells (intracellular compartments). The method of AAS – atomic absorption spectrophotometry – was used to read the values of the respective electrolytes. The procedure involved the sampling of 5 ml of heparinized blood centrifuged at 3,000 rounds per minute and plasma was used for dilution of electrolytes.

Plasma

To determine sodium and potassium in plasma, 1:100 dilution (0.1 ml plasma/10 ml water) was prepared. To determine calcium and magnesium, $LaCl_3$ – 0.2% (5 ml) was added for precipitation of phosphates and prevention of formation of potassium and magnesium phosphates which could have interfered with their values.

Red blood cells

Red blood cells were washed three times by 0.9% NaCl in 1:1 ratio (2 ml WBC and 2 ml saline) and used for preparation of electrolyte dilution. Upon centrifuging it, supernatant was decanted and the procedure was repeated three times. Thereafter, washed erythrocytes were diluted by deionized water. For sodium and potassium determination, 1:100 dilution (0.1 ml erythrocytes/10 ml water) and 1:1,000 dilution was prepared, respectively. Calcium and magnesium were diluted in 1:100 ratio with addition of $LaCl_3$, as in plasma.

For dilutions prepared in this way, electrolytes were determined by reading the values on the AAS according to standards of known concentrations. The AAS SP 192 produced by Pye Uncima Ltd. functions as flame spectrophotometry. Solution elements in contact with a burner (acetylene) are transferred to free atoms. The light source emission is characteristic for each element at specific wave, lengths. Accordingly, magnesium is determined at 285.2 nm, calcium at 422.7 nm, sodium at 589 nm, and potassium at 766.5 nm.

Reference values for plasma were as follows: Na^+ (135-148 mmol/l), K^+ (4.09-4.73 mmol/l), Mg^{2+} (0.65-1.05 mmol/l), Ca^{2+} (2.12-2.62 mmol/l). Reference values for red blood cells were: Na^+ (17 ± 2.10 mmol/L), Mg^{2+} (2.30 ± 0.24 mmol/l), K^+ (125 ± 10.6 mmol/l), Ca^{2+} (0 + 0.10 mmol/l). The values were directly obtained by expression in mmol/l of blood.

Statistical data processing included: descriptive parameters (mean value, standard deviation, standard error and median), Student's t-test (with probability $p < 0.01$ and $p < 0.05$; χ^2 test ($p < 0.05$), Fisher's exact test, Mann-Whitney U-test ($p < 0.05$), and McNemar's test.

Results

The mean age of pregnant women with premature delivery was 25.14 ± 4.40 years, and 26.11 ± 5.10 years (t = 1.092) in the controls. In both groups, the majority of pregnant women was primipara (76/67.7% of the study group and 20/55.6% of the controls), χ^2 test = 1.705. In multiparas, former pregnancies in the experimental group were terminated at gestational week 39.03, and in the controls at gestational week 38.33 (t = 1.453).

The results revealed that the average gestational week at the time of delivery in the study group was 33.60 ± 2.05 (the least being week 27), and in the controls 39.17 ± 1.48 weeks, t = 15.015 ($p < 0.01$).

The concentration of the analyzed bivalent magnesium ion within the extracellular space in the premature delivery group was at the lowest normal limits (0.93 ± 0.14 mmol/l), while measured values in pregnant women with term delivery were within optimal physiological values (1.12 ± 0.11 mmol/l). In spite of the fact that the obtained results were reviewed rather strictly within tolerable limits, statistical analysis yielded quite different figures, i.e. t = 7.435 ($p < 0.01$).

Measured values of magnesium in red blood cells are illustrated in Table 1; values in subjects with premature delivery were far lower than physiological values, accounting for 0.86 ± 0.22 mmol/l. In control subjects, the values of intracellular magnesium were 2.19 ± 0.12 mmol/l. The Mann-Whitney U test recorded U = 36.00, Z = 9.770. Such result indicated that intracellular magnesium level in subjects with premature delivery was significantly lower in relation to intracellular level in the group of subjects with term delivery (Table 1).

Analysis of the results, as presented in Table 2, demonstrated no significant difference of calcium concentration in the extracellular compartment in either group.

Cellular calcium level was 0.63 ± 0.18 mmol/l in subjects with premature delivery, and 0.47 ± 0.18 mmol/l in the controls. The measurements of intracellular calcium showed that in both groups there was a significantly higher calcium level compared with physiological values, and further analysis of the obtained results found a difference between these two groups ($p < 0.01$), meaning that significantly higher values were recorded in subjects with premature delivery (Table 2).

The results of serum sodium concentrations were within physiological limits in both groups and there was no significant difference (154.62 mmol/l vs 147.51 mmol/l).

The obtained results of sodium concentrations in the cell, as demonstrated in Table 3, showed that there was a significant difference ($p < 0.01$), with lower values in subjects with premature delivery. Considering that blood samples were collected from both groups at the time of delivery, i.e., at the time of full activity, the obtained sodium values in the intracellular space were the result of depolarizing activity of the cell membrane which generally precedes the contraction of all cells, even the uterine muscle cells (Table 3).

The values of major intracellular monovalent ion - K ion (potassium), which determines the positivity of the external cell membrane surface and magnitude of the resting membrane potential, were within physiological limits in both groups (4.44 ± 0.60 mmol/l in the experimental and 4.28 ± 0.63 mmol/l in the controls).

Measurements of potassium concentrations in the cell revealed that the values were lower in both groups in relation to reference physiological values, but a significant difference was found between these two groups. In ery-

Table 1. — *Magnesium intracellular level (RBCs)*.

		Group	
		Study	Control
Mg^{2+} RBC mmol/l	N	N = 105	N = 36
	X	0.86	2.96
	SD	0.22	0.29
	Median	0.85	2.92
	Minimum	0.40	2.36
	Maximum	1.80	3.60

(U = 36.00; Z = 8.770; $p < 0.01$).

Table 2. — *Calcium plasma level*.

		Group	
		Study	Control
Ca^{2+} serum mmol/l	N	N = 105	N = 36
	X	2.15	2.19
	SD	0.21	0.12
	Median	2.15	2.15
	Minimum	1.40	1.96
	Maximum	2.60	2.50

(t = 0.634).

Table 3. — *Sodium red blood cell (RBC) levels*.

		Group	
		Study	Control
Na^+ RBC mmol/l	N	N = 105	N = 36
	X	39.15	68.19
	SD	10.99	13.21
	Median	38.00	70.50
	Minimum	21.00	35.00
	Maximum	80.00	83.00

(t = 12.979; $p < 0.01$).

throcytes of women with preterm delivery, the values were 56 ± 11.08 mmol/l, and in the controls 91.56 ± 12.08 mmol/l (t = 16.104; $p < 0.01$). The results supported the role of depolarized activity during the action potential as the trigger of contractions of the uterine muscle cells.

Discussion

Premature delivery is one of the most important individual problems of perinatal medicine. Multiple facts with a predominating impact can affect the occurrence of preterm delivery [15, 16]. One of the most frequent unrecognized and untimely, diagnosed electrolyte disorders is magnesium deficit. Plasma magnesium level is a poor indicator of magnesium status in the body [17].

The obtained results showed that the level of plasma magnesium was within physiological limits in both groups, and they themselves could not indicate hypomagnesemia. The fact that these results could not be relied on was confirmed by findings of the cell compartment – red blood cells. Intracellular space is the only valid indicator of the actual status, i.e., electrolytic level of magnesium, because this bivalent cation is predominantly intracellular electrolytes, and, in normal serum magnesium concentrations, intracellular hypomagnesemia may be, and often is, present [18, 19]. Our sample verified the aforementioned.

The results suggested that in case of idiopathic premature delivery, intracellular hypomagnesemia was present in borderline values of serum magnesium; given the known role of magnesium in intracellular processes, it may cause a series of reactions giving rise to elevated cell excitability. The role of magnesium is seen in neuronal activity, cardiac excitability, vasomotor tonus, and muscular contractions, which is all the result of its modulatory potential.

One of the modulatory potentials of magnesium is the control of cellular calcium level [20]. Magnesium by magnesium-dependent enzymes directly affects calcium influx through cell membranes as well as the release of calcium from intracellular depots through membranes of the sarcotubular system [21, 22].

In both groups, serum calcium values were at lower physiological limits, which entirely corresponded with the fact that the subjects were giving birth, meaning that uterine activity (muscular contractions) was present.

So far, it has been only concluded that there is a rise of intracellular calcium, which by a known mechanism brings about muscular contractions, but the etiological factor causing the development has been completely neglected [23]. Our results showed that there was a significant increase of intracellular calcium concentrations in both studied groups, but also that there was a significant difference between these groups. It supports the observation that increased intracellular calcium concentration is necessary for muscular contractions, but the trigger of the uterine contraction mechanism is different in preterm and term deliveries.

Cellular processes proceed as chain reactions, and therefore any disorder cannot be observed as an isolated phenomenon. It is known that magnesium affects the cell membrane and significantly changes its action potential. Changed levels of sodium and potassium, particularly in the intracellular space, are the direct result of cellular hypomagnesemia and the effect of magnesium on Na and K biochemistry. The presence of hypomagnesemia causes the stimulation of transport systems of Na-channels, K-channels, Na^+, K^+ and Cl^--transport, K^+ Cl^-, co-transport, and Na/H exchange [24].

In red blood cells, there is a direct relation between K^+/Mg^{2+} and Na^+/Ca^{2+} concentrations.

In our study, plasma sodium levels were within physiological limits in both groups. What attracts special attention is the level in intracellular sodium. In both groups, there was a significant rise in sodium concentration. More marked hypernatremia was found in subjects with term deliveries. Such result supported and correlated with the finding that there was intracellular hypercalcemia to a lesser degree of this group in comparison with subjects having premature delivery. Significantly lower intracellular sodium values in preterm delivery showed that depolarization activity had a certain but not decisive role in the initiation of the uterine muscular cell contractions, with constant focus on recorded higher intracellular calcium level and low level of its physiological antagonist – magnesium. It verifies a predominant effect of action potential to pathogenetic mechanisms of term delivery [25].

The analysis of results of K+ level in red blood cells

revealed a higher degree of hypokalemia in the group of subjects with premature delivery, what was certainly the result of the hypomagnesemia effect on Na^+ and K^+ membrane transport.

Hypomagnesemia is always followed by varying degrees of hypokalemia, and it is beyond doubt that the role of magnesium and its activity as an intracellular metabolism controller and modulator of enzymatic catalyzed processes is an important individual causative agent of higher cell excitability of the excitable tissues [26].

Conclusion

Hypomagnesemia in intracellular space increases the excitability of uterine smooth muscle, altering the permeability of cell membrane for Ca^{2+}, N^+, and K^+, and enhancing the release of calcium from the intracellular depots thus preventing its re-accumulation. In term delivery, the increase of intracellular sodium concentration interferes with action potential which is the major factor – trigger of initiation of the uterine contraction. If an adequate level of intracellular magnesium were provided during pregnancy, it would certainly result in fewer premature deliveries caused by initiation of the uterine contractions due to electrolytic imbalance in the cell.

The magnesium ion is a crucially important element for biochemical processes in the cell.

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Address reprint requests to:
A. MITROVIC-JOVANOVIC, M.D., Ph.D
University Clinic of Gynecology and Obstetrics "Narodni front"
School of Medicine, Belgrade
Kraljice Natalije Street 62
11000 Belgrade (Serbia)
e-mail: anamitrovicjov@gmail.com