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Red Cell Deformability and Serum Magnesium Level in Non-pregnant and Normal Pregnant Women

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The study was carried out to determine red cell deformability (RCD) and serum magnesium (Mg^{2+}) level in normal pregnancy, in comparison with non-pregnant subjects and to find out relationship between these two parameters in different gestational ages of normal pregnancy. This cross-sectional study was carried out in non-pregnant women (n=25) and 1st, 2nd, 3rd trimesters of normal pregnant women (n=15, 30 and 30, respectively). The RCD was determined by whole blood filtration method and serum Mg^{2+} level by colourimetric test with Chlorophosphonazo III method. The RCD was expressed as the flow rate (ml/min) as well as red cell deformability index (RCDI). Flow rate and RCDI of non-pregnant women, 1st, 2nd and 3rd trimesters normal pregnant women were 0.45±0.09, 0.35±0.07, 0.33±0.08, 0.23±0.06 ml/min and 0.18±0.03, 0.13±0.02, 0.11±0.03, 0.08±0.02, respectively. The RCD of 1st, 2nd and 3rd trimester normal pregnant women was significantly lower than that of non-pregnant women ($p<0.001$). Serum Mg^{2+} levels of non-pregnant, 1st, 2nd and 3rd trimesters of normal pregnant women were 0.88±0.11, 0.74±0.11, 0.73±0.06 and 0.67±0.14 mmol/l, respectively. Serum Mg^{2+} levels were significantly lower in all 3 trimesters compared with non-pregnant women ($p<0.001$). Both RCD and serum Mg^{2+} level progressively decrease as the pregnancy approaches term. There was a significant positive correlation between two parameters in 2nd trimester ($r=0.42$, $n=30$, $p<0.05$) and 3rd trimester of pregnant women ($r=0.47$, $n=30$, $p<0.01$). In the present study, 33% of normal pregnant women in 2nd trimester and 50% in 3rd trimester were Mg^{2+} deficient. It could be concluded that reduction in Mg^{2+} level might be related with reduction in RCD during normal pregnancy.

Key words: Red cell deformability, Serum magnesium, Pregnancy

INTRODUCTION

Pregnancy is a unique state where the physiology of a woman is greatly altered to accommodate the newly developing organ-the fetus. The normal human pregnancy has a large impact on the wellbeing of a woman without any underling medical disorder.[1] Numerous physiological changes occur in pregnant women that affect blood rheology. Some significant features are: (a) the formation of a new uteroplacental vascular network with a large maternal fetal exchange surface area at the level of intervillous space where the blood flow depends basically on the haematocrits (Hct), cell aggregation and erythrocyte deformability, (b) changes in the plasma protein composition especially fibrinogen level which affects the blood flow and plasma flow.[2]

Red cell deformability (RCD) is the important haemorrheologic parameter. Red blood cells with a diameter of ~8 µm can pass through capillaries that have a diameter of 3-4 µm, allowing oxygen uptake and release.[3] This ability of red blood cells to deform is essential for the microcirculation. In pregnancy, maintenance of an efficient

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placenta blood flow plays a vital role for normal fetal growth and development. Many studies have been carried out to investigate the changes in RCD in normal pregnancy.

It was reported that the red cell deformability index (RCDI) was elevated in normal pregnancy compared with the non-pregnant value.[4, 5] In contrast, other studies showed that erythrocytes from normal pregnant women were significantly less filterable than those from non-pregnant women.[6, 7]

With the growth of both maternal and fetal tissues during pregnancy, higher magnesium (Mg^{2+}) intake is required throughout pregnancy. Studies from different regions reported a decline in Mg^{2+} levels during pregnancy. The frequency of Mg^{2+} deficiency in pregnancy has been variously reported to be between 4.6%[8] and 48%.[9] There is an oxidative stress of the maternal organism even in uncomplicated pregnancies, which could lead to a reduction of RCD towards the end of pregnancy.[10] It was described the beneficial effect of Mg^{2+} on RCD in non-preeclamptic pregnancies with normal RCD after intravenous Mg^{2+} administration. They suggested that Mg^{2+} as a calcium-antagonist is able to antagonize the effect of inflammatory response in normal pregnancy, resulting in an increase of RCD.[11]

Therefore, the present study aimed to determine the serum Mg^{2+} level and RCD in different gestational ages of normal pregnancy and examine whether there is any relationship between these two parameters in different gestational ages of normal pregnancy.

MATERIALS AND METHODS

Study population

A total of 75 normal pregnant women including 15 subjects in 1st trimester (<13th week), 30 subjects in 2nd trimester (13th-28th week), 30 subjects in 3rd trimester (>28th-40th week) were recruited from Out-patient Department of Central Women's Hospital, Yangon. Age matched apparently healthy non-pregnant women (n=25) residing in Kamayut Township were recruited.

Determination of red cell deformability

Red cell deformability was determined by simple filtration method as mentioned below. Under standard conditions, whole blood was allowed to pass through a membrane filter using a negative pressure of 20 cm water. The time for 1 ml of blood to flow was recorded by stop watch. From which, flow rate (FR) was calculated as the volume of the blood filtered per minute. In order to eliminate the influential effect of packed cell volume (PCV) on flow rate, red cell deformability index (RCDI) was expressed as FR (ml/min) multiplied by packed cell volume before filtration (PCV_{bf}) in proportion.

Red cell deformability index (RCDI)=FRx PCV_{bf}[12]

Determination of serum magnesium level

Five milliliters of blood were collected in plain tubes. Then, blood samples were centrifuged at 3000 rpm for 15 minutes for separation of serum. The serum was kept in aliquots and then transported in ice packs to the Pathology Research Division of National Health Laboratory for determination of serum magnesium level and stored at -20ºC until analysis. The frozen patients' samples were thawed at the room temperature for about 30 minutes. Serum Mg^{2+} level was determined by Colourimetric test with Chlorophosphonazo III.[13] The Cobas C 111 system automatically calculates the analyte concentration of each sample.

Statistical analysis

Data were analyzed by using the Statistical Package for Social Science (SPSS) software version 22. The results were expressed as mean±SD. Comparison of variables (serum Mg^{2+} level and red cell deformability) among non-pregnant and 1st, 2nd, 3rd trimesters of normal pregnant women was assessed by one way ANOVA and Post Hoc Bonferroni test. Pearson's correlation coefficient was calculated to assess the relationship between the variables. In this study, p value <0.05 was regarded as significant.

RESULTS

Baseline parameters of non-pregnant women and 1st, 2nd and 3rd trimesters of normal pregnant women are shown in Table 1.

Table 1. Baseline parameters of the study population

Baseline parameters (Mean±SD)	Non-pregnant women (n=25)	Normal pregnant women Trimester (week)		
		1st (<13th) (n=15)	2nd (13th-28th) (n=30)	3rd (>28th-40th) (n=30)
Age (year)	28.96 ±4.39	26.93 ±5.16	29.40 ±5.53	29.30 ±5.30
BMI (kg/m^2)	21.97 ±2.23	22.05 ±3.56	23.98* ±3.99	26.55*** ±4.23
Gravida	-	1±0	2±1	2±1
Gestational age at sampling (weeks)	-	9.64 ±1.91	20.33 ±3.75	33.70 ±3.52
SBP (mmHg)	108.40 ±10.28	106.00 ±9.26	107.33 ±7.39	107.00 ±9.15
DBP (mmHg)	69.60 ±7.34	65.33 ±10.60	68.00 ±6.64	69.00 ±7.12
MAP (mmHg)	82.53 ±7.83	74.53 ±4.50	77.93 ±6.18	79.53 ±5.77

*indicates significant difference ($p<0.05$)

***indicates significant difference ($p<0.001$)

BMI=Body max index, SBP=Syotolic blood pressure, DBP=Diastolic blood pressure, MAP=Mean arterial pressure

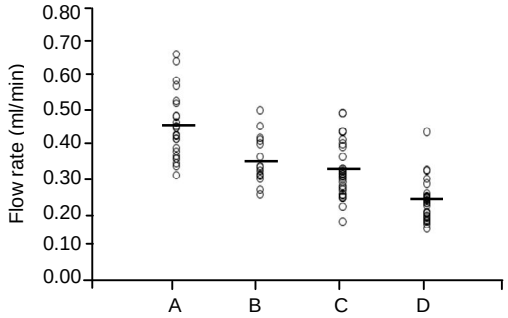
The mean flow rate, red cell deformability index and serum magnesium level among non-pregnant women, 1st, 2nd and 3rd trimesters of normal pregnant women are shown in Table 2.

Table 2. Flow rate, red cell deformability index and serum magnesium level among non-pregnant women, 1st, 2nd and 3rd trimesters of normal pregnant women

Parameters (Mean±SD)	Non-pregnant women (n=25)	Trimester (week)		
		1st (<13th) (n=15)	2nd (13th-28th) (n=30)	3rd (>28th-40th) (n=30)
Flow rate (ml/min)	0.45±0.09	0.35±0.07	0.33±0.08	0.23±0.06
RCDI	0.18±0.03	0.13±0.02	0.11±0.03	0.08±0.02
Serum Mg^{2+} level (mmol/l)	0.88±0.11	0.74±0.11	0.73±0.06	0.67±0.14

RCDI=Red cell deformability index

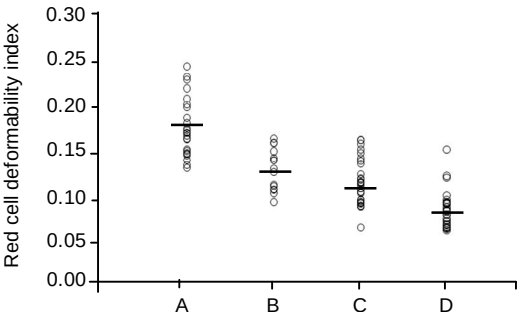
There was a significant decrease in flow rate and RCDI of 1st, 2nd and 3rd trimesters of normal pregnant women as compared to that of non-pregnant women ($p<0.001$). The flow rate and RCDI of 3rd trimester was significantly lower than that of 1st and 2nd trimesters of normal pregnant women ($p<0.001$) (Fig. 1 & Fig. 2).



A=Non-pergnant women (n=25) C= 2nd trimester (n=30)
B= 1st trimester (n=15) D= 3rd trimester (n=30)

Solid line (—) indicates mean of different groups.

Fig. 1. Comparison of flow rate among non-pregnant women, 1st, 2nd and 3rd trimesters of normal pregnant women

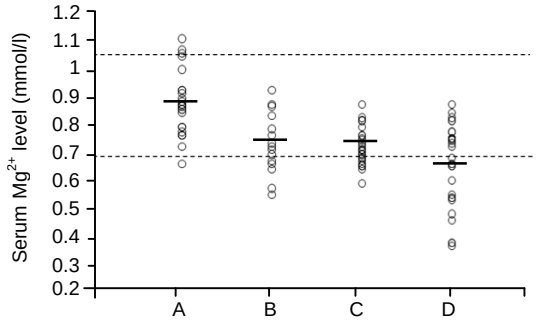


A=Non-pergnant women (n=25) C= 2nd trimester (n=30)
B= 1st trimester (n=15) D= 3rd trimester (n=30)

Solid line (—) indicates mean of different groups.

Fig. 2. Comparison of red cell deformability indices among non-pregnant women, 1st, 2nd and 3rd trimesters of normal pregnant women

Serum Mg^{2+} levels of 1st, 2nd and 3rd trimesters of normal pregnant women were significantly lower than that of non-pregnant women ($p<0.001$). There was no significant difference in serum Mg^{2+} levels between 1st and 2nd trimesters of normal pregnant women and between 1st and 3rd trimesters of normal pregnant women. But, the mean serum Mg^{2+} level was significantly lower in 3rd trimester than 2nd trimester of normal pregnancy ($p<0.05$) (Fig. 3).



A=Non-pergnant women (n=25) C= 2nd trimester (n=30)
B= 1st trimester (n=15) D=3rd trimester (n=30)

Solid line (—) indicates mean of different groups.
Upper dash line: Upper limit of normal serum Mg^{2+} level (1.05 mmol/l)
Lower dash line: Lower limit of normal serum Mg^{2+} level (0.7 mmol/l)

Fig. 3. Comparison of serum magnesium level among non-pregnant women, 1st, 2nd and 3rd trimesters of normal pregnant women

In non-pregnant and 1st trimester of normal pregnant women, there was no significant correlation between red cell deformability index and serum Mg^{2+} level. There was a significant positive correlation between red cell deformability index and serum Mg^{2+} level in 2nd trimester ($p<0.05$) and 3rd trimester of normal pregnancy ($p<0.01$). Significant correlation between flow rate and serum Mg^{2+} level was found only in 2nd trimester of normal pregnancy ($p<0.05$).

DISCUSSION

In the present study, RCDI of non-pregnant women (n=25) was 0.18±0.03 and that of normal pregnant women (n=75) was 0.11 ±0.03. Flow rate of non-pregnant women (n=25) was 0.45±0.09 ml/min and that of normal pregnant women (n=75) was 0.29 ±0.08 ml/min. One study reported that RCDI of non-pregnant and normal pregnant women were 0.24±0.05 and 0.10±0.02, respectively, and that the flow rates of non-pregnant and normal pregnant women were 0.63±0.17 and 0.27±0.06 ml/min, respectively. It was found that RCDI and flow rate of normal pregnant women participated in the present study was comparable with those of previous Myanmar study.[14]

In the present study, RCD of normal pregnant women was statistically lower than that of non-pregnant control and RCD reduced with the progression of pregnancy from 1st to 3rd trimester. Similar findings were reported by some studies in which RCD of 1st, 2nd and 3rd trimesters of normal pregnancy was significantly lower than that of the non-pregnant women and red blood cells of pregnant women are found to be progressively less deformable as the pregnancy advanced.[15, 16] Red cell de-formability was determined by erythrocyte sedimentation rate method and osmotic haemolytic method in these previous studies.[15, 16] Even though the methods used for RCD were different, the observed findings were similar to those of the present study.

Some research explained reduced RCD of normal pregnancy from the view of oxidative stress because oxidative stress was definitely present in normal pregnancy and the membrane of RBC itself was very sensitive to free radicals of various types. One of the limitations of the present study was that oxidative stress marker was not determined. According to a study,[14] metabolite of lipid peroxidation, malondialdehyde level (MDA) was significantly higher in normal pregnant women than non-pregnant women and MDA level of 3rd trimester of normal pregnant women was significantly higher than that of 2nd trimester. Moreover, RCD decreased with increasing concentration of fibrinogen *in vitro*.[17] Therefore, it could be stated that the increase in fibrinogen concentration in the surrounding media of the RBC increases the viscosity of the surrounding media resulting in prolongation of the filtration time (decreased RCD). It was well-known that the higher level of fibrinogen was observed in normal pregnant women.[18, 19] Therefore, decreased RCD observed in the present study might probably be due to increased reactive oxygen species and/or increased fibrinogen during pregnancy. In the present study, among the normal pregnant women, 40% (30 out of 75 subjects) had lower serum Mg^{2+} level than the normal value i.e., 0.7-1.05 mmol/l.[20] Five out of 15 pregnant

women (33%) in 1st trimester, 10 out of 30 pregnant women (33%) in 2nd trimester and 15 out of 30 pregnant women (50%) in 3rd trimester were found to be Mg^{2+} deficient. But, there was normal serum Mg^{2+} level in all non-pregnant control except the only one subject. Serum Mg^{2+} level of normal pregnant women was significantly lower than that of non-pregnant control in this study.

It was found that there was a significant decrease in serum Mg^{2+} level during normal pregnancy. There are many explanations behind this reduction. It was suggested that inadequate dietary intake of Mg^{2+} contributes greatly to the negative Mg^{2+} balance associated with pregnancy.[21] One of the limitations of the present study is that dietary history was not taken. But, all normal pregnant women are recommended to take vitamin and mineral supplementation as antenatal care. Therefore, it could be assumed that the normal pregnant women included in this study had adequate dietary intake.

Therefore, hypomagnesaemia during pregnancy might probably be due to increased demand of nutrients particularly Mg^{2+} for the growing fetus and pregnant mother and physiological haemodilution effect during 2nd and 3rd trimesters. Increased renal excretion of magnesium as a result of an increase in glomerular filtration rate during pregnancy could be another contributing factor for hypomagnesaemia. In the present study, there was no correlation between red cell deformability index (RCDI) and serum Mg^{2+} level in 1st trimester of normal pregnancy. However, a significant positive correlation between RCDI and serum Mg^{2+} level was found in 2nd and 3rd trimesters of pregnant women. There is the limited evidence to compare these findings of the present study.

The percentage of Mg^{2+} deficient in 2nd and 3rd trimesters of normal pregnant women were 33% and 50%, respectively. When RCDI of Mg^{2+} deficient subjects in both trimesters were compared with that of the subjects who had normal Mg^{2+} level, it was also found that Mg^{2+} deficient subjects had significantly lower RCDI than Mg^{2+} sufficient subjects. It is well known that Mg^{2+} acts as a calcium channel antagonist. By competing with calcium for membrane binding sites and by stimulating calcium sequestration by endoplasmic reticulum, Mg^{2+} helps maintain a low intracellular free calcium concentration which is important in many cellular functions including deformation of RBC.[20] Thus, it seems that Mg^{2+} deficiency in 2nd and 3rd trimesters of pregnancy might result in an intracellular calcium overload, reducing RCD.

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