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Red Cell Deformability and Serum Magnesium Level in Pre-eclampsia

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The study was conducted to find out the changes of red cell deformability (RCD) and serum magnesium (Mg^{2+}) level with severity of pre-eclampsia (PE) and relationship between these two parameters in mild and severe PE patients. This cross-sectional study was carried out in 32 mild PE patients and 32 severe PE patients. The RCD was determined by whole blood filtration method and serum Mg^{2+} level was determined by colorimetric test with Chlorophosphonazo III method. The RCD was expressed as flow rate (volume of blood filtered per minute) and red cell deformability index (RCDI) (flow rate multiplied by PCV before filtration in proportion). Flow rate and RCDI of severe PE patients were significantly lower than those of mild PE patients (0.10±0.04 *vs.* 0.18±0.07 ml/min and 0.03±0.01 *vs.* 0.06±0.02) ($p<0.001$). Serum Mg^{2+} level of severe PE patients was significantly lower than that of mild PE patients (0.63±0.17 *vs.* 0.71± 0.16 mmol/L, $p<0.05$). In this study, 50% of all PE, 37.5% of mild PE patients and 62.5% of severe PE patients had hypomagnesaemia. There was a significant association between hypomagnesaemia and severity of PE ($\chi^2=4$, $p<0.05$). Flow rate and RCDI between hypomagnesaemic and normomagnesaemic group of all PE patients showed significant difference (0.11±0.06 *vs.* 0.16±0.06 ml/min; $p<0.01$ and 0.04±0.02 *vs.* 0.06±0.02; $p<0.01$). There was a significant positive correlation between serum Mg^{2+} level and RCD in severe PE patients ($r=0.48$, $n=32$, $p<0.01$) and mild PE patients ($r=0.40$, $n=32$, $p<0.05$). It could be concluded that the changes in RCD and serum Mg^{2+} level might be involved in the pathophysiology of severity of PE.

Key words:

INTRODUCTION

Pre-eclampsia (PE) is a pregnancy-specific disorder of widespread vascular endothelial malfunction and vasospasm, characterized by new onset hypertension and proteinuria with or without pathologic edema, occurring after 20 weeks gestations.[1] It is the commonest medical complication of pregnancy, which is associated with substantial morbidity and mortality of both mother and fetus.[2] In Myanmar, data collected during 2006-2011 at Central Women's Hospital (CWH), Yangon have found that PE is the second commonest cause of maternal mortality.[3] If erythrocytes exist in conditions of flowing blood, they undergo deformation which depends on the nature of the blood flow, size of the blood vessels and fluidity of the red cell membrane.

In the small uterine vessels and in the intervillous space of placenta, deformation may result in erythrocytes changing from their usual biconcave disk into an umbrella shape, with a cylindrical diameter approximately that of the vessel or the diameter between the villi. Such deformation is essential to the normal blood flow. When red cell deformability (RCD) is decreased, blood viscosity is significantly

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increased, and this may be associated with a tendency to microvascular thrombosis and a shorter life span of red cells with a reduced tissue oxygen supply.[4, 5]

In women with PE, utero-placental ischemia causes persistent hypoxia. Under hypoxic conditions, increased amount of unsaturated fatty acids will be oxidized to lipid peroxides, leading to new distribution and loss of asymmetry of phospholipids, to increased cell-cell interaction and to damages of red cell membrane.[4, 6] A significant decrease in RCD in pre-eclamptic pregnant women compared with normal pregnant controls was observed and this decrease being more pronounced as gestation approaches term.[4, 7] But some studies reported no significant reduction of RCD in PE.[8, 9] Moreover, it is not still exactly known whether there is a difference in RCD between mild and severe pre-eclamptic patients.

Previous studies have shown a significant reduction in serum magnesium (Mg^{2+}) in pre-eclamptic pregnant mothers compared with normal pregnant mothers.[10, 11] However, it was revealed no significant differences in serum Mg^{2+} concentration between normal pregnant women and pre-eclamptic pregnant women.[12] It was also reported the mean serum Mg^{2+} concentration in severe pre-eclamptic women was less than normal pregnant women and mild pre-eclamptic pregnant women.[13]

Mild pre-eclampsia is defined as (i) de novo hypertension after 20 weeks' gestation, (ii) properly documented proteinuria of greater than 300 mg/24 hours, and (iii) both hypertension and proteinuria disappearing postpartum.[2] Severe pre-eclampsia is defined by the presence of severe hypertension in association with proteinuria or if there is hypertension in association with severe proteinuria (≥5 g/24 hours period) or if there is multiorgan involvement such as pulmonary edema, seizures, oliguria (<500 ml/24 hours period), thrombo cytopenia (platelet count <100,000/mm^3), abnormal liver enzymes in association with persistent epigastric or right upper quadrant pain, or persistent severe central nervous system symptoms (altered mental status, headaches, blurred vision, or blindness).[2] It was shown the beneficial effect of Mg^{2+} on RCD in pre-eclamptic pregnant women.[14] A clinical study revealed that intravenous dosage of magnesium sulfate (1 g/hour) over a period of 24 hours improved the RCD in pre-eclamptic pregnant women.[15] It was also examined the effect of Mg^{2+} as calcium antagonist on the RCD of pre-eclamptic patients *in vitro* and found that *in vitro* incubation with Mg^{2+} enhanced RCD.[14]

The reasons for the reduced RCD of PE are not still known. Some evidences showed that an increase in intracellular calcium (Ca^{2+}) concentration leads to a reduction in RCD as intracellular Ca^{2+} concentration plays a key role in regulating red blood cell mechanical properties.[15] Moreover, PE is a state of enhanced neutrophil action and of increased amounts of reactive oxygen species which tend to interfere with membrane lipids, resulting in changes of the membrane properties and stiffen RBCs.[14, 15] This change of membrane properties may also lead to elevated intracellular Ca^{2+}, which is known to reduce RCD.[7] It was found that Mg^{2+} (i.e. calcium-antagonist) is able to antagonize the effect of increased intracellular Ca^{2+} on RCD, resulting in an increase of RCD.[14]

Therefore, the purposes of the present research study were to determine RCD and serum Mg^{2+} con-centration in mild and severe PE patients and to find out the relationship between RCD and serum Mg^{2+} concentration in mild and severe PE patients.

MATERIALS AND METHODS

Study population

A cross-sectional, analytical study was conducted to 32 mild and 32 severe PE patients who admitted Obstetric Wards of Central Women's Hospital, Yangon.

Determination of red cell deformability

Red cell deformability was determined by simple filtration method mentioned. 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 PCV on flow rate, red cell deformability index (RCDI) was expressed as FR (ml/min) multiplied by packed cell volume (PCV_{bf}) before filtration in proportion.

Red cell deformability index=$FRxPCV_{bf}$[16]

Determination of serum magnesium level

Five millilitre of blood was collected in plain tubes. Then, blood sample was 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 serum magnesium level and stored at -20°C 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.[17] The Cobas C 111 system automatically calculates the analyze concentration of each sample.

Statistical analysis

Data were analyzed by using the Statistical Package for Social Science (SPSS) soft-ware version 22. Values were expressed as mean±SD. Student's 't' test (unpaired 't' test) was applied to compare two parameters (serum Mg^{2+} and RCD) between mild and severe pre-eclampsia. Pearson's correlation coefficient was calculated to assess the correlation between the variables. p value <0.05 was regarded as significant.

RESULTS

The detail baseline characteristics of the study population are shown in Table 1. Apart from clinical presentation of PE, other baseline parameters were found to be comparable between mild and severe PE patients. The mean value of flow rate, red cell deformability index (RCDI) and serum magnesium level of both groups were shown in Table 2 and these values were significantly lower in severe PE patients than mild PE patients (Fig. 1, Fig. 2 & Fig. 3).

Table 1. Baseline parameters in mild and severe pre-eclamptic pregnant women

Baseline parameters	Pre-eclamptic pregnant women (n=32)	
	Mild	Severe
Age (years)	31.91±5.53	31.75±6.22
Weight (kg)	71.89±12.53	74.97±14.45
Height (m)	1.52±0.04	1.52±0.05
BMI (kg/m^2)	31.28±5.04	32.49±5.96
Gravida	2±1	2±1
Maturity by date at the time of sampling (wk)	36.94±3.16	36.52±2.69
Maturity by scan at the time of sampling (wk)	36.35±2.74	35.33±2.89
Systolic blood pressure (mmHg)	149.06±6.47	178.38±10.15***
Diastolic blood pressure (mmHg)	95.06±5.54	111.94±5.65***
Mean arterial pressure (mmHg)	113.06±5.01	134.08±6.25***
Pulse rate (times per minute)	85.25±9.39	87.25±9.58
Proteinuria	++	+++

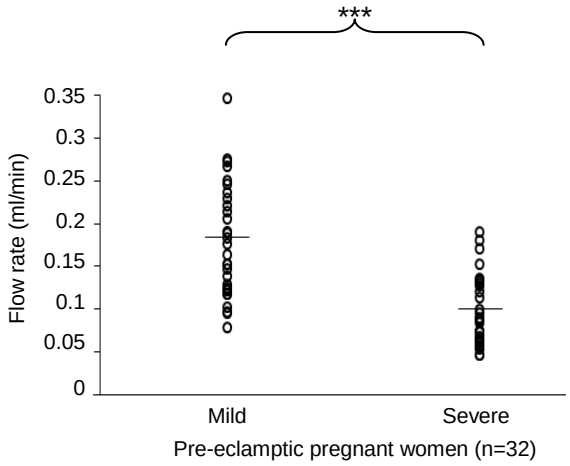
***indicates significant difference between two groups ($p<0.001$)

Table 2. Flow rate, red cell deformability index and serum magnesium level in mild and severe pre-eclamptic pregnant women

	Pre-eclamptic pregnant women (n=32)	
	Mild	Severe
Flow rate (ml/min)	0.18±0.07	0.10±0.04***
RCDI	0.06±0.02	0.03±0.01***
Serum Mg^{2+} level (mmol/l)	0.71±0.16	0.63±0.17*

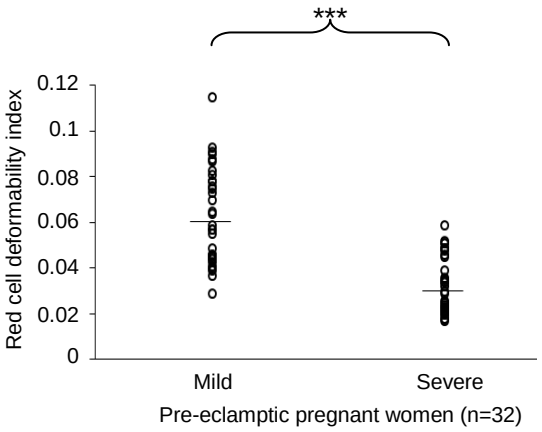
*indicates significant difference between two groups ($p<0.05$), ***indicates significant difference between two groups ($p<0.001$)

The results of the present study showed flow rate was positively correlated with serum Mg^{2+} level in PE patients, this correlation being stronger in the severe



Solid line (—) indicates mean of flow rate
***indicates significant difference (p<0.001)

Fig. 1. Comparison of flow rate between mild and severe pre-eclamptic pregnant women

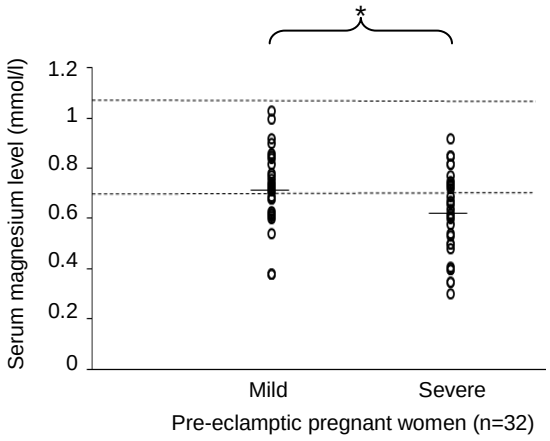


Solid line (—) indicates mean of red cell deformability index
***indicates significant difference (p<0.001)

Fig. 2. Comparison of red cell deformability index between mild and severe pre-eclamptic pregnant women

Table 3. Correlation between flow rate, RCDI and serum Mg^{2+} level in different study groups

Study group	Flow rate r value	p value	RCDI r value	p value
All PE (n=64)	0.47	p<0.001	0.47	p<0.001
Severe PE (n=32)	0.45	p<0.01	0.48	p<0.01
Mild PE (n=32)	0.40	p<0.05	0.40	p<0.05



Solid line (—) indicates mean of serum magnesium level, Upper dash line: Upper limit of normal serum magnesium level (0.70-1.05 mmol/l), Lower dash line: Lower limit of normal serum magnesium level (0.70-1.05 mmol/l)
*indicates significant difference (p<0.05)

Fig. 3. Comparison of serum magnesium level between mild and severe pre-eclamptic pregnant women

PE group (Table 3). In all PE patients, there was a significant positive correlation between RCDI and serum Mg^{2+} level. Thecorrelation was stronger in severe PE patients than mild PE patients (Table 3).

DISCUSSION

In the present study, RCDI was significantly lower in severe PE patients than mild PE patients. In addition, flow rate between severe PE patients and mild PE patients showed significant difference. This finding was in agreement with the findings of Heilmann[18] who reported that FR of PE patients with DBP ≤100 mmHg (0.49± 0.26 ml/min) was significantly higher than that of PE patients with DBP >100 mmHg (0.31±0.29 ml/min). Even though there was a difference in the disease state of the subjects participated in the studies, methods and population, RCD was found to be lower in PE patients and more pronounced in severe PE patients. Therefore, the findings of the present study indicated that reduction in RCD might be associated with the severity of PE.

Some researchers explained that reduction of RCD in PE may be due to oxidative stress because oxidative stress was definitely present in PE[6] and oxidative stress is involved in reduction of RCD by two means: directly by damaging the membrane structure[19] and indirectly through by damaging the Ca^{2+}-ATPase.[20] A previous study in Myanmar carried out by Su Su Htwe[21] reported that there was a significant negative correlation between plasma malondialdehyde level (oxidative stress marker) and RCD in normal and pre-eclamptic pregnant women. In addition, it was reported that the degree of oxidative stress is closely related to the clinical severity of PE.[22] Therefore, it could be concluded that the observed findings in the present study (i.e. reduction of RCD in relation to the severity of PE) might be due to oxidative stress.

Regarding serum Mg^{2+} level, the present study showed that serum Mg^{2+} of mild PE patients was significantly higher than that of severe PE patients. This finding was in agreement with Sandip, *et al.*[13] who reported that serum Mg^{2+} level in mild PE patients (0.89±0.07 mmol/l) was greater than severe PE patients (0.67±0.14 mmol/l).

The present study demonstrated that there was a significant positive correlation between flow rate and serum Mg^{2+} level in PE patients. There was a significant positive correlation between flow rate and serum Mg^{2+} level in both mild and severe PE patients. Red cell deformability index was also positively correlated with serum Mg^{2+} level in PE patients. Moreover, a significant positive correlation between RCDI and serum Mg^{2+} level was found in both mild PE patients and severe PE patients. These correlations were found to be more significant in severe PE patients than mild PE patients.

The effect of Mg^{2+} on RCD could be explained by two possible mechanisms: Ca^{2+} and reactive oxygen species. Some studies showed that PE is a state of inflammatory state and increased amounts of activated oxygen species which are known to interfere with membrane lipids, leading to changes of the red cell membrane properties.[7, 23] In addition, oxidase stress can inhibit Ca^{2+} ATPase and this may lead to elevated intracellular Ca^{2+}, which is known to reduce RCD[24] by enhancing tubulin polymerization.[25] It is well-known that magnesium is a Ca^{2+} antagonist which might block the effects of increased intracellular Ca^{2+} on the tubulin polymerization, thus improving RCD.[15] It was also reported that the effect of Ca^{2+} antagonists (Mg^{2+} or Nifedipine) on RCD from PE patients *in vitro* is the additional finding pointing to a Ca^{2+}-driven reduction of RCD.[14]

In conclusion, these results showed that reduction of RCD and serum Mg^{2+} level were more pronounced in severe PE patients than mild PE patients. Reduction in serum Mg^{2+} level could contribute to reduction in RCD in PE patients. It could be concluded that the changes in RCD and serum Mg^{2+} level might involve in the pathophysiology of severity of PE.

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