



EVALUATION OF MATERNAL SERUM CALCIUM, MAGNESIUM, AND THE CA:Mg RATIO AS EARLY PREDICTORS OF PREECLAMPSIA SEVERITY

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ABSTRACT

Background: Preeclampsia is a pregnancy-specific hypertensive disorder with severe outcome closely related to systemic endothelial dysfunction and trace element imbalances. The study was aimed to evaluate maternal serum calcium, magnesium and calcium-to-magnesium (Ca:Mg) ratio as early biochemical predictors of severity of preeclampsia.

Methods: This was a prospective observational case control study conducted over a period of 6 months (July 2014 to December 2014) in Tertiary care center in Central India. The study included 100 pregnant women (>20 weeks gestation) divided into normotensive control group (n=50) and preeclampsia case group (n=50). To assess severity of the disease, the preeclampsia group was further divided into mild and severe groups (n = 30 and n = 20, respectively). Total calcium and magnesium concentrations were measured in maternal serum using photometric assays and the mathematical ratio of Ca:Mg was calculated.

Results: Mean serum calcium and serum magnesium were significantly lower in women with preeclampsia (8.16 ± 0.74 vs. 9.42 ± 0.58 mg/dL, $p < 0.001$ and 1.62 ± 0.40 vs. 2.10 ± 0.32 mg/dL, $p < 0.001$ respectively) than the normotensive controls. However, Ca:Mg ratio was significantly increased in preeclampsia group (5.23 ± 0.88 vs. 4.54 ± 0.65 , $p < 0.001$). There was a progressive statistically significant increase in the Ca:Mg ratio with the severity of disease and was significantly higher in severe preeclampsia than in mild preeclampsia (5.72 ± 0.94 vs. 4.90 ± 0.72 , $p = 0.002$).

Conclusion: Relative hypocalcemia and hypomagnesemia are the main features of preeclampsia in Central Indian population. The Ca:Mg ratio is a very sensitive dynamic biochemical marker which accurately reflects the progressive vasospasm and escalation of the disease and provides a robust cost effective screening tool for early risk stratification.

Key words: Preeclampsia, Serum Calcium, Serum Magnesium, Calcium-Magnesium Ratio, Biomarkers

INTRODUCTION

Preeclampsia is a multisystem hypertensive disorder specific to pregnancy and is one of the leading

causes of maternal and perinatal morbidity and mortality worldwide. It is responsible for a large proportion of adverse obstetric outcomes, especially in developing countries like India, where maternal health resources are often limited [1]. Hypertensive disorders of pregnancy are one of the most important causes of severe maternal outcomes and the World Health Organization (WHO) has recommended strong measures for early detection and intervention [2]. Preeclampsia is diagnosed by clinical evidence of the development of hypertension (systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg) and proteinuria after 20 weeks of gestation. Not treating this disease can quickly lead to serious complications such as eclampsia, HELLP (Hemolysis, Elevated Liver enzymes, Low Platelet count) syndrome, placental abruption and fetal growth restriction [1, 3]. Preeclampsia is a pregnancy disorder that has been studied extensively for decades, but the exact cause has not been determined. However, the current “two-stage model” suggests the pathogenesis starts with abnormal placentation. Impaired invasion of the maternal spiral arteries by trophoblasts leads to narrow, high resistance vessels resulting in persistent placental ischemia and hypoxia [3]. The localized ischemic environment initiates the second step which leads to the release of circulating anti-angiogenic factors such as soluble fms-like tyrosine kinase-1 (sFlt-1) and various pro-inflammatory cytokines into the maternal circulation [4]. This cascade results in generalized maternal systemic endothelial dysfunction clinically manifesting as hypertension, proteinuria and end-organ damage [4]. In recent years, the central role of trace elements and micronutrients in the maintenance of endothelial integrity and the modulation of preeclampsia pathogenesis has been of considerable scientific interest [5]. Normal pregnancy is associated with marked physiological changes including hemodilution, altered renal clearance and exponentially increasing fetal nutritional demands. Calcium and magnesium are the most important trace elements for the regulation of vascular smooth muscle tone and systemic blood pressure [6]. Calcium is the main intracellular messenger of smooth muscle contraction and thus of vasoconstriction. Adequate calcium intake and serum levels in pregnancy is important to reduce vascular resistance [6]. But magnesium is a physiologic antagonist of calcium. It is a natural calcium channel blocker that competes for the same binding sites to antagonize calcium-dependent smooth muscle contraction [7]. Magnesium also improves the release of powerful vasodilators such as prostacyclin and nitric oxide from the endothelium and decreases oxidative stress [7, 8]. Magnesium sulfate has important neuroprotective and vasodilator effects that are important in the setting of severe preeclampsia and related to its therapeutic effect in the prevention of eclamptic seizures [8, 9].

The relation of serum calcium and magnesium is finely balanced and accurately controlled in normal pregnancies so that blood pressure is maintained within normal limits despite the increased intravascular volume. In preeclampsia this biological balance is said to be profoundly disturbed usually in favor of an intra-cellular dominant state of calcium with relative extra-cellular depletion of magnesium [10]. Measurement of absolute serum concentrations of these elements is of interest, but interpretation of individual values is difficult because of normal gestational hemodilution. The Ca:Mg ratio could be a much more sensitive, mathematically normalized and dynamic representation of the underlying endothelial biochemical environment. The biological plausibility for the role of these essential minerals in the pathophysiology of preeclampsia is strong but the existing global literature provides conflicting evidence. These differences are largely due to regional dietary differences, genetic predisposition, variation in baseline maternal health and methodology between studies. Hence, the present study aimed to evaluate the levels of maternal serum calcium, magnesium and certain Ca:Mg ratio and their use as early, cheap biochemical predictors of severity of preeclampsia among pregnant women.

MATERIALS AND METHODS

Study Design and Setting

This prospective, observational case-control study was conducted in the Department of Obstetrics and Gynecology at Tertiary care center in Central India. The study was carried out over a strict duration of 6 months, from July 2014 to December 2014.

Study Population

A total of 100 pregnant women attending the routine antenatal clinic and the obstetric emergency triage were recruited for the study. The sample size was strategically divided into two distinct, matched groups based on rigorous diagnostic criteria endorsed by obstetric guidelines [11]:

- Group A (Control): 50 healthy, normotensive pregnant women undergoing routine antenatal care.
- Group B (Cases): 50 pregnant women definitively diagnosed with preeclampsia.

To precisely evaluate the capacity of biochemical markers to reflect disease severity, Group B was further stratified into two clinical sub-categories:

1.Mild Preeclampsia (n=30): Defined by a blood pressure $\geq 140/90$ mmHg but $< 160/110$ mmHg on two separate occasions at least 4 hours apart, accompanied by proteinuria of $\geq +1$ on a standard urine dipstick.

2.Severe Preeclampsia (n=20): Defined by a persistently elevated blood pressure $\geq 160/110$ mmHg, profound proteinuria of $\geq +3$, or the presence of specific severity features such as unrelenting headache, visual disturbances, epigastric pain, or oliguria [11].

Inclusion and Exclusion Criteria

Women aged 18 to 35 years with a confirmed singleton pregnancy at > 20 weeks of gestation were included in the study. To ensure that the biochemical alterations observed were strictly attributable to preeclampsia rather than pre-existing conditions, a stringent set of exclusion criteria was applied. Patients with pre-existing chronic hypertension, pre-gestational type 1 or type 2 diabetes mellitus, chronic renal disease, established hepatic dysfunction, overt thyroid disorders, or a history of metabolic bone diseases were completely excluded. Furthermore, women who had already commenced therapeutic intravenous magnesium sulfate or who were on specific high-dose oral calcium/magnesium supplementation beyond standard prenatal multivitamins prior to the initial blood sample collection were excluded to eliminate direct biochemical confounding.

Data and Sample Collection

Following explicit, written informed consent, detailed demographic data, obstetric history, and past medical histories were recorded using a standardized proforma. Standardized blood pressure measurements were taken in a seated position after a minimum of a 10-minute resting period using a properly calibrated mercury sphygmomanometer. For the biochemical analysis, 5 mL of venous blood was drawn from the antecubital vein of each participant under strict aseptic precautions using a tourniquet applied for less than one minute to prevent hemoconcentration. The samples were collected in plain, sterile vials without anticoagulants, allowed to clot undisturbed at room temperature for 30 minutes, and subsequently centrifuged at 3000 rpm for 10 minutes to separate the serum.

Statistical Analysis

All raw clinical and biochemical data were compiled, cross-verified, and coded using Microsoft Excel spreadsheets. Formal statistical analysis was executed utilizing appropriate statistical software. A predefined p-value of < 0.05 (two-tailed) was considered statistically significant for all tests.

Results

Table 1: Baseline Demographic and Clinical Characteristics of the Study Population

Parameter	Normotensive Group (n = 50) Mean $\pm$ SD	Preeclampsia Group (n = 50) Mean $\pm$ SD	p-value
Maternal Age (years)	24.6 $\pm$ 3.4	25.1 $\pm$ 3.8	0.490

Gestational Age (weeks)	33.2 ± 2.6	32.8 ± 3.1	0.485
Systolic BP (mmHg)	114.2 ± 8.6	158.4 ± 14.2	< 0.001*
Diastolic BP (mmHg)	74.6 ± 6.2	104.8 ± 11.5	< 0.001*
BMI (kg/m ²)	23.4 ± 2.1	24.8 ± 3.2	0.014*

*Statistically significant (p < 0.05)

Table 1 shows a systematic overview of the demographic baseline and main clinical characteristics of the study population (n=100). Mean maternal age (24.6 versus 25.1 years, p = 0.490) and mean gestational age at the time of clinical presentation (33.2 versus 32.8 weeks, p = 0.485) were not statistically significantly different between the normotensive control women and the preeclamptic cohort. This means that the groups were very well matched and comparable. As expected from the study design and diagnostic criteria, systolic and diastolic blood pressures were significantly higher in the preeclampsia group compared with the healthy controls (p < 0.001). The maternal body mass index (BMI) was also significantly higher in the preeclamptic group.

Table 2: Comparison of Serum Calcium, Magnesium, and Ca:Mg Ratio Between Groups

Biochemical Marker	Normotensive Group (n = 50) Mean ± SD	Preeclampsia Group (n = 50) Mean ± SD	p-value
Serum Calcium (mg/dL)	9.42 ± 0.58	8.16 ± 0.74	< 0.001*
Serum Magnesium (mg/dL)	2.10 ± 0.32	1.62 ± 0.40	< 0.001*
Ca:Mg Ratio	4.54 ± 0.65	5.23 ± 0.88	< 0.001*

Core biochemical analysis revealed dramatic statistically significant changes in trace element profiles of the preeclamptic group compared to the healthy normotensive controls (Table 2). Mean serum calcium level was significantly lower in preeclamptic women (8.16 mg/dL vs. 9.42 mg/dL) indicating a definite state of relative physiological hypocalcemia. The mean serum magnesium levels were also significantly lower in the preeclamptic group (1.62 mg/dL vs. 2.10 mg/dL). The mathematical Ca:Mg ratio was significantly higher in women with preeclampsia because of a greater decrease in magnesium than the decrease in calcium (5.23 vs 4.54, p < 0.001).

Table 3: Comparison of Biochemical Markers Based on Preeclampsia Severity

Biochemical Marker	Mild Preeclampsia (n = 30) Mean ± SD	Severe Preeclampsia (n = 20) Mean ± SD	p-value
Serum Calcium	8.35 ± 0.61	7.88 ± 0.84	0.024*

(mg/dL)			
Serum Magnesium (mg/dL)	1.74 ± 0.35	1.44 ± 0.38	0.008*
Ca:Mg Ratio	4.90 ± 0.72	5.72 ± 0.94	0.002*

*Statistically significant ($p < 0.05$)

The preeclamptic group was classified into mild and severe preeclampsia according to clinical severity so as to assess the predictive and diagnostic value of these biochemical markers in determining the clinical severity of the disease in real life (Table 3). Serum total calcium and serum magnesium showed a highly objective, progressive decline with increasing severity of preeclampsia. In severe preeclamptics the serum magnesium levels were low (1.44 mg/dL) and mean Ca:Mg ratio was high (5.72). The Ca:Mg ratio in mild and severe preeclampsia was statistically highly significant ($p=0.002$) firmly establishing its potential as a dynamic biological marker for disease escalation and impending vascular crisis.

DISCUSSION

Preeclampsia is indeed one of the most clinically unpredictable and inherently dangerous obstetric complications faced in modern medicine. It is very important to identify and validate readily available, inexpensive biomarkers to identify women at high risk for progression from mild symptomatology to life-threatening severe preeclampsia. A 6 month prospective study carried out at Tertiary care center in Central India concluded that pregnant women with preeclampsia had significantly lower levels of maternal serum calcium and magnesium compared with normotensive and healthy pregnant women. More importantly, our results suggest the Ca:Mg ratio as a robust mathematically normalized correlate of severity of preeclampsia, which increases progressively with worsening clinical phenotype. The biochemical observations depend on interdependent antagonistic roles of calcium and magnesium in maintenance of normal vascular architecture and basal endothelial tone. Calcium is the principal agent in smooth muscle cell contraction. Magnesium is a direct competitive antagonist at the cellular level, effectively blocking transmembrane transit of calcium to the intracellular matrix and promoting necessary vasodilatation [7, 10]. Critically deficient circulating serum magnesium effectively abolishes this protective inhibitory blockade and results in unopposed rapid intracellular calcium influx. This biochemical cascade is directly implicated in the profound systemic endothelial vasospasm characteristic of preeclampsia [7]. These findings are highly in agreement with early and fundamental pathophysiological findings of Sukonpan and Phupong who firmly reported that both serum calcium and serum magnesium levels were significantly and measurably decreased in the preeclamptic cohorts versus normally progressing pregnancies [12]. This has been echoed strongly in regional demographic-specific studies. Kanagal had conducted a detailed survey of the coastal population of India and found very low serum calcium levels in preeclamptic women which is indicative of a profound pattern of nutritional and biochemical deficiency in the Indian subcontinent [13]. Jain et al. also observed a significant correlation between hypocalcemia and hypomagnesemia and hypertensive disorders in pregnancy, supporting the hypothesis of trace element depletion as a basic pathological bedrock [14]. The dietary profiles of the central Indian populations like the cohort observed are very unique and are predominantly plant based and highly vegetarian. However, diets high in some macronutrients often lack highly bioavailable essential trace minerals such as magnesium and calcium due to the high phytate content of local grains inhibiting intestinal absorption [15]. The Ca:Mg ratio seems to have a special diagnostic and prognostic value and this is the most clinically interesting finding of our present study. The normal physiological haemodilution that occurs with increasing rapidity during the late second and third trimesters of pregnancy often leads to equivocal

or borderline clinical interpretation of individual trace element levels in isolation. These fluid volume changes are mathematically, effectively, normalized via the Ca:Mg ratio. We observed a significantly higher and alarming Ca:Mg ratio in preeclampsia women (5.23 Vs 4.54). Our data clearly show that the ratio is significantly and progressively divergent at the disease progression from mild to severe clinical stage. The clinical importance of applying this ratio in the daily practice is very high. Routine monitoring of the Ca:Mg ratio by readily available low-cost photometric assays in standard third-trimester antenatal care would seamlessly achieve early stratification of maternal risk. This would give exactly the right direction for timely therapeutic interventions including increased fetal surveillance, prophylactic trace mineral correction or timely delivery to mitigate severe maternal morbidity.

CONCLUSION

The characteristic feature of preeclampsia in pregnant women of Central India is severe hypomagnesemia with significant physiological hypocalcemia. The calculated Ca:Mg ratio seems to be a very significant, dynamic and reliable biochemical marker that accurately reflects the underlying pathophysiological vasospasm that characterizes the disorder. The ratio is so clearly associated with increasing severity of disease that its routine protocol-driven evaluation in antenatal care has enormous promise. It provides an early low cost predictor that allows important risk stratification, guides specific therapeutic interventions and eventually reduces the high toll of maternal and perinatal mortality.

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