International Journal of Research in Medical Sciences
Biswas S et al. Int J Res Med Sci. 2016 Jun;4(6):1879-1883
www.msjonline.org
pISSN 2320-6071 | eISSN 2320-6012
DOI: http://dx.doi.org/10.18203/2320-6012.ijrms20161482

Research Article

Comparative study of copper, zinc, iron, ferritin, calcium and magnesium levels in pregnancy induced hypertension and normotensive primigravida mothers

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Received: 12 May 2016
Accepted: 20 May 2016

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ABSTRACT

Background: Exact pathophysiology of pregnancy induced hypertension (PIH) is still unknown but there is a definite relationship between trace elements and preeclampsia. Several studies in this context have conflicting reports. So, a comparative study of serum levels of copper (Cu), zinc (Zn), iron (Fe), ferritin, calcium (Ca) and magnesium (Mg), in PIH and normotensive primipara mothers was conducted.
Methods: Study was conducted in Department of Biochemistry & Department of Gynaecology and Obstetrics, R. G. Kar Medical College & Hospital, Kolkata over 1 year from September 2013 to August 2014 on fifty PIH patients as cases and fifty normotensive primipara mother without proteinuria as controls, both having ages ranging between 15-35 years in 2nd and 3rd trimesters.
Results: Serum Ca, Mg, Cu and Zn levels were found to be significantly reduced (<0.05) in the PIH group compared to the normal pregnant group. Serum ferritin was markedly increased in the cases (mean 90.41±47.39, p<0.00001). No significant correlation was found in serum Fe levels.
Conclusions: Alteration of serum Cu, Zn, Ca, Mg and ferritin levels can be considered to have a role in the etiopathogenesis and severity of PIH.

Keywords: Copper, Zinc, Iron, Ferritin, Calcium, Magnesium, Pregnancy induced hypertension

INTRODUCTION

Pregnancy induced hypertension (PIH) is defined as the hypertension that develops as a direct result of gravid state having blood pressure (BP) ≥140/90 mm Hg measured twice at least 6 hours apart but not more than 7 days.[1] PIH complicates at least 10% pregnancies worldwide.[2] In spite of reduction in maternal mortality in many developed countries, PIH is still one of the leading causes of maternal death worldwide.[3]

Trace elements calcium (Ca) and magnesium (Mg) are essential micronutrients while iron (Fe), zinc (Zn) and copper (Cu) have antioxidant properties. These elements should be supplemented as a daily requirement in pregnant women. Deficiency or disruption of metabolism of these elements can complicate pregnancy and compromise fetal growth.

These days, there has been worldwide upsurge in the incidence of PIH. PIH affects multisystem including coagulation pathways and contributes substantially to perinatal morbidity and mortality of both mother and newborn. High level suspicion about PIH can be guided by epidemiological and clinical risk factors, yet with no specific biomarkers.[4] Its exact pathophysiology is still

unknown but there is a definite relationship between trace elements and preeclampsia. Several studies in this context have conflicting reports. Some of them show that, changes in the levels of serum trace elements in preeclamptic patients have role in its pathogenesis while others have failed to show any association.[2,5]

Calcium has role in vascular smooth muscles functions. Alteration of plasma Ca level leads to raised blood pressure. Mg acts as cofactor for essential enzymes like sodium potassium ATPase and in peripheral vasodilatation. Some studies show that Ca and Mg have a relaxant effect on the blood vessels of pregnant women.[6] Cu is a cofactor in cytochrome oxidase, catalase and superoxide dismutase (SOD).[4] Serum Cu level gets raised in pregnancy normally due to increased level of ceruloplasmin, a Cu binding protein with antioxidant ferroxidase properties.[2,5] Cu participates in single electron reactions and catalyses generation of free radicals, including hydroxyl radicals which causes oxidative stress characteristic of preeclampsia. So, though Cu/Zn SOD functions as an antioxidant, Cu by itself has pro-oxidant character. Zn is a cofactor in >200 metalloenzymes of carbohydrate and protein metabolism, nucleic acid synthesis, antioxidant Cu/Zn SOD and performs important role in cell differentiation and embryogenesis.[6] Plasma zinc concentrations declines in pregnancy. Reduced level of Zn in both serum and amniotic fluid of preeclamptic mothers compared to normal healthy pregnant mothers has been documented in various studies.[7,8] Iron and folic acid supplementation are universally given in pregnancy. But role of ferritin in PIH and iron as potentiators of pro-oxidants has been documented in literature.[9,10]

METHODS

It was a comparative cross sectional study of one year, September 2013 to August 2014, conducted in the Dept. of Biochemistry, Dept. of Gynaecology and Obstetrics, R. G. Kar Medical College & Hospital, Kolkata. The study involved two groups of subjects- group I (controls) was 30 healthy pregnant women and group II (cases) was 30 PIH women registered in antenatal clinic of R. G. Kar MCH. Data of cases and controls were matched for age, gestational age, anthropometrics and socioeconomic status. Inclusion criteria were: primigravida of gestational age 2nd & 3rd trimester, willing to participate in the study. The exclusion criteria were: known diabetic, known hypertensive, history of cardiovascular and/or renal insufficiency, chronic liver disease, infective hepatitis, HIV, TORCH, alcohol consumption, smoking.

Fasting blood samples (10 ml) were collected at 8-9 a.m. into polypropylene tubes. Serum was separated within 2 hours and aliquots were kept frozen at -20°C until trace element analysis. All laboratory wares including pipette tips and autosampler cups were cleaned thoroughly with detergent and tap water, rinsed with distilled water, soaked in dilute nitric acid and then rinsed thoroughly with deionized distilled water. Determination of these elements performed on a semiautoanalizer using the following methods in Table 1.

Table 1: Methods used for estimation of trace elements.

Parameters	Methods
Cu	Di-Br-PAES colorimetric method
Zn	Nitro-PAPS colorimetric method
Iron	Ferrozine method
Ferritin	ELISA
Ca	O-Cresolphthalein Complexone method
Mg	Calmagite method

The accuracy of the measurement was evaluated based on recovery studies and analysis of quality control material (QCM) (Seronorm TM Trace Elements, serum, Level 1, Art. No. 201405, Norway).

Informed consent was obtained from all participants. The study was approved by Institutional Ethical Committee. Statistical evaluation was carried out by using the SPSS 20 Version for Windows (USA, Houston). All results were expressed as mean values±SD. Group means comparisons were tested for significance by student's t-test. Statistical significance was defined as $p<0.05$.

RESULTS

Most subjects in both groups were in the age range of 18-30 years. The pregnancy age at the time of sampling was 16-40 weeks for most subjects in both groups. The mothers' mean (SD) age was 26.77±4.77 years in normal pregnancy group and 26.37±4.93 years for the PIH group. As it was expected, the results of t-test showed that the two groups were matched in mothers' age and pregnancy age at the time of sampling (Table 2).

Table 2: Basic parameters.

Parameters	Group I (controls)	Group II (cases)	p value
Age (years)	26.77±4.77	26.37±4.93	0.791
BMI (Kg/m^2)	24.65±3.70	29.73±1.58	<0.00001
Gestational age (weeks)	28.87±4.61	32.30±3.72	0.002
Hb (g/dl)	10.28±1.12	10.90±1.34	0.058
Mean Systolic BP (mm Hg)	106.73±7.85	147±10.49	<0.00001
Mean Diastolic BP (mm Hg)	65.40±5.66	95.13 ±4.35	<0.00001

The serum Zn level was above 50 mg/dl in all subjects, 126.60±42.14 for controls and 78.81±15.15 for cases with highly significant p value (<0.001). Ca, Mg, Cu levels were significantly increased in normal pregnancy than

PIH ($p<0.01$, $p<0.001$ and $p<0.001$ respectively). No statistically significant variations were observed in serum Iron concentration ($p>0.23$), while Ferritin significantly increased in PIH mothers ($p<0.001$) (Table 3).

Table 3: Serum parameters.

Parameters	Cases	Controls	p value	Significance
Copper	103.30± 14.61	177.87± 24.44	<0.001	Highly significant
Zinc	78.81± 15.15	126.60± 42.14	<0.001	Highly significant
Iron	196.07 ±65.49	216.67± 65.47	0.23	Not significant
Ferritin	90.41± 37.39	25.71± 11.38	<0.001	Highly significant
Calcium	8.66± 0.71	9.15± 0.46	<0.01	Significant
Magnesium	1.67± 0.56	2.09± 0.30	<0.001	Highly significant

SBP was negatively correlated with all serum parameters except ferritin which was also statistically significant. Iron was found to be negatively correlated with SBP but positively correlated with DBP. Only the negative correlation of Zn with SBP was statistically significant (Table 4).

Table 4: Pearson's bivariate correlation.

Parameters	Systolic BP (r)	p value	Diastolic BP (r)	p value
Copper	-0.0935	0.623 (NS)	-0.2908	0.119 (NS)
Zinc	-0.6595	<0.05 (S)	-0.0100	0.958 (NS)
Ferritin	0.5079	0.004 (S)	0.0660	0.729 (NS)
Iron	-0.0368	0.847 (NS)	0.1908	0.312 (NS)
Calcium	-0.1968	0.297 (NS)	-0.1843	0.330 (NS)
Magnesium	-0.0914	0.631 (NS)	-0.2014	0.286 (NS)

DISCUSSION

Copper is an essential cofactor for a number of enzymes like catalase, Cu/Zn SOD and cytochrome oxidase. It is involved in metabolic reactions, angiogenesis, oxygen transport and antioxidation. It is also essential for embryonic development.[6] Cu gets transferred across placenta via high-affinity copper transporter (CTR1) and is related to iron transport by an unknown mechanism.[11] Approximately 96% of plasma Cu remains strongly bound to ceruloplasmin, a major copper-binding protein with ferroxidase properties.[2,5] Ceruloplasmin level rises during pregnancy due to altered levels of oestrogen and in response to increased lipid peroxidation.[8,12] Subsequently, Cu requirements also increase. Another reason for this increase is due to blockade in the transfer of Cu to fetus by the placenta.[13] High level of maternal Cu participate in single electron reactions and free radicals, including undesirable hydroxyl radicals which contributes to oxidative stress characteristic of preeclampsia.[12] So role of Cu is both pro-oxidant and antioxidant.

Zinc takes part in carbohydrate, protein and nucleic acid metabolism, antioxidant functions, cell division and differentiation, making it essential for successful embryogenesis. Its requirement during the third trimester is approximately twice as high as that in nonpregnant women.[14] Zn deficiency has been associated with preeclampsia including adolescent pregnancies in the 1980s.[15,16] Low serum Zn concentrations in preeclampsia mothers have been suggested to be partly due to reduced oestrogen and zinc binding-protein levels.[17] Placental Zn concentration has also been shown to be lower in preeclampsia in cross-sectional retrospective studies with placental Zn values positively correlating with birth weights.[18,19] More recently lower serum concentrations of Zn in preeclampsia compared to controls have been shown in two relatively small retrospective studies from Turkey (mean ± SD: 10.6±4.4 versus 12.7±4.1 µg/L, respectively).[12,20] A retrospective study from India has reported that serum Zn is less in preeclamptic mothers compared to controls which compromises the antioxidant protection and raises blood pressure.[21] Amniotic fluid Zn concentrations have also been reported to be decreased in preeclamptic women delivering preterm (33-36 weeks gestation) in a small retrospective cross-sectional study from USA.[22]

Over decades, many studies have been conducted to evaluate iron status in pregnancy and its possible contributory role in oxidative stress in preeclampsia, but there were different conclusions. In our study there was no significant difference in maternal age between normal pregnant and preeclamptic women. Several Western studies have found no difference in haemoglobin concentration and haematocrit in preeclamptic group while in our study, it was observed that hemoconcentration occurs in preeclampsia and that altered hemodynamics may play a partial role in causing hyperferritinemia.[23,24] Normal women has a decrease in serum iron and ferritin during the third trimester of pregnancy as their stores of iron are depleted because of fetoplacental demand and expansion of red cell mass.[25] However, elevated level of serum iron in pre eclamptic as compared to normal pregnant women have also been reported.[26] Local iron excess and iron mediated oxidative stress have been demonstrated in the intestinal mucosa, liver spleen, bone marrow and placenta and the production of hydroxyl and methoxyl radicals in gastrointestinal tract proves the role of iron in free radical damage.[27] The possible contribution of released iron free radicals from ischemic placenta in preeclampsia may contribute to its etiology.

Serum ferritin is a reliable indicator of total body iron status in non-diseased individuals, with low concentration diagnostic of iron deficiency. However a high ferritin does not always signify iron excess. Elevated serum ferritin occurs in a variety of clinical conditions with non-utilization of iron and destruction of tissues such as in hemolytic anemia, hepatic damage or to suppression of erythropoiesis leading to accumulation of storage iron.[28] In our study, serum ferritin was found to be elevated in preeclamptic group, which is in agreement with study conducted by Raman L et al.[26] One American prospective observational study performed on 450 women found that high ferritin was associated with increased risk for preterm delivery and neonatal asphyxia, while the lower ferritin level was associated with decreased risk of preeclampsia, prelabour rupture of membranes.[29] Increased concentration of serum ferritin during third trimester may be part of an acute phase response, which suggests maternal infection and increased risk of poor pregnancy outcome.[25] Iron supplements and increased iron stores have recently been linked to maternal complications e.g. gestational diabetes and increased oxidative stress during pregnancy.[30] So, while iron supplementation may improve pregnancy outcome in iron deficient mother, there is also possibility of increased risk of preeclampsia when there is no iron deficiency. Estimates of gestational iron requirements and of the proportion of iron absorbed from different iron supplemental doses suggest that with present supplementation schemes the intestinal mucosal cells are constantly exposed to unabsorbed iron excess and oxidative stress.[31]

Calcium takes part in muscle contraction and regulation of cellular water balance.[32] In our study, there was statistically significant reduced levels of Ca in cases compared to controls. Similar findings were reported in other studies conducted in India and abroad.[6,33,34] Low serum Ca stimulates parathyroid hormone and rennin release which then increase intracellular Ca in vascular smooth muscle.[33] This causes vasoconstriction, increase of vascular resistance and rise in blood pressure in preeclamptic mother.[2,6]

Magnesium increase prostacyclin release from the endothelial cells of blood vessels, which acts as potent vasodilator.[2] Hypomagnesemia in preeclampsia is generally associated with hemodilution, altered renal clearance and consumption by growing fetus.[32] Low Mg levels potentiates contractile response of vascular smooth muscle to angiotensin II and nor adrenaline. The low levels of Ca and Mg during pregnancy are exaggerated in preeclampsia.[2] That's why $MgSO_4$ is used for prevention and treatment of convulsions.

CONCLUSION

Natural homeostasis tends to maintain normal calcium, magnesium, copper and zinc levels. But our study population had low levels of these elements due to inadequate dietary intake owing to poor socioeconomic status. So, chronic deficiency has a definite role in the pathophysiology of PIH. Serum ferritin increases in PIH mother and has significant positive correlation with SBP. Unnecessary routine iron supplementation can further worsen the situation. Whether monitored and regulated supplementation of calcium, magnesium, copper, zinc and iron in pregnancy can prevent PIH is a scope for further research.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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Cite this article as: Biswas S, Roy A, Biswas S. Comparative study of copper, zinc, iron, ferritin, calcium and magnesium levels in pregnancy induced hypertension and normotensive primigravida mothers. Int J Res Med Sci 2016;4:1879-83.