ORIGINAL ARTICLE

Evaluation of serum copper, zinc and magnesium in pre-eclampsia and gestational diabetes in Calabar, Cross River State, Nigeria

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ABSTRACT

Objectives: Alterations in zinc (Zn), copper (Cu) and magnesium (Mg) levels in blood have been linked to gestational diabetes mellitus (GDM) and pre-eclampsia. This study aimed at evaluating the levels of some serum minerals in women with pre-eclampsia and gestational diabetes mellitus in order to understand their relationship with these pregnancy disorders.

Methods: Ninety (90) pregnant women (18-45 years) attending ante-natal clinic at General Hospital, Calabar were recruited into this case control cross sectional study. Thirty (30) were clinically diagnosed with gestational diabetes mellitus, 30 clinically diagnosed with pre-eclampsia and 30 were apparently healthy women. Serum Zn, Cu and Mg were determined using Atomic Absorption Spectrometry. Random blood glucose (RBG) was estimated using a glucometer. Urine protein estimation was done turbidimetrically with trichloroacetic acid. Data was analysed using SPSS version 22.0 with ANOVA and Pearson's correlation. P <0.05 was considered statistically significant.

Results: The zinc levels were significantly lower in gestational diabetes mellitus and pre-eclamptic group compared to the control group ($p<0.001$). No significant difference was found in mean serum copper and magnesium levels. A positive correlation ($r=0.422$, $p=0.020$) was observed between magnesium and random blood glucose in the gestational diabetes mellitus group. A negative correlation was observed between zinc and systolic blood pressure ($r=-0.471$, $p=0.001$), diastolic blood pressure ($r=-0.485$, $p=0.001$) and urine protein ($r=-0.399$, $p=0.001$) in all study participants.

Conclusion: Significant changes in serum zinc was demonstrated in pre-eclampsia suggesting a relationship with pathogenesis of this disorder. There was however no significant difference in levels of copper and magnesium, thus no relationship between them and gestational diabetes mellitus and pre-eclampsia.

Keywords: Pre-eclampsia, gestational diabetes Mellitus, Copper, Zinc, Magnesium.

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INTRODUCTION

Pregnancy initiates many metabolic and physiological changes over a period of 40 weeks, and may have complications such as hyperemesis gravidarum, gestational diabetes (GDM), intrauterine growth restriction (IUGR) and pre-eclampsia (1). Nigeria's approximate 40 million women of child-bearing age (between 15-49 years) suffer a disproportionately high level of health issues contributing 10% of global deaths for pregnant mothers (2). Gestational diabetes mellitus (GDM) and pre-eclampsia are both serious complications of pregnancy threatening both mother and foetus's life (3).

Gestational diabetes is defined as glucose intolerance resulting in hyperglycaemia of variable severity with onset or first recognition during pregnancy. According to the most recent International Diabetes Federation (IDF) estimate GDM affects approximately 14% of pregnancies worldwide, representing approximately 18 million births annually (4). Pre-eclampsia is a pregnancy specific disorder characterised by hypertension, significant proteinuria with or without oedema. It complicates 2-7% of all pregnancies. It remains one of the most common medical complications of pregnancy and is a major cause of maternal and perinatal mortality worldwide (5).

The exact pathogenic mechanism of both pre-eclampsia and GDM is uncertain however disturbances in mineral status have been suggested to play essential or contributory roles in the pathogenesis and progression of these disorders (6). The important role of zinc and copper in robust antioxidant capacity and magnesium's vital role in blood pressure regulation and signal transduction of insulin make them important in understanding suggested roles of serum minerals in the pathogenesis of both disorders. Previous studies have established significant alterations of serum zinc, copper and magnesium levels in pre-eclampsia and GDM (6, 7) suggesting they may contribute to these disorders. However, reports describing their associations vary greatly (7, 8).

This research was therefore designed to determine whether abnormal levels of these trace elements in pregnant women exist in these disorders.

MATERIALS AND METHODS

Study design/subject selection

A total of 90 women attending ante-natal clinic of General Hospital, Calabar were recruited into this case control cross sectional study. They were aged between 18-45 years. Out of the 90 pregnant women, 30 were clinically diagnosed with gestational diabetes Mellitus (GDM), 30 clinically diagnosed with pre-eclampsia and 30 were apparently healthy women (controls). Ethical clearance was obtained from the Cross River State Ministry of Health. A well-structured questionnaire was administered to obtain demographic data and their informed consent to participate in the study was obtained. The study was conducted between June 2021 and August 2021.

Inclusion criteria

Apparently healthy multiparous pregnant women in their second and third trimesters were recruited as controls and pregnant women diagnosed with pre-eclampsia and GDM as test subjects. They were within the age range of 18 and 45 years.

Exclusion criteria

Those excluded from the study were those whose informed consent were not obtained, those who did not fall within the specified age range (18-45 years), Pregnant women in their first trimester. Pregnant women diagnosed with other conditions, non-pregnant females and those on iron supplements.

Sample size

A sample size of 18 was obtained using the formula described by Naing *et al* (9) allowing an expected proportion of occurrence of 1.2% from data of the study by Koofreh *et al* (10) with 95% confidence limit. However, the sample size was increased to 30 for better representation of the study population and reduction in error margin.

Sample collection

Using aseptic technique, 5mL of blood was collected via venepuncture into a clean dry plain sample container and kept from sunlight. The sample was allowed to clot, dislodged and then spun in a centrifuge at 3000rpm for 5 minutes to obtain serum. The serum was transferred into a 5mL plain container, correctly labelled and stored frozen at -20^{o}C until time of analysis. On the spot urine was also collected into a sterile universal container, correctly labelled and stored frozen (-20^{o}C) until time of analysis.

Measurement of blood pressure

Blood pressure was measured in a well-seated and relaxed position after resting about 10 minutes, using a digital blood pressure monitor from OMRON HEALTHCARE LTD, United Kingdom. Two readings were taken from each subject and the systolic and diastolic blood pressures were recorded after computing the average of the two readings.

Measurement of weight and height

The measurement of weight was achieved using a bathroom weighing scale. The participants were made to stand erect bare -footed and facing front. The values were read to the nearest 0.1kg. A stadiometer was used in the measurement of height. Each participant was instructed to stand erect, without any shoes and cap against a wall. Their heights were read in metres.

Determination of body mass index

Body Mass Index (BMI) was obtained by finding the ratio of weight (kilograms) to the square of height (metres^2). This is expressed in kg/m^2.

Laboratory assays

Serum copper, zinc and magnesium were estimated for both the test and control samples using Atomic Absorption Spectrometry (AAS) with instrument from Agilent Technologies. Santa Clara, USA. Random blood glucose was estimated with the aid of a glucometer (Fine Test by Osang Healthcare). Urine protein analysis was by the turbidimetric technique using trichloroacetic acid (11). Qualitative analysis of protein and glucose in urine was done using combi-2 test strip (Medi-test by Macherey-Nagel Inc, Allentown, USA).

Statistical analysis

Data collected was entered into Microsoft excel spreadsheet and analysed using Statistical Packages for Social Science (SPSS) version 20.0 for determination of mean, standard deviation and comparison of variables was done using analysis of variance (ANOVA) and correlation analysis with Pearson's correlation, with a $p<0.05$ considered statistically significant.

RESULTS

A comparison of the age, BMI, blood pressure and biochemical parameters among the three groups studied showed no statistically significant variation in the mean values of age ($p=0.208$), magnesium ($p=0.344$) and Copper ($p=0.253$). However, there were statistically significant variations in the mean values of zinc, body mass index (BMI), random blood glucose (RBG), urine protein, systolic and diastolic pressures ($p<0.05$) among the groups. The mean zinc values of the pre-eclamptic and GDM groups were significantly lower than those of the controls. However, the mean zinc values of the pre-eclamptic group were significantly lower than the GDM group.

The Pre-eclamptic group also had significantly higher BMI, systolic and diastolic pressures and urine protein compared to other groups. GDM subjects had significantly higher RBG compared to other groups (Table 1). The blood pressure and urine protein were significantly higher in the pre-eclamptic group compared to the controls while Zn was significantly lower. The GDM subjects had significantly higher Zn and RBG levels, and significantly lower BMI, Blood pressures and urine protein levels compared to the pre-eclamptic subjects (Table 2). A significant positive correlation ($r=0.422$, $p=0.020$) was observed between magnesium and random blood sugar in the GDM group (Table 3).

Table 1: Comparison of mean physical and biochemical parameters of normotensive, gestational diabetes mellitus and pre-eclamptic women.

Parameter	Control n=30	Pre-eclampsia n=30	Gestational Diabetes n=30	F-cal	p-value
Age (years)	30.0 ± 5.39	28.70 ± 5.27	31.1 ± 4.94	1.600	0.208
Magnesium (mmol/L)	0.93 ± 0.39	0.81 ± 0.17	0.91 ± 0.35	113.41	0.344
Zinc (ug/L)	106.1 ± 16.6	80.0 ± 14.2	97.9 ± 16.9	23.026	<0.001*
Copper (ug/L)	116.9 ± 19.4	117.8 ± 18.2	124.3 ± 17.9	32.178	0.253
BMI (kg/m^2)	30.33 ± 3.61	33.39 ± 6.57	30.32 ± 4.19	14.937	0.027*
Systolic BP (mmHg)	116.6 ± 4.84	138.7 ± 11.4	115.3 ± 5.71	0.312	<0.001*
Diastolic BP (mmHg)	73.10 ± 4.70	93.00 ± 8.37	75.33 ± 5.71	1.363	<0.001*
Urine Protein (g/L)	0.037 ± 0.024	0.161 ± 0.071	0.03 ± 0.012	134.0	<0.001*
RBG (mmol/L)	5.6 ± 0.68	5.3 ± 0.69	9.1 ± 0.95	80.54	<0.001*

*Significant at $p<0.05$

Table 2: Comparison of mean physical and biochemical parameters of normotensive, gestational diabetes mellitus and pre-eclamptic women using LSD post-hoc analysis.

Parameter	Groups		Mean Difference	p-value
	Control	Pre-eclampsia		
Zinc (ug/L)	106.1 ± 16.6	80 ± 14.2	26.05	<0.001*
BMI (kg/m^2)	30.33 ± 3.61	33.4 ± 6.6	-3.057	0.021*
Systolic BP (mmHg)	116.6 ± 4.84	139 ± 11.4	-22.11	<0.001*
Diastolic BP (mmHg)	73.10 ± 4.70	93 ± 8.37	-19.89	<0.001*
Urine Protein(g/l)	0.04 ± 0.02	0.16 ± 0.07	-0.124	<0.001*
	Control	Gestational Diabetes		
RBG(mmol/l)	5.6 ± 0.68	9.1 ± 0.95	-3.55	<0.001*
	Gestational Diabetes	Pre-eclampsia		
Zinc (ug/L)	97.9 ± 16.9	80 ± 14.2	17.90	<0.001*
BMI (kg/m^2)	30.32 ± 4.19	33 ± 6.6	-3.07	0.019*
Systolic BP (mmHg)	115.3 ± 5.71	139 ± 11	-23.3	<0.001*
Diastolic BP (mmHg)	75.33 ± 5.71	93 ± 8.37	-17.67	<0.001*
RBG(mmol/L)	9.1 ± 0.95	5.3 ± 0.69	3.780	<0.001*
Urine Protein(g/L)	0.03 ± 0.012	0.16 ± 0.07	-0.131	<0.001*

*Significant at p<0.05

Table 3: Correlation of serum magnesium with systolic blood pressure, diastolic blood pressure, random blood sugar and urine protein across the groups.

Parameters	Control n=30 r (p-value)	Preeclampsia n=30 r (p-value)	GDM n=30 r (p-value)
SBP	0.088(0.650)	0.051(0.788)	0.226(0.230)
DBP	0.345(0.067)	-0.225(0.231)	0.085(0.657)
RBG	-0.015(0.939)	0.007(0.971)	0.422(0.020)*
Urine Protein	-0.059(0.760)	-0.137(0.472)	0.227(0.228)

*Significant at p<0.05

DISCUSSION

The reduced levels of zinc observed in the GDM compared to the controls, is similar to previous report of Mishu *et al* (12) but in contrast to previous report of Hamdan *et al* (13) which did not show any significant difference in mean zinc levels between pregnant women with GDM and controls. The serum zinc levels were also significantly lower in pre-eclampsia which agrees with previous study by Farzin and Sajadi, (14). Contrary to the present study, Mahomed *et al* (15) reported significantly high serum zinc levels as compared to controls. Studies suggested that lower serum zinc levels in gestational diabetes and pre-eclampsia was related to low oestrogen and Zn-binding protein levels caused by oxidative stress (16).

The disproportional elevation of plasma volume, increased zinc requirement, the reduced dietary bioavailability and the ultra-high dietary iron or copper content competing with zinc for the absorption sites were also suggested to cause reduced serum zinc levels (17). The increased urine protein observed in the pre-eclamptic subjects compared to other groups may be due, in part, to impaired integrity of the glomerular filtration barrier and altered tubular handling of filtered proteins (hypofiltration) leading to increased non-selective protein excretion. This observation agrees with the findings of previous studies who reported elevated urine protein levels in pregnant women with pre-eclampsia (18, 19). As expected, the BMI was increased in the pre-eclamptic pregnant women compared to the controls as the amniotic fluid and foetal body weights

increased. A positive correlation was observed between magnesium and random blood sugar in the GDM group which agrees with the study of Ertberg *et al* (20).

It has been established that Mg participates directly in glucose metabolism disorders in humans and has also been shown to function in hyperglycaemia, hyperinsulinemia and insulin resistance, although its exact role has not been elucidated (21).

CONCLUSION

It is concluded that low serum zinc levels may play a significant role in the pathogenesis of pre-eclampsia. Therefore, dietary intake or supplementation of zinc in pre-eclamptic patients may be a potential area for further studies.

LIMITATION OF STUDY

A relatively small sample size was used in this study.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to declare

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REFERENCES

1. Spong CY. Defining ''term'' pregnancy: recommendations from the Defining ''Term'' Pregnancy Workgroup. JAMA 2013; 309 (23): 2445-2446.
2. United Nations International Children Emergency Fund (UNICEF). Situation of Women and Children in Nigeria, 2019. Accessed June 20, 2021.
3. Glazener CMA, Abdalla M, Stroud P, et al. Extent Causes, Prevention and treatment of preeclampsia. *Br J Obstet Gynaecol* 2015; 102(4): 282-287.
4. International Diabetes Federation (2017) IDF Diabetes Atlas, 8th ed.; IDF: Brussels, Belgium, 2017. Assessed on July 2nd, 2021.
5. Lorquet S, Pequeux C, Munaut C, Foidart JM. Aetiology and physiopathology of preeclampsia and related forms. *Acta Clin Belg* 2010; 65(4): 237–241.
6. Roshanravan N, Mesri Alamdari N, Ghavami A, et al. Serum levels of copper, zinc and magnesium in pregnant women with Impaired Glucose Tolerance test: a case control study. *Progress in Nutrition* 2018; 20(1-5): 325-329.
7. Abdulmageed E, Hamdan HZ, Elsheikh AH, et al. Serum Calcium, magnesium, zinc and copper levels in sudanese women with pre-eclampsia *PLoS One* 2016; 11(12) e0167495.
8. Al-jameil N, Tabassum H, Al-mayouf H, et al. Analysis of Serum Trace Elements, Copper, Manganese and Zinc in Pre-eclampsia: A perspective case-controlled study in Riyadh, Saudi Arabia. *Int J Clin Exp Pathol* 2014; 7(5): 1900-1910.
9. Naing L, Winn T, Nordin R. Practical issues in calculating the sample size for prevalence studies. *Archives of Orofacial Science* 2006; 1: 9-14.
10. Koofreh ME, Ekoh M, Ekpoudom DO. The Prevalence of Pre-eclampsia among pregnant women in the University of Calabar Teaching Hospital, Calabar. *Saudi Journal for Health Sciences* 2014; (3): 133-136.
11. Henry RJ, Sobel C, Segalove M. Turbidimetric determination of proteins with sulfosalicylic and trichloroacetic acids. *Proc Soc Exp Biol Med* 1956; 92: 748 -751.
12. Mishu F, Muttalib M, Sultana B. Serum copper levels in gestational diabetes in tertiary care hospital of Bangladesh. *BIRDEM Medical Journal* 2017; 8(1): 52-55.
13. Hamdan HZ, Elbashir LM, Hamdan SZ, et al. Zinc and Selenium with Gestational Diabetes Mellitus At Medani Hospital, Sudan. *J Obstet Gynecol* 2014; 34: 567-570.
14. Farzin L, Sajadi F. Comparison of serum trace element levels in patients with or without pre-eclampsia. *J Res Med Sci* 2012; 17(10): 938–941.
15. Mahomed K, Williams MA, Woelk GB, et al. Leukocyte selenium, zinc, copper concentrations in preeclamptic and normotensive pregnant women. *Biol Trace Elem Res* 2000; 75: 107-118.
16. Kumru S, Aydin S, Simsek M, et al. Comparison of serum copper, zinc, calcium, and magnesium levels in preeclamptic and healthy pregnant women. *Biol Trace Elem Res* 2003; 94(2): 105–112.
17. Luo J, Wu W, Zhang P, et al. Zinc levels and birth weight in pregnant women with gestational diabetes mellitus: A matched cohort study in China. *J Clin Endocrinol Metab* 2020; 105(7): e2337–e2345.
18. Moran P, Lindheimer MD, Davison JM. The renal response to preeclampsia, *Semin Nephrol* 2004; 24: 588-595.
19. August P, Sibai BM. Preeclampsia: clinical features and diagnosis. Retrieved from https://www.uptodate.com/contents/preeclampsia-clinical-features-and-diagnosis, Accessed on March 10, 2022.
20. Ertberg P, Norgadi P, Bang L, Nyholm H, Rudrick M. Magnesium in gestational diabetes. *Magnes Res* 2005; 17: 35-38.
21. Morais J, Severo JS, de Alencar G, et al. Effect of magnesium supplementation on insulin resistance in humans: A systematic review. *Nutrition* 2017; 38: 54–60.