



Maternal Serum Zinc, Copper, Magnesium, and Iron in Spontaneous Abortions

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Abstract Twenty five percent of pregnant women have some degree of vaginal bleeding during the first trimester, and about 50% of those pregnancies end in spontaneous abortion (SA) because the fetus is not developing typically. As studies have reported that inadequacies of trace metals such as Copper (Cu), Zinc (Zn), Magnesium (Mg) can predispose to various adverse pregnancy outcomes (PO); multiple micronutrient (MMN) supplementations are given without justifying their deficiency and toxicities on the fetus. Earlier studies on effects of MMN supplementations during pregnancy have not considered the need, duration, dose, and time of initiation of supplementations leading to inconclusive results. So, there is a need to optimize this to prevent their abuse and side effects. This study can help in establishing critical cut-offs of these minerals in maternal serum that can forecast future pregnancy outcomes. Study measured the serum Zn, Cu, Mg, and Fe in pregnant women who presented with ($n = 80$) and without ($n = 100$) SA at 5–2 weeks of pregnancy using iron -ferrozine

method, magnesium-calmagite method, zinc reaction with nitro-PAPS, copper reaction with Di-Br- PAESA methods, respectively. Data analyzed using the student t test and cutoff value was established using Receiver Operating Characteristic (ROC) by SPSS software. Maternal serum Cu, Mg, Fe, and Zn levels measured were significantly lower in SA as compared to that of controls ($p < 0.005$) (Fig. 1) and maternal age and Body mass index were not statistically significant different among study group. Maternal serum Cu, Mg, Zn and Iron (Fe) measured in 5–12 weeks of pregnancy has the potential to forecast future occurrence of SA. The study has been registered under “The Clinical Trials Registry- India (CTRI),” -REF/2020/01/030393.

Keywords Spontaneous abortion · Serum micronutrients · Pregnancy · Zinc · Iron · Copper · Magnesium

Introduction

The World Health Organization defines Spontaneous abortion (SAb) as the termination or removal of a fetus of weight ≤ 500 g before the 20th week of gestation. [1–3] Maternal malnutrition during pregnancy impairs embryonic and fetal growth and development, resulting in harmful outcomes. [4] Trace elements are known to protect cells from toxic superoxide anion and ensure normal fetal growth, cell energy metabolism and immune function. Maternal and child undernutrition and micronutrient deficiencies affect approximately half of the world’s population. Studies have reported that inadequacies of trace metals such as Cu, Zn, magnesium, and increased levels of Ca/Mg ratio can predispose to various adverse pregnancy outcomes, including spontaneous abortion [5–7].

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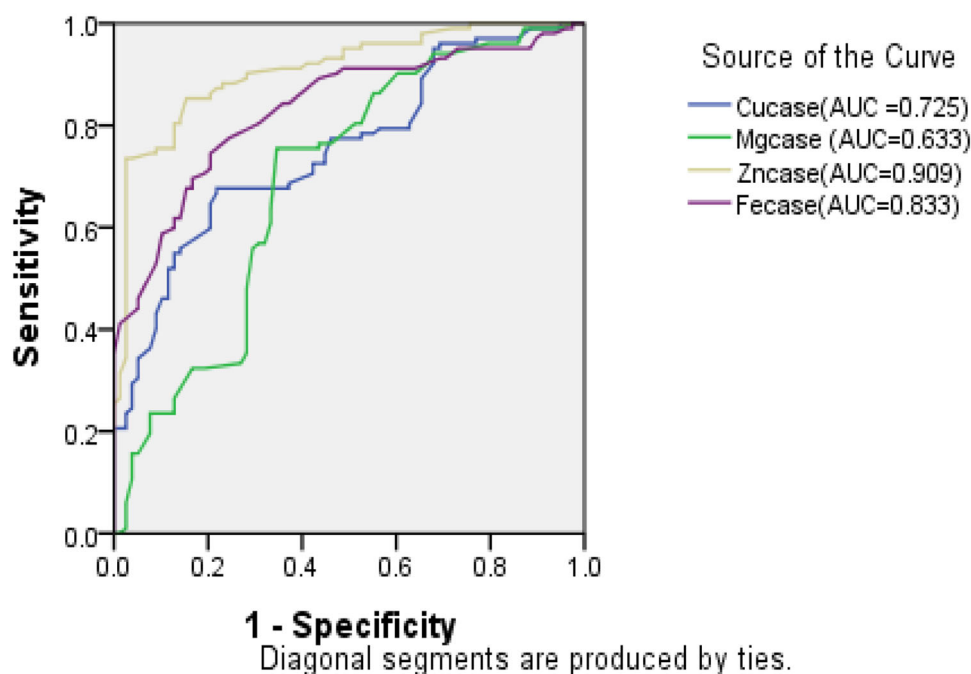
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Fig. 1 ROC curve for maternal serum copper, magnesium, zinc, and iron in predicting spontaneous abortion



Micronutrients other than iron and folic acid, have not been evaluated for their role in adverse pregnancy outcomes. Also, clinically multiple micronutrient supplementations are given without justifying their deficiency and possible toxicities on the growing fetus. Metals even though are essential various biochemical and physiological processes they could also negatively affect endometrial functionality, compromising the decidualization process and disrupting endometrial regeneration and embryo adhesion in toxic concentrations [8]. Most of the earlier studies have been confounded as they have not considered the need for supplementation, variations with respect time of initiation of supplements, duration and formulations of supplements leading to inconclusive results. So, there is a need to optimize the supplementations of these trace elements in pregnancy to prevent their abuse and side effects [9]. No studies have established any critical cutoffs for serum zinc, magnesium, copper, and iron in maternal serum during the first trimester that could foresee future spontaneous abortion. This study measured serum zinc, copper, magnesium, and iron in the first trimester of pregnancy to evaluate their potential role in predicting spontaneous abortion.

Materials and Method

Study was conducted in Kasturba hospital Manipal after taking clearance from institutional ethics committee (IEC:182/2018), a total of 180 pregnant women in first trimester (5–12 weeks of pregnancy) aged 18–40 years who consented to participate were included in the study.

The study excluded pregnancy with chronic hypertension, overt diabetes, IVF pregnancy and multifetal gestation. Serum sample was collected at (5–12 weeks) first trimester of pregnancy from all the participants during regular antenatal checkup before the occurrence of any adverse pregnancy outcome. Out of them 80 had spontaneous abortions, the remaining 100 patients were grouped separately as control who had normal pregnancy without any complications. Serum iron was measured by ferrozine method, magnesium by magnesium-calmagite method, zinc was measured using zinc reaction with nitro-PAPS and copper reaction with Di-Br-PAESA using the spectrophotometer. [10, 11] Samples were treated with 1% fresh hypochlorite solution for 30 min before they were discarded. Means were used to compare cases and controls. Data was compared using student *t* test by SPSS software. A receiver operating characteristic (ROC) curve was used to determine the serum micronutrient cut-off level that best discriminated between cases and controls.

Results

Serum copper, iron, magnesium and zinc levels in spontaneous abortion cases were significantly lower as compared to that of Comparison group ($p < 0.005$) (Table 1).

Table 1 Serum micronutrients in normal pregnancy and spontaneous abortion

Variables	Control(N = 100) Mean \pm SD	Cases(N = 80) Mean \pm SD	P value*
Age(yrs.)	28.19 \pm 3.09	27.4 \pm 4.4	0.518
BMI (kg/m ²)	24.32 \pm 7.9	21.78 \pm 2.8	0.167
Copper (μ g/dL)	302.5 \pm 95.2	222.5 \pm 60.5	0.0006
Iron (μ g/dL)	124.6 \pm 24.8	94.02 \pm 21.39	0.0022
Magnesium(mEQ/L)	1.19 \pm 0.24	1.04 \pm 0.24	0.0005
Zinc (μ g /dL)	81.6 \pm 20.3	51.7 \pm 10.4	0.0000244

(*paired student *t* test)**Table 2** Cut-off points of micronutrients based on highest area (AUC) under the receiver operating characteristics curves in predicting spontaneous abortion with their corresponding sensitivity and specificity

Parameter	AUC	Cut off	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Copper (μ g/dl)	0.753	237.5	77	65	58	64
Zinc (μ g/dl)	0.692	54.7	91	65	65	76
Iron (μ g/dl)	0.909	98.8	84	65	50	57
Magnesium (mEQ/L)	0.633	1.11	60	67	55	70

Discussion

Even though spontaneous abortion is the most common adverse pregnancy outcomes occurring in approximately between 12 and 15% of clinically recognized pregnancies, the main reason is unknown. Although several spontaneous abortions are caused by chromosomal abnormalities, maternal nutritional status, also shown to play an important role by animal and human studies [12, 13]. Due to lack of standardization with respect to time of onset and dose of micronutrient supplements during pregnancy, studies have reported inconclusive findings with respect to possible adverse effects related to over supplementations. Considering the above facts this study estimated the maternal serum zinc, copper, magnesium, and iron in first trimester to evaluate their potential to forecast future spontaneous abortion. This will also help to establish critical cut offs, positive and negative predictive values for these minerals to prevent their abuse/ unwanted supplementations.

Copper is an essential element required for normal human metabolism. Studies indicated that serum Copper deficiency in pregnant women can have been associated with some adverse effects such as Premature Rupture of Membrane (PROM), abortion, fetus growth restriction etc [14]. Zinc (Zn) is essential for cell growth, development and differentiation, embryonic development, and the function of the mother's immune system. Several earlier reports have demonstrated increased recurrent spontaneous abortions, preterm delivery, stillbirth, and fetal neural tube defects linked to maternal zinc deficiency [15]. Magnesium status has been linked to fetal development and risk of preterm labor [16]. Insulin-dependent diabetic pregnant

women are at risk for spontaneous abortion induced by increased urinary magnesium losses [17]. Hence, reviews published based on randomized control trials have shown multiple micronutrient supplementation may be beneficial than supplementation of selected vitamin/mineral in averting early pregnancy loss or still births [12, 18, 19]. But one of the main confounders of these findings is due to variations in the timing of initiation, dose, and duration of supplementation. A recent study also found that raised Hb levels in early pregnancy can predispose to miscarriages due to increased blood viscosity impeding the uteroplacental circulation, causing placental infarction. This suggests a need to revise the criteria for iron and nutrient supplementations in non-anemic pregnant women [18, 19].

ROC curve was developed for each of these minerals to establish a critical cut off for these minerals for prevention of spontaneous abortions (Fig. 1). This will help in identifying the patient requiring supplementation and thus preventing their abuse. Study results showed first trimester serum levels of copper, iron, magnesium and zinc in spontaneous abortion cases were significantly lower as compared to that of normal pregnancy (Table 1). The specificity of all the minerals was comparable to that of iron, Copper, Zinc, and magnesium, all of them showed greater positive predictive value for spontaneous abortion as compared to iron (Table 2). Iron even though used as a routine test in antenatal screening and being prescribed for all pregnant women, it showed poor positive predictive value for spontaneous abortion as compared to other minerals implying its overuse in clinical practice (Table 2). Thus, there is a need to identify and develop a panel of minerals rather than iron alone for regular antenatal

screening for enabling its optimum supplementation to avoid possible adverse effects on pregnancy.

Conclusion

Maternal serum plasma copper, iron, magnesium, and zinc measured during first trimester have a potential to forecast future occurrence of spontaneous abortions.

Limitation

Out of 80 abortion patients included in the study, 10% ($N = 8$) had previous abortion. But the cause could not be ascertained as these patients were referred from other hospitals and reports were not available for these patients.

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Author's Contribution S-Protocol/project development, Data collection or management, Data analysis, Manuscript writing/editing, Patient recruitment. KP-Protocol/project development, Manuscript writing/editing. VGP-Protocol/project development, Patient recruitment. SS-Protocol/project development. MR- Protocol/project development. AK-Statistical analysis.

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Declarations

Conflict of interest There is no conflict of interest.

Consent to Participate Informed consent was obtained from all individual participants included in the study.

Ethical Approval This study was approved by the appropriate institutional ethics committee- Kasturba medical college and the hospital institutional ethical committee (IEC:182/2018) and the study was performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. The study has been registered under "The Clinical Trials Registry- India (CTRI)," -REF/2020/01/030393.

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