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Original Article

Deficiency of calcium and microelements predict the risk of fetal growth restriction

Fengying Xu[1], Shuifang Xu[1], Xiulan Shao[1], Min Wu[1], Ying Xu[1], Lihua Wang[2]

[1]*Department of Obstetrics and Gynecology, Tinglin Hospital of Shanghai Jinshan District, Shanghai, China;* [2]*Department of Gynaecology, International Peace Maternity and Child Health Hospital, Shanghai, China*

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Abstract: Our study aimed to investigate the correlation between the concentrations of calcium (Ca), iron (Fe), copper (Cu), zinc (Zn), iodine (I) in peripheral blood of different pregnancy trimesters and fetal growth restriction (FGR) risk. A total of 233 pregnant women were recruited in this cohort study. The blood specimens were obtained from all the pregnant women at early pregnancy (pregnancy within 13 weeks), middle pregnancy (between 14^{th} and 27^{th} weeks) and late pregnancy (above 28 weeks). And the concentrations of Ca, Fe, Cu, Zn, I were subsequently determined. Among 233 analyzed patients, 25 cases were diagnosed as FGR with incidence rate 10.7%, which were divided into FGR group, while other 198 cases were included in non-FGR group. Despite of no difference in Cu concentration in middle pregnancy ($P=0.184$), levels of Ca, Fe, Cu, Zn and I markedly decreased in FGR group compared to non-FGR group in early, middle and late pregnancies (all $P<0.05$). Receiver Operating Characteristic (ROC) curves were performed to evaluate the predictive value of candidate elements for FGR risk. When combined the five elements, the area under curve (AUC) for FGR was 0.875 (95% CI: 0.790-0.960) in early pregnancy, 0.895 (95% CI: 0.837-0.953) in middle pregnancy and 0.919 (95% CI: 0.850-0.988) in late pregnancy. Our study manifested that combined measurement of Ca, Fe, Cu, Zn and I could be a promising biomarker for predicting the risk of FGR.

Keywords: Calcium, iron, copper, zinc, iodine, fetal growth restriction

Introduction

Fetal growth restriction (FGR), also known as intrauterine growth restriction (IUGR), is a complex perinatal disease that mainly describes an abnormal fetus growth intrauterine, which means the fetus could not grow to its potential [1]. Among all pregnancies, FGR was found in 30 million fetuses per year worldwide, and about 5-10% pregnancies with FGR result in poor perinatal outcomes of stillbirth or neonatal death [1, 2]. Neonates with FGR exhibits serious possibilities of having worse postnatal outcomes such as cognitive impairment and cerebral palsy [3, 4]. FGR not only influences the growth and development of fetus, but also leads to the damage of physical and mental development in adolescence as well as an elevating incidence of cardiovascular system and nervous system disease in adulthood [4].

Microelement plays an important role to the health of human body [5]. During critical periods of growth, deficiency of microelement has become an important health issue in both high-income and low-income countries, and the physiologic roles of microelements in pregnancies have been discovered over years [6]. Microelements are essential to the physiology of human organisms, for fetuses and mothers, for instance, certain microelements were observed to participate in fetal bone development and reduce complications during pregnancies [7, 8]. So far, there are 14 essential microelements in human physiology, including iron (Fe), zinc (Zn), copper (Cu), iodine (I), selenium, fluorine, drill, chromium, and so on. Calcium (Ca) is a predominant content of skeletons in human, and Ca functions as a mediator in multiple physiological processes such as hormone synthesis and blood coagulation [9]. Some microelements and macroelements such as Fe, Zn, Cu, Ca and I take part in lots of biochemical processes in human's body, and are essential elements of metabolic active compounds [5].

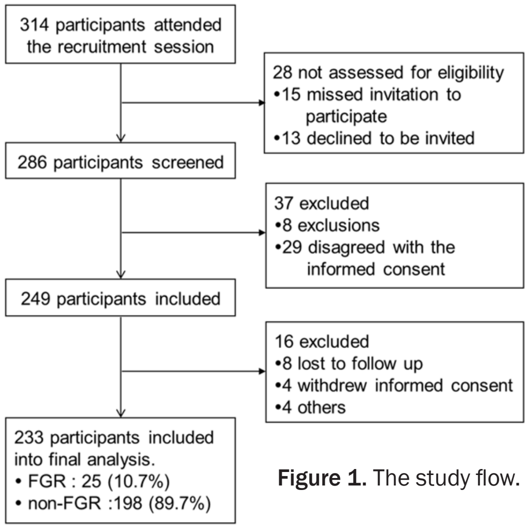

Figure 1. The study flow.

Table 1. Baseline demographic and clinical characteristics of pregnant women

Parameters	FGR Group	Non-FGR Group	*P* value
Number of cases	25	198	--
Maternal age (years)	27.4±4.2	29.0±4.6	0.100
Maternal BMI (kg/m^2)	19.5±2.2	20.2±2.5	0.183
Primiparity (count %)	21 (84%)	147 (74%)	0.286
Smoking (count %)	2 (8%)	6 (3%)	0.208
Drinking (count %)	3 (12%)	13 (6%)	0.321

Data was presented as mean value ± SD or counts (%). Significance of the comparison was determined by Student test or Chi-squared test. *P* Value <0.05 was considered significant. FGR, fetal growth restriction; BMI, body mass index.

However, the roles of Fe, Zn, Cu, Ca and I in FGR are still obscure.

Therefore, our study aimed to investigate the correlation between the concentrations of Ca, Fe, Cu, Zn, I in peripheral blood of different pregnancy trimesters and FGR risk.

Materials and methods

Participants

A total of 233 pregnant women were recruited in this cohort study from Nov. 2013 to Dec. 2015 in Tinglin Hospital of Shanghai Jinshan District. The inclusion criteria were: (1) Age between 20 and 35 years; (2) Pregnancy within 8 weeks. While the exclusion criteria were as follows: (1) Suffering from pregnancy complications, such as hypertensive disorder complicating pregnancy, gestational diabetes mellitus, and nephropathy; (2) Previous reproductive system surgery; (3) Previous tumor, severe infection; (4) Drug addiction history; (5) Cognitive impairment, or poor adherence. This study was approved by the Ethics committee of Tinglin Hospital of Shanghai Jinshan District, and each participant provided signed informed consent.

Samples and candidate elements determination

The blood specimens were obtained from all the pregnant women at early pregnancy (pregnancy within 13 weeks), middle pregnancy (between 14th and 27th weeks) and late pregnancy (above 28 weeks). Collecting fasting venous blood 4 ml with disposable separating gel tubes without anticoagulant was used to detect I, after 30 min centrifuged with 3000 r/min, and then stored at -20°C. The detection of other elements was used fasting venous blood 1 ml collected in heparin lithium blood collection tube and stored at room temperature.

The samples were diluted 3 times with double distilled water and oscillated 1 min, recorded the test results. Double distilled water was used as blank control to determine the concentration of each candidate element (Ca, Fe, Cu, Zn, I). In this detection, controlled standard 1 time every 5 samples.

Fetal growth restriction

FGR was defined as fetal birth weight less than 2500 g, or less than two fold standard deviation (SD) of normal newborns with matched sex and maternal age, or less than 10% body weight of normal newborns with matched sex and maternal age according to criteria of FGR in Obstetrics and Gynecology (2nd edition, People's medical publishing house).

Statistics

All statistical analysis was performed using the software of SPSS 21.0. The data were summarized and presented as mean ± SD, median (25-75th quarter) or count (percentage). Comparison between two groups was determined by Student test, Wilcoxon rank sum test or Chi-square test. Receiver Operating Characteristic (ROC) curve was performed to evaluate the predictive value of candidate elements for FGR. *P*

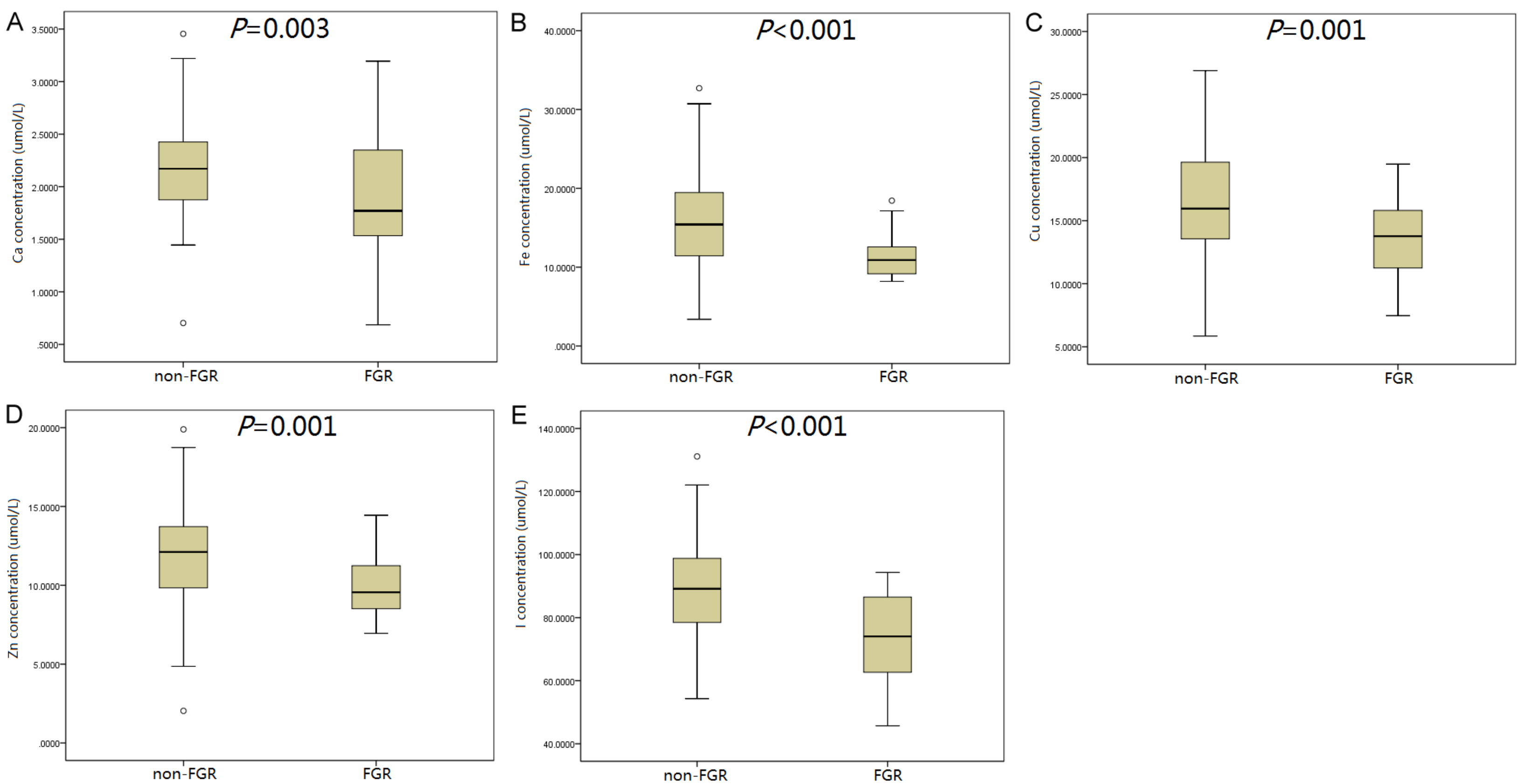

Figure 2. The concentrations of Ca, Fe, Cu, Zn and I of early pregnancy in FGR group and non-FGR group. A. Concentrations of Ca of early pregnancy in FGR group and non-FGR group; B. Concentrations of Fe of early pregnancy in FGR group and non-FGR group; C. Concentrations of Cu of early pregnancy in FGR group and non-FGR group; D. Concentrations of Zn of early pregnancy in FGR group and non-FGR group; E. Concentrations of I of early pregnancy in FGR group and non-FGR group. Comparison between two groups was determined by Wilcoxon rank sum test. $P<0.05$ was considered as significant.

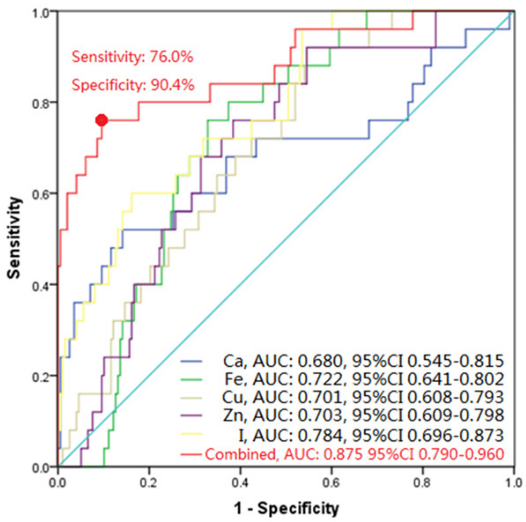

Figure 3. ROC curve of Ca, Fe, Cu, Zn and I of early pregnancy in FGR diagnosis. The analysis was determined by ROC curve analysis.

value <0.05 was considered statistically significant in this study.

Results

Study flow

314 participants attended the recruitment session, among which 28 participants were not assessed for eligibility (15 missed invitation to participate and 13 declined to be invited); the remaining 286 participants were screened for eligibility and 37 participants were excluded (8 for exclusion criterion, 29 disagreed with the informed consents); after that 249 participants were included with 16 excluded (8 were lost to follow up, 4 withdrew informed consents and 4 for other reasons). The remaining 233 participants were included in our analysis (**Figure 1**). Among 233 analyzed patients, 25 cases were diagnosed as FGR with incidence rate 10.7%, which were divided into FGR group, while other 198 cases were included in non-FGR group.

Characteristics

As shown in **Table 1**, the FGR group of 25 participants has a mean maternal age of 27.4±4.2 years and a mean value of maternal BMI of 19.5±2.2 kg/m^2; while the non-FGR group of 198 patients has a mean maternal age of 29.0±4.6 years and a mean value of BMI of 20.2±2.5 kg/m^2. The other characteristics of participants were presented in **Table 1**, and no difference in maternal age, maternal BMI, primiparity percentage, smoking and drinking were found between two groups.

Concentration of candidate elements in early pregnancy

In early pregnancy, the concentrations of Ca (P=0.003), Fe (P<0.001), Cu (P=0.001), Zn (P=0.001) and I (P<0.001) decreased in FGR group compared to the non-FGR group (**Figure 2**). In order to further investigate the diagnostic value of Ca, Fe, Cu, Zn and I in early pregnancy of FGR, ROC curve was performed. The area under curve (AUC) of Ca, Fe, Cu, Zn and I were 0.680 (95% CI: 0.545-0.815), 0.722 (95% CI: 0.641-0.802), 0.701 (95% CI: 0.608-0.793), 0.703 (95% CI: 0.609-0.798) and 0.784 (95% CI: 0.696-0.873), respectively. When combined the five elements, the AUC was 0.875 (95% CI: 0.790-0.960) with the sensitivity of 76.0% and specificity of 90.4% at the best cut off point (**Figure 3**).

Concentration of candidate elements in middle pregnancy

As shown in **Figure 4**, in middle pregnancy, the concentrations of Ca (P=0.001), Fe (P=0.001), Zn (P=0.001) and I (P<0.001) reduced in FGR group compared to the non-FGR group. However, there was no difference in the concentrations of Cu (P=0.184) between two groups. To further investigate the diagnostic value of Ca, Fe, Zn and I in middle pregnancy of FGR, ROC curve was performed. The area under curve (AUC) of Ca, Fe, Zn and I were 0.702 (95% CI: 0.607-0.796), 0.699 (95% CI: 0.612-0.786), 0.700 (95% CI: 0.609-0.792) and 0.758 (95% CI: 0.628-0.889), respectively. When combined the four elements, the AUC was 0.895 (95% CI: 0.837-0.953) with the sensitivity of 96.0% and specificity of 71.2% at the best cut off point (**Figure 5**).

Concentration of candidate microelements in late pregnancy

In late pregnancy, the concentrations of Ca (P<0.001), Fe (P<0.001), Cu (P=0.016), Zn (P<0.001) and I (P<0.001) was lessened in FGR group compared to the non-FGR group (**Figure 6**). Similarly, in order to further investigate the diagnostic value of Ca, Fe, Cu, Zn and I in late

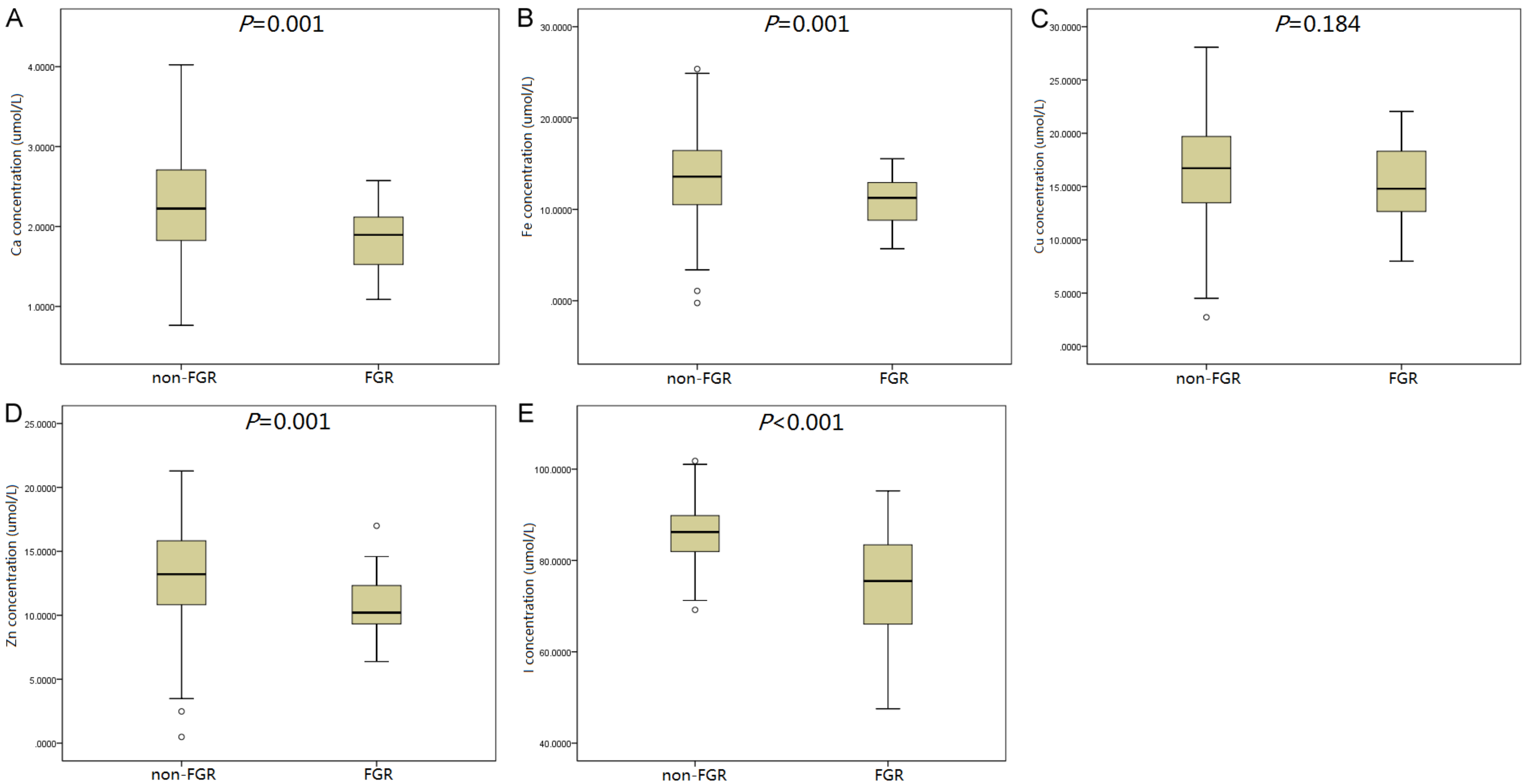

Figure 4. The concentrations of Ca, Fe, Cu, Zn and I of middle pregnancy in FGR group and non-FGR group. A. Concentrations of Ca of middle pregnancy in FGR group and non-FGR group; B. Concentrations of Fe of middle pregnancy in FGR group and non-FGR group; C. Concentrations of Cu of middle pregnancy in FGR group and non-FGR group; D. Concentrations of Zn of middle pregnancy in FGR group and non-FGR group; E. Concentrations of I of middle pregnancy in FGR group and non-FGR group. Comparison between two groups was determined by Wilcoxon rank sum test. P<0.05 was considered as significant.

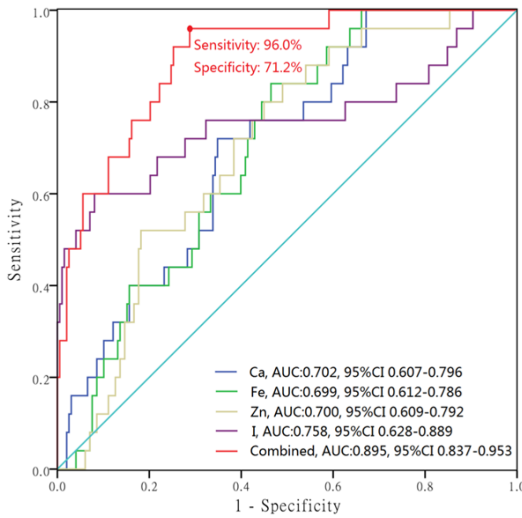

Figure 5. ROC curve of Ca, Fe, Cu, Zn and I of middle pregnancy in FGR diagnosis. The analysis was determined by ROC curve analysis.

pregnancy of FGR, ROC curve was performed. The area under curve (AUC) of Ca, Fe, Zn and I are 0.716 (95% CI: 0.624), 0.721 (95% CI: 0.649-0.793), 0.648 (95% CI: 0.541-0.755), 0.760 (95% CI: 0.676-0.844) and 0.801 (95% CI: 0.691-0.911), respectively. When combined the five elements, the AUC is 0.919 (95% CI: 0.850-0.988) with the sensitivity of 92.0% and specificity of 83.8% at the best cut off point, which was higher than that in the early and middle pregnancies (**Figure 7**).

Discussion

The results of our study presented that (1) Despite of no difference in Cu concentration in middle pregnancy, levels of Ca, Fe, Cu, Zn and I markedly decreased in FGR group compared to non-FGR group in early, middle and late pregnancies; (2) Combined measurement of Ca, Fe, Cu, Zn, I in early, middle and late pregnancies predict the risk of FGR; and the AUC in late pregnancy of the combined measurement of Ca, Fe, Cu, Zn and I is especially high.

Fetuses with FGR are more likely to born with neuro development deficits, and the susceptibility of diabetes as well as cardiovascular disease is investigated to be elevated in adults born with FGR [1, 10]. Perinatal morbidity and mortality of FGR is high and about a quarter of the stillbirths is growth restricted [11]. FGR generally falls into two categories: early-onset and late-onset forms, and early-onset FGR is considered to be strongly correlated with perinatal death [12, 13]. Although prior study has demonstrated a preventive value of Doppler ultrasonography in FGR, diagnosis is still a tough issue in FGR management [14, 15].

Microelements, mainly obtained from food, are essential elements occupy only less than 0.01 percent of human weight [5]. Among the four microelements investigated in this study, Fe is an important component of hemoglobin, a large amount of Fe is needed in pregnant women to meet the needs of fetal growth [16]. In early pregnancy and middle pregnancy, Fe deficiency results in inhabitations of embryo growth and development of cardiovascular system; in later period, Fe causes anemia, low birth weight, and damage of the nervous system [17]. It is reported that iron-deficiency anemia is a dominant type of anemias in China [17, 18]. Zn is fundamental in cell growth, differentiation and survival [19], and the result of a cohort study among pregnant women demonstrated a low maternal Zn level led to the elevation of FGR risk [20]. In addition, Pathak P et al. reported that low level during pregnancy was related with an increased risk of low birth weight and preterm delivery [21]. Assessment of Cu in pregnant women in order to evaluate the nutrient condition is necessary [22]. Decreased Cu level was found in pregnant women with preeclampsia in a comparative study [23]. I, an essential microelement involved in thyroid hormone synthesis and intrauterine neuro development, is suggested to be extra ingested in pregnancy for the maternal I lose and the additional need of I in fetal growth [24]. Low I during pregnancy reduces the production of fetal thyroid hormone, which affects especially the fetal brain development [25]. Our study validated that concentrations of Fe, Cu, Zn and I markedly decreased in all trimesters of pregnancies with FGR and separate concentrations of Fe, Cu, Zn and I in early, middle and late pregnancies could predict the risk of FGR, the results were generally consistent with the former studies [17, 20, 21, 23, 25].

While Ca, which is a macroelement, usually increased in a normal fetus to play a preventive role in the occurrences of tetany, arrhythmias and seizures [8]. Ca has been studied by large amount of studies on pregnancies over decades

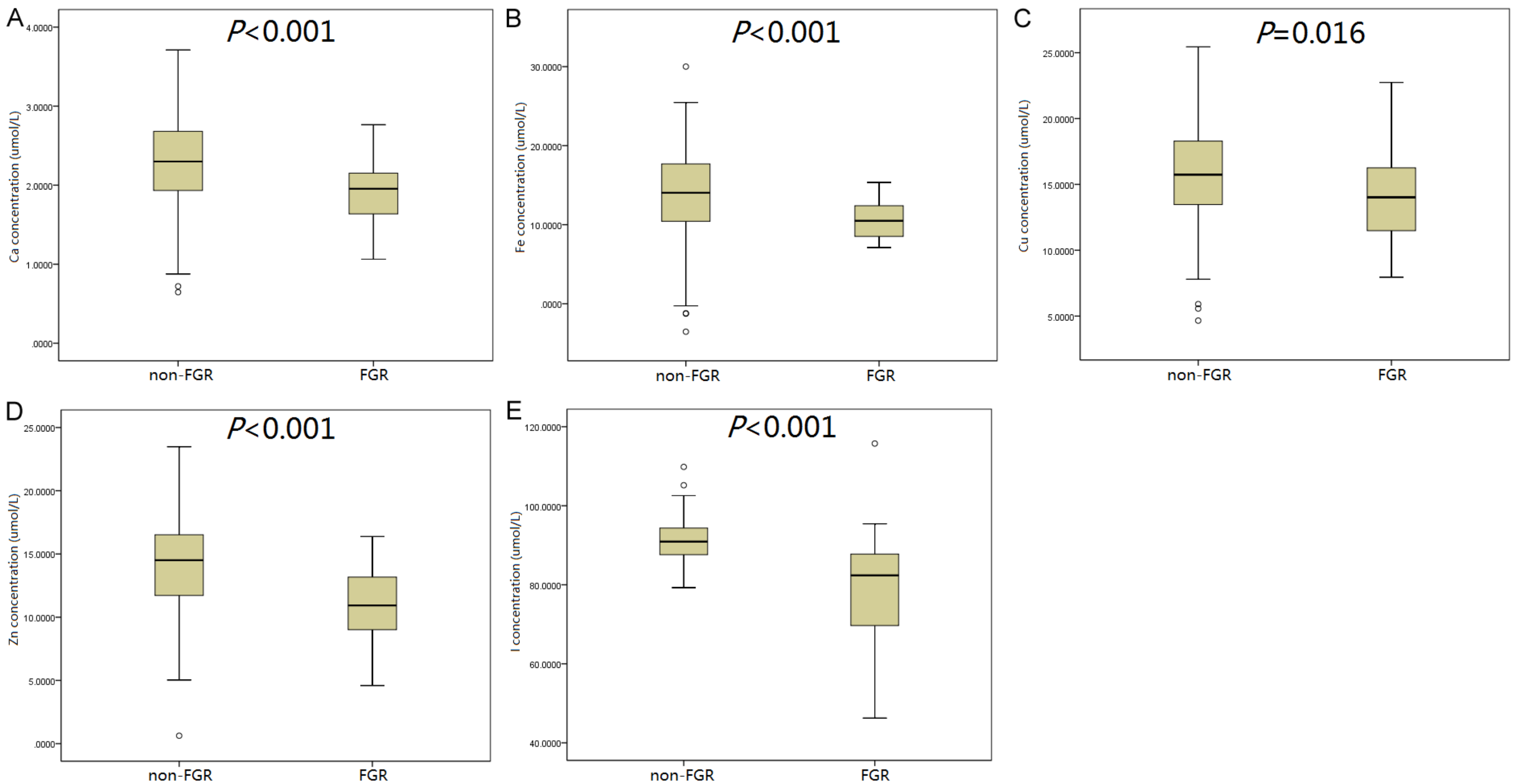

Figure 6. The concentrations of Ca, Fe, Cu, Zn and I of late pregnancy in FGR group and non-FGR group. A. Concentrations of Ca of late pregnancy in FGR group and non-FGR group; B. Concentrations of Fe of late pregnancy in FGR group and non-FGR group; C. Concentrations of Cu of late pregnancy in FGR group and non-FGR group; D. Concentrations of Zn of late pregnancy in FGR group and non-FGR group; E. Concentrations of I of late pregnancy in FGR group and non-FGR group. Comparison between two groups was determined by Wilcoxon rank sum test. P<0.05 was considered as significant.

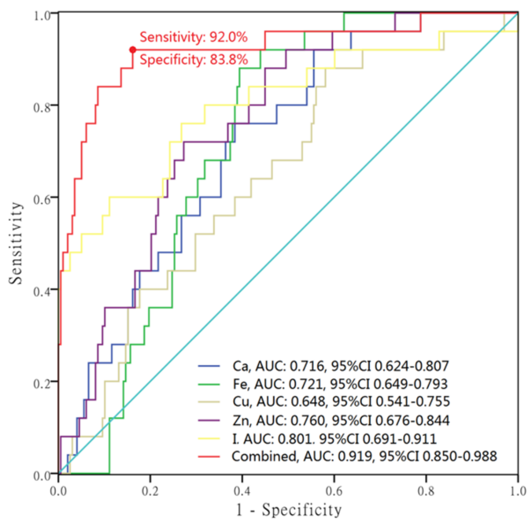

Figure 7. ROC curve of Ca, Fe, Cu, Zn and I of late pregnancy in FGR diagnosis. The analysis was determined by ROC curve analysis.

and confirmed a high concentration and indispensable functions in skeletons and muscles [26]. Hofmeyr GJ et al. illustrated that Ca supplementation during pregnancy significantly reduces the mortality of pregnant women and incidence of pregnancy with serious disease [27]. Imdad A et al. also showed that in developing countries Ca supplementation during pregnancy reduce the risk of neonatal death and premature birth [28]. In our study, Ca was discovered decreased in FGR group and the concentrations of Ca in early, middle and late pregnancies were proved to predict the risk of FGR. It might due to the preventive roles of high Ca level in pregnancies [8, 27, 28].

Our data disclosed that combination of Ca, Fe, Cu, Zn and I in early, middle and late pregnancies predict the risk of FGR, and most importantly the combination of the five elements concentration in late pregnancy is of the highest predictive value with the largest AUC 0.919 (95% CI: 0.850-0.988), which may result from: firstly, the late pregnancy is the stage that elements mainly help with the fetal growth on the size; secondly, when the deficiencies of elements appeared in early and middle pregnancy, according elements were usually supplemented that reduce the influence on FGR [29, 30].

Several limitations existed in this study: (1) the sample size of 233 participants is relatively small for a cohort study; (2) several potential risk factors of FGR are not documented, such as small maternal size and radiation exposure [31, 32]. Thus a large sample size study with more potential risk factors excluded should be carried out to further investigate the cellular and molecular mechanisms of Ca, Fe, Cu, Zn and I in FGR.

In conclusion, our study manifested that combined measurement of Ca, Fe, Cu, Zn and I could be a promising biomarker for predicting the risk of FGR.

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Disclosure of conflict of interest

None.

Address correspondence to: Lihua Wang, Department of Gynaecology, International Peace Maternity and Child Health Hospital, 1961 Huashan Road, Shanghai, China. Tel: 86-21-64073896; Fax: 86-21-64073896; E-mail: lihuawangfk@163.com

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