INTERNATIONAL CONFERENCE ON BIOLOGICAL RESEARCH AND APPLIED SCIENCE (IBRAS)

JINNAH UNIVERSITY FOR WOMEN, PAKISTAN

Mehnaz Akhtar[1], Irfan Zia Qureshi[2] Email: maha.aina83@gmail.com

[1]Quaid-I-Azam University Islamabad.

Ca
- Concentration Lowered from 1st to 3rd trimester

Mg
- Concentration Lowered from 1st to 3rd trimester

Zn
- Concentration Lowered from 1st to 3rd trimester

Fe
- Concentration Lowered from 1st to 3rd trimester

Cr
- Concentration Lowered from 1st to 3rd trimester

Cu
- Concentration elevated from 1st to 3rd trimester

Ni
- Concentration Lowered from 1st to 3rd trimester

Mn
- Remained non significantly different

Co
- Concentration increased from 1st to 3rd trimester

Methods and Materials

MATERIALS AND METHODS

This study was planned, designed and conducted in the Laboratory of Animal and Human Physiology, Department of Animal Sciences, Quaid-i-Azam University, Islamabad, Pakistan.Approval of study was obtained from "Bioethics Committee for research on Human Subjects", Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad, Pakistan and "Administrator, Mother and Child Hospital", Chakwal, Punjab.

This was a randomized and case control study. Forty healthy pregnant women between the ages of 20 to 40 years were recruited for this study. These women were followed through whole gestation period (three trimesters). A control group of 20 non pregnant females between the ages of 18 to 45 were also recruited.

Pregnant women below the age of 40 having no history of hypertension, diabetes mellitus or any other major disease were recruited.

A questionnaire (Appendix-I) was designed which was consisted of open and close ended questions about the age of mother, gestational age of fetus at the time of blood collection, education, residence (rural/urban), previous history of hypertension/ diabetes mellitus/ any other disease, family background, diet, supplement intake, number of deliveries and history of previous pregnancies.

The first trimester height and weight were considered as pre pregnancy readings and then BMI was calculated accordingly.

Gestational age was estimated from the date of last menstrual period or based on ultrasonography in the first trimester.

The study group and control group were further divided in to two sub groups according to their age. The women having 20-30 years age (young) were placed in group I while others having 30-40 years age (middle-aged) were placed in group II.

Blood sampling

About 2 ml venous blood was drawn from the antecubital vein by venipuncture into plain vacutainers. Samples were drawn from each subject in 1st, 2nd and 3rd trimester (3 times).

All sample containers, polyethylene plastic bottles and glassware were carefully washed with non-ionic detergent followed by tap water to remove any residues. All glassware and plastic bottles were then soaked in 10% (v/v) nitric acid for 48h, rinsed with distilled water and completely dried before use.

Sample preparation for FAAS (Flame Atomic Absorption Spectrophotometry)

All samples were kept in refrigerator at 4 °C till further processing. Blood was transferred from vacutainers to test tubes. 10 ml of 65% extra pure nitric acid (Sigma Aldrich) was added and allowed to stand overnight for pre digestion.

Predigested samples were shifted to digestion flask and heated on a hotplate at 400 °C without charring. Digestion was carried out manually in a fume hood. When heating was started, dark brown fumes appeared which were then changed into dense white fumes and a homogenized solution was formed. Samples were cooled to room temperature and filtered through 125 mm Whattman filter paper. Final volume of the filtrate was raised to 25 ml by adding deionized H_2O and stored in graduated polyethylene plastic bottles. These prepared samples were subjected to flame atomic absorption spectrophotometry (FAAS).

Flame Atomic Absorption Spectrophotometry

Flame Atomic Absorption Spectrophotometry (FAAS) is used to determine the concentration of elements in different tissues.

Preparation of standard solutions

Standard solutions for each metal having concentration of 1000 ppm was prepared by adding 1 g/L or 0.1 g/100 ml of salt in double distilled water. 1 mg/L is equal to 1000 ppm, by applying unitary method standard solutions of known concentrations were prepared. For the calibration of the instrument, further dilutions of 2.5 ppm, 5 ppm and 10 ppm were made from stock solution by using the formula

$$N_1V_1 = N_2V_2$$

N_1= the normality of stock solution which is 1000ppm

V_1= the volume of stock solution required for making standards of different concentrations

N_2= required normality

V_2= required volume

Determination of metal concentration

Elemental concentrations were determined on an Atomic Absorption spectrophotometer (Varian, AA240 FS, USA). By using the standard solutions of different concentrations, calibration curves were prepared to determine the concentration of metal in prepared sample.

Digest of blood was subjected to Air/ Acetylene fast sequential flame atomic absorption spectrophotometer (Varian, AA240 FS, USA). Concentrations of micronutrients: Cobalt (Co), Copper (Cu), Zinc (Zn), Manganese (Mn), Nickel (Ni), Iron (Fe), Chromium (Cr), Cadmium (Cd) and macronutrients including Calcium (Ca) and Magnesium (Mg) were determined. The obtained concentrations of these metals were in ppm (mg/L) and were converted in to µg/ml by using the following formula:

$$\text{Metal concentration (µg/ml)} = \frac{\text{Metal concentration (ppm)} \times \text{Final volume of solution}}{\text{Sample weight/volume}}$$

Statistical Analysis:

Data are presented as mean ± SEM (Standard error of mean). ANOVA (One way analysis of variance) was applied using Sigma plot (version 12.0, California USA) to compare the concentrations of metals in control and study groups. Data was analyzed using two tailed t-test and Pearson´s correlation by using SPSS version 20 (SPSS ver.20.0, Microsoft Inc. USA). P<0.05 was considered statistically significant.

Table 1. Comparison of trace elements in whole blood of pregnant women and non-pregnant controls in Group 1. Values are expressed as mean±SEM

Metals	Controls (n=15)	1st Trimester (n=33)	2nd Trimester (n=33)	3rd Trimester (n=33)
Zn	8.02±1.234	6.95±0.721	2.14±0.309a*b*	2.84±0.307a*c*
Mg	58.94±4.720	41.13±3.805a*	27.88±1.293a*	27.22±1.381a*b*
Fe	534.67±63.885	550.51±43.499	323.13±30.357a*b*	354.70±43.167a*b*
Cu	0.91±0.155	0.50±0.0569	0.74±0.066	2.79±0.336a*b*c*
Cr	5.04±1.728	10.06±0.957	0.85±0.125a*b*	1.49±0.170b*
Ni	1.31±0.134	1.08±0.153	0.34±0.046a*b*	0.54±0.040a*
Co	1.82±0.417	0.94±0.129	1.53±0.142b*	2.65±0.171a*b*c*
Mn	0.32±0.077	0.49±0.073	0.48±0.055	0.36±0.046
Ca	233.16±33.133	248.17±23.776	111.06±9.239a*b*	154.79±11.592

*p<0.05; **p<0.01; ***p<0.001
[a] Significantly different from control
[b] Significantly different from 1st trimester
[c] Significantly different from 2nd trimester
↓Significantly decreased
↑Significantly increased

Table 2. Comparison of trace elements in whole blood of pregnant women and non-pregnant controls in Group 2. Values are expressed as mean±SEM.

Metals	Control (n=05)	1st Trimester (n=07)	2nd Trimester (n=07)	3rd Trimester (n=07)
Zn	7.87±0.603	6.41±0.656	2.70±0.910a***b**	3.10±0.846a**b*
Mg	54.01±15.101	44.89±7.207	28.45±3.171	20.79±3.400b*
Fe	482.96±74.652	571.65±54.714	359.91±63.928	269.70±51.287b**
Cu	0.80±0.169	0.60±0.104	0.60±0.133	3.52±0.841b*c*
Cr	1.28±0.397	10.74 ±1.625	0.95±0.388b*	0.98±0.334b*
Ni	1.13±0.142	1.15±0.154	0.37±0.148a**b**	0.53±0.097a*b*
Co	1.56±0.379	0.62±0.129	1.05±0.272	2.18±0.192b*
Mn	0.68±0.156	0.56±0.126	0.43±0.076	0.45±0.109
Ca	187.16±22.517	257.84±45.709	89.91±12.254b**	129.01±19.985b*

*p<0.05; **p<0.01; ***p<0.001
[a] Significantly different from control
[b] Significantly different from 1st trimester
[c] Significantly different from 2nd trimester
↓ Significantly decreased
↑Significantly increased

Results

Anthropometric status of both pregnant and control females expressed in BMI (Body Mass Index) was calculated through their height (inches) and weight (pounds). Women were classified into four subgroups according to the calculated values of Body Mass Index (BMI): Underweight (BMI<18.5), Normal (18.5<BMI<25), Overweight (25<BMI<30) and Obese (30<BMI). Anthropometric status of both control and study group is presented in Fig.

Iron

Blood Fe concentration was significantly lowered in group I pregnant women in 2nd trimester and 3rd trimester (p<0.05) as compared to non-pregnant controls. Fe concentration was significantly lowered in 3rd trimester as compared to 1st trimester (p<0.05) in group I pregnant women.

In group II pregnant women, the concentration of Fe was siginificantly decreased in 3rd trimester (p<0.008) as compared to 1st trimester.

Zinc

Significantly decreased levels of Zn were observed in 2nd and 3rd trimester (p<0.05) of group I pregnant women as compared to controls. Zn level was significantly lowered in 2nd trimester (p<0.05) of group I pregnant women as compared to 1st trimester and in 3rd trimester as compared to 2nd trimester (p<0.05).

In group II pregnant women, the level of Zn was highly significantly decreased in 2nd trimester (p<0.001) and significantly decreased in 3rd trimester (p<0.01) as compared to controls. Level of Zn was also significantly decreased in 2nd (p<0.01) and 3rd trimester (p<0.05) as compared to 1st trimester .

Calcium

In group I pregnant women, the levels of Ca were significantly lowered in 2nd trimester (p<0.05) as compared to non-pregnant controls and 1st trimester. In group II pregnant women, the level of Ca is significantly lowered in 2nd trimester (p<0.01) as compared to 1st trimester and significantly lowered in 3rd trimester (p<0.05) as compared to 1st trimester

Magnesium

The levels of Mg in group I pregnant women were significantly decreased in 1st trimester (p<0.05), 2nd trimester (p<0.05) and 3rd trimester (p<0.05) as compared to non-pregnant controls. In 3rd trimester, the level of Mg was significantly lowered (p<0.05) as compared to 1st trimester.

In group II pregnant women, the level of Mg was significantly decreased in 3rd trimester (p<0.05) as compared to 1st trimester .

Manganese

In group I pregnant women, the levels of Mn remained non-significantly different in 2nd and 3rd trimester as compared to non-pregnant controls and 1st trimester. In group II pregnant women, Mn levels also remained non- significantly different in 2nd and 3rd trimesters as compared to non-pregnant controls and 1st trimester.

Copper

Cu levels of group I pregnant women were significantly elevated (p<0.05) in 3rd trimester as compared to non-pregnant controls, 1st trimester and 2nd trimester. In group II pregnant women, Cu levels were also significantly elevated (p<0.05) in 3rd trimester as compared to 1st and 2nd trimester

Cobalt

In group I pregnant women, the levels of Co were significantly elevated in 2nd trimester (p<0.05) as compared to 1st trimester. In 3rd trimester, a significant increase (p<0.05) as compared to non-pregnant controls, 1st trimester and 2nd trimester was observed.

In group II pregnant women, the levels of Co were significantly increased in 3rd trimester (p<0.05) as compared to 1st trimester.

Chromium

Cr levels in group I pregnant women were significantly lowered in 2nd trimester (p<0.05) as compared to non-pregnant controls and 1st trimester. In 3rd trimester the level of Cr was significantly lowered (p<0.05) as compared to 1st trimester.

In group II pregnant women, Cr levels were significantly lowered in 2nd trimester (p<0.05) as compared to 1st trimester and significantly lowered in 3rd trimester (p<0.05) as compared to 1st trimester.

Nickel

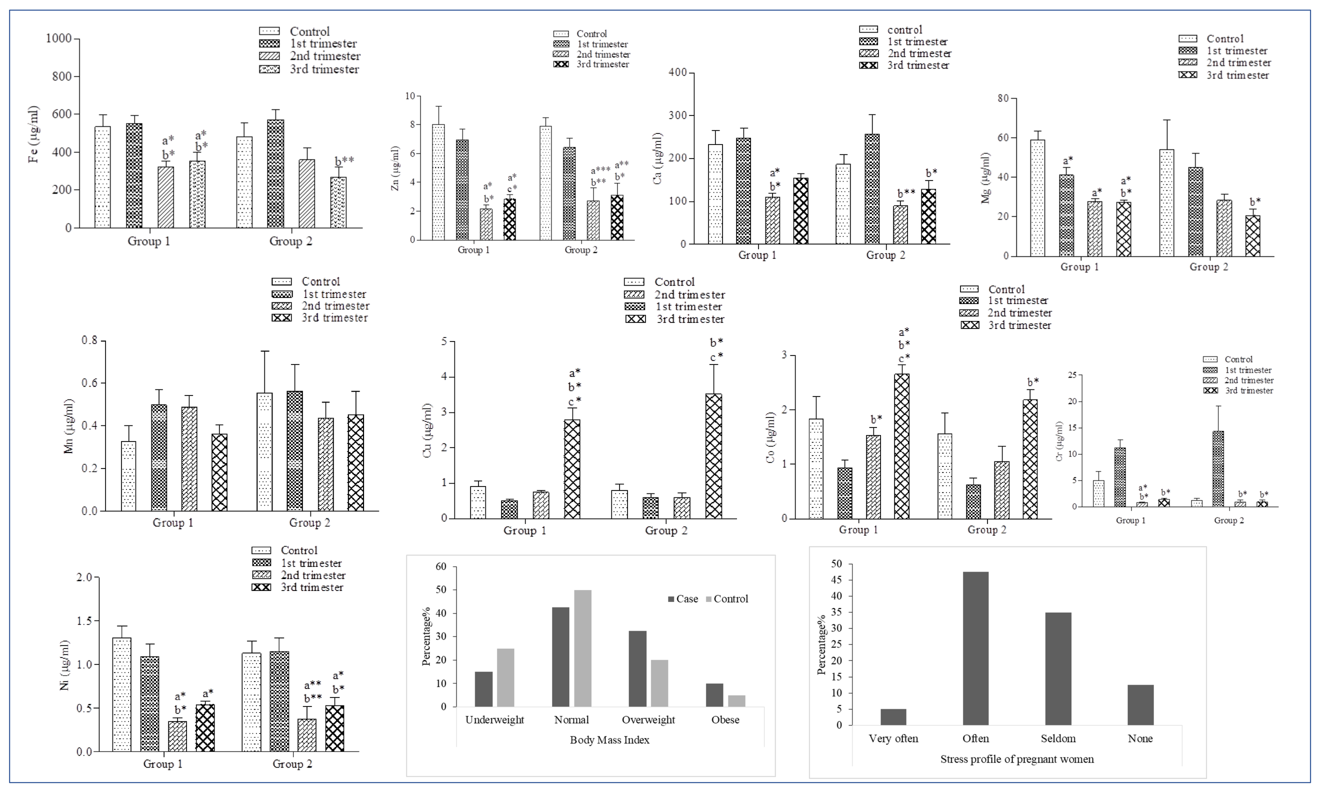
Ni levels in group I pregnant women were significantly lowered in 2nd trimester (p<0.05) as compared to non-pregnant controls and 1st trimester. In 3rd trimester the levels of Ni were significantly reduced (p<0.05) as compared to non-pregnant controls.

In group II pregnant women, the levels of Ni were significantly lowered in 2nd trimester (p<0.01) as compared to non-pregnant controls and 1st trimester. In 3rd trimester, the levels of Ni were significantly reduced (p<0.05) as compared to non- pregnant controls and 1st trimester

Cadmium

Cd is a toxic ultra-trace element added in our food through contamination and exposure. In the present study Cd was also estimated in all pregnant and non- pregnant samples but it was not detected in any of them.

Figure 1. Label in 28pt Calibri.



Discussion

DISCUSSION

During pregnancy, the micronutrient requirement of women is increased due to the progressive changes in the physiology to support the increased metabolic demand of the growing fetus (King, 2000). Any increase or decrease in their quantity may lead to severe health issues or physiological dysfunctions (Starchan, 2010). In pregnancy, the need for balanced level of micronutrients is increased because it can affect the outcomes of pregnancy as investigated in many previous studies (Mori, 2012). The reference ranges of trace elements were estimated in Chinese (Xiaobing et al., 2017) and Korean (Rhiwa et al., 2016) pregnant women using serum samples but no such study was performed on the whole blood samples of pregnant women.

The present study was conducted and designed to analyze the trace element concentration and other minerals concentrations in the whole blood samples of pregnant women. The values were compared for three trimesters and also with healthy non-pregnant control subjects to determine any rise or fall in the concentrations during the whole gestation period. Trace element levels showed marked alterations in relation to trimesters and in comparison with non-pregnant controls. Although the trace element concentrations were determined in pregnant women in different countries but no such study was performed in Pakistani population according to our knowledge. Moreover it is evident that no such study had been performed on same subjects for the whole gestation period (three trimesters) by estimating their trace elements and metal concentrations trimester-wise and concluding their final comparison.

Comparison between control and pregnant women showed significant decrease in Fe concentration. These results are in accordance with previous studies which have reported decrease in Fe concentration during different trimesters (Xiaobing et al., 2017). As maternal Fe concentration tends to decrease with increasing demand of fetus in 3rd trimester, Fe supplements are prescribed to fulfill the demand and to keep the mother healthy.

Significantly decreased levels of Zn were found in 2nd trimester as compared to 1st and also in 3rd trimester as compared to 2nd in both groups. On the other hand, the levels of Zn in pregnant women were lowered as compared to controls. Similar findings have been reported in previous studies which show a well-documented fall in Zn concentration throughout a human pregnancy (Campbell, 1988). Likewise, Alvarez (2007) has also reported the progressive decline in serum Zn concentration during gestation in Spanish women.

As the concentration of Zn tends to decrease during pregnancy, Zn deficiency could be harmful for mother and fetus. This is due to the fact that Zn deficiency is associated with growth retardation of fetus, complications and preterm labor and it may cause congenital anomalies (Campbell, 1988).

In current study, Cu level showed significant elevation in the 3rd trimester as compared to 1st and 2nd trimesters and non-pregnant control in both age groups. Previously, similar results have been observed in Korean (Rhiwa et al., 2016) and Chinese (Xiaobing et al., 2017) populations. Interestingly, increased Cu concentration during pregnancy has an impact over the absorption of Zn leading to its decreased circulating levels (Gibson, 2007).

Current investigations have demonstrated the significant decrease in Ca level during 2nd trimester in both age groups but the level was stabilized in 3rd trimester because the subjects started taking Ca supplements recommended by the gynecologist. Similar decline in Ca levels throughout pregnancy has been found by (Pitkin et al., 1979).

The level of Mg was significantly lowered in pregnant women in comparison to controls as well as, decreased from 1st to 3rd trimester of pregnancy in both age groups. Mg is not metabolically linked to any hormone but a decrease in the level of Mg can cause an increase in the level of PTH which can affect kidneys, intestine and bones. Different studies regarding Mg level have reported different results.

The level of Mn remained non-significantly different in 2nd and 3rd trimesters as compared to 1st trimester and control group. In group I, it showed a non-significant increase in pregnant as compared to control but in group II, there was a slight difference. In contrast, Eum et al., (2014), have reported that Mn concentration increases during pregnancy (Eum et al., 2014).

Our results exhibit significantly elevated level of Co during pregnancy in both age groups which is in agreement with the previous study conducted on Chinese women. The increase in Co concentration might be due to a certain release of trace elements into the blood stream due to metabolic and physiological changes during pregnancy (Xiaobing et al., 2017). Co is an ultra-trace element and is studied least in relation to pregnancy.

Significantly decreased Cr levels were observed in 2nd and 3rd trimesters as compared to 1st trimester in both age groups. This decline observed in current study might be due to the increased excretion of Cr in urine as described by Morris et al. (2000).

In the present study, the level of Ni significantly decreased in both age groups. Similarly, Rubányi et al. (1982) have also found that the serum level of Ni were lowered in pregnant women as compared to healthy non-pregnant females (Rubányi et al., 1982). Ni is least studied in relation to pregnancy and less data is available.

The present study is the very first study performed on a sample from Pakistan to estimate the levels of trace elements and first ever study to follow the same subjects throughout their pregnancy. Our findings will provide a great help in monitoring the maternal health status and fetal growth.

Conclusions

The present study is the very first study performed on a sample from Pakistan to estimate the levels of trace elements and first ever study to follow the same subjects throughout their pregnancy. Our findings will provide a great help in monitoring the maternal health status and fetal growth. However, further detailed and comprehensive studies are still needed in this regard. Although the study has provided a data on whole blood levels of trace elements concentrations of Pakistani women. There are, however, some limitations that must be noted. Firstly, the concentrations of some ultra-trace elements such as Co and Cr are at very low levels, thus the risk of contamination during blood collection, sample preparation and determination has to be seriously considered. Secondly, we have recruited only healthy females having no major disease, hypertension or diabetes mellitus but in future more studies can be performed recruiting pregnant females and comparing the levels of trace elements in diseased and healthy.

Figure 1. Pregnancy A New Start.

Figure 2. Flame Atomic Absorption Spectrophotometry

References

1. **Eum, J. H., Cheong, H. K., Ha, E. H., Ha, M., Kim, Y., et al.** (2014). Maternal blood manganese level and birth weight: a MOCEH birth cohort study. *Environmental Health,* **13**:31.
2. **Gibson, R. S.** (2007). The role of diet- and host-related factors in nutrient bioavailability and thus in nutrient-based dietary requirement estimates. *Food and Nutrition Bulletin,* **28**:S77–S100
3. **King, J. C.** (2000). Physiology of pregnancy and nutrient metabolism. *The American Journal of Clinical Nutrition,* **71**:1218S-25S.
4. **Mori, R., Ota, E., Middleton, P., Tobe-Gai, R., Mahomed, K., Miyazaki, C., and Bhutta, Z. A.** (2015). Zinc supplementation for improving pregnancy and infant outcome. *Cochrane Database Systematic Review,* **2015**:2.
5. **Pitkin, R. M.,** (1985). Calcium metabolism in pregnancy and the perinatal period: a review. *American Journal of Obstetrics and Gynecology,* **151**:99–109.
6. **Rubányi, G., Britalan, I., Gergley, A., and Kovách, A.G.** (1982). Serum Nickel concentrations in women during pregnancy, during parturition, and post partum. *American Journal of Obstetrics and Gynecology,* **143**(2):167-9.
7. **Strachan, S.** (2010). Trace elements. *Current Anaesthesia and Critical Care,* **21**:44–48.
8. **Xiaobing, L., Zhang, Y., Jianhua, P., Degian, M., Yajie, L., Weidog, L., Lichen, Y., and Xiaoguang, Y.** (2017). Reference values of 14 trace elements for pregnant Chinese women. *Nutrients,* **9**(3):309.