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Maternal Nutrient Intake and Maternal Serum Micronutrients and Their Relation to Birth Weight-A longitudinal study

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ABSTRACT

Micronutrients are well known to play an important role in the maintenance of health. Alterations in maternal-fetal disposition of some essential micronutrients could be a potential health risk for mother as well as the fetus. A longitudinal study was conducted in Khoy city located in North West of Iran to investigate maternal nutrient intake and maternal serum micronutrients and their relation to birth weight. Nutrient intake was computed based on 24 hour recall method. During the three trimesters of pregnancy, blood specimens were collected from 162 healthy pregnant women aged 16-40 years and from cord blood of their neonates. The mean age of studied pregnant women was 26 ± 5 years, and the mean birth weight of neonates was 3.3 ± 0.4 kg. Maternal serum levels of calcium, iron, zinc and copper were determined by an inductively couple plasma mass spectrometer (ICP/MS). The results showed that majority (41%) of pregnant women were in age group 26-36 years Fifty-five percent had high school and diploma levels of education and the total income of a majority of them was Rials 3-5 million per month. Anthropometric measurements, namely, height, weight, body mass index and upper mid arm during three trimesters of pregnancy showed significant differences except height according to different trimesters. The Mean energy, protein, calcium, iron, zinc, and copper were 2186 kcal, 74.5 g, 855.6 mg, 79.69, 12.3 mg and 1.58 respectively. Percentage adequacy of energy and protein intakes with reference to RDA recommendation showed 85% and 80% of subjects had sufficient RDA intakes, while calcium and zinc intake considered as insufficient. Energy, protein, calcium, zinc and iron intakes in the third trimester were significantly associated with birth weight of neonates. Regarding micronutrients, results indicate that iron levels decreased significantly from first to second trimester and significantly increased in third trimester. Serum zinc levels of subjects significantly decreased gradually during the first, second and third trimester. Serum copper levels increased significantly with increasing the gestational period. Calcium serum levels during three trimesters were constant. Maternal calcium, iron and zinc serum levels were associated with birth weight of neonates. Using Binary test the findings showed that calcium, protein, iron and energy intake as a predictor intake of pregnant women could be considered as primary "predictor factors" for birth weight of neonates.

Keywords: Nutrient intake, Micronutrients, Longitudinal study, Birth Weight

Introduction

Nutrition is an important health determinant that can affect the course of pregnancy and its outcomes. Optimal nutrient intake during pregnancy is reflected not only in the improved health of the mother, but also in the improved health of the infant [1]. Maternal diets during pregnancy need to provide energy and nutrients for the mother as well as for fetal growth [2]. Poor maternal nutrition during pregnancy, particularly during the third trimester, is a major cause of low birth weight in developing countries [3].

Trace elements are well known to play an important role in the maintenance of health. Alterations in maternal-fetal disposition of some essential trace elements could be a potential health risk for mother as well as the fetus [4]. Pregnancy is associated with increased demand of all the nutrients like calcium, iron, zinc, copper, and other micronutrients. Deficiency of any of these could affect pregnancy, delivery and outcome of pregnancy [5]. Calcium, iron, zinc, and copper are essential elements required for the normal growth and development of the fetus [6].

The role of calcium in intermediary metabolism and skeletal development in pre-natal and post natal periods have been established [7]. Calcium plays an important role in muscle contraction and regulation of water balance in cells.

Iron is an important micronutrient and is necessary for hemoglobin synthesis and several other important functions in the body. Iron deficiency can result not only in reduced oxygen carrying capacity due to lowered hemoglobin levels, but can also affect immunity and growth and development. More than half the women of reproductive age in developing countries are anemic, primarily

due to iron deficiency as a result of poor diets and increased requirements [8]. During pregnancy, iron needs are usually very high to meet the requirements for the fetus, placenta, and maternal red cell expansion. Worldwide, poor pregnancy outcome has been most commonly associated with anemia caused by low plasma levels of iron [9].

Inadequate zinc during the prenatal period has been particularly linked with low birth weight [10]. Zinc is required for cellular division and differentiation, and is an essential nutrient for normal embryogenesis [11].

Copper is an essential trace element for enzyme systems, such as the catalase, superoxide dismutase and cytochrome oxidase systems, and its deficiency can lead to a variety of nutritional and vascular disorders [4]. In human adults, severe copper deficiency is relatively rare, whereas signs of moderate copper deficiency were observed in human infants under a variety of conditions [12].

The micronutrient profile in maternal body will influence the normal growth and development of the fetus in the womb. In the present study, energy and protein intake, some mineral intake of pregnant women during pregnancy, maternal and cord blood levels of calcium, iron, zinc, and copper were determined to assess the relationship between these parameters in pregnant mothers and birth weight of their neonates.

Material and Methods

Selection of area, hospitals and subjects

Khoy, a city of Western Azarbaijan province located in North West of Iran, was selected for research work, as it is the home town of the investigator. Seven urban health centers was

selected. In the present study a total of 450 healthy pregnant women, aged between 18-40 years, attending in public health centers for their routine prenatal care were selected as subjects of present study in different social economic status. From 450 subjects 162 pregnant women were voluntary for giving blood sample to analyze the microelements in serum. All subjects were invited by the head midwife of the center to take part in this longitudinal Study. The inclusive criteria were age group (16 to 40 years) and who continuously visited for health care during the three trimesters of pregnancy in selected urban health care centers areas of Khoy city. The pregnant women with diabetes mellitus and cardio vascular disease (CVD), multiple pregnancies, mothers with placenta previa and placenta abruptia were excluded from this study.

Since the investigator had registered for Ph.D in University of Mysore, the study was approved by the Human Ethical Committee of the University of Mysore and Urmia Medical University (home town of investigator) as all the health centers and hospitals in Khoy city are affiliated to Urmia Medical University. Written consent letter from all of subjects was obtained and they accepted to be the subjects to continue until the birth of the babies. The study was carried out in the year 2009 to 2010. The required information about various aspects proposed to study was provided by questionnaires.

Selection and Description of the Tools Used for the Study

- Questionnaire:

A Questionnaire is a tool or device for securing answers to a set of questions, by respondent who fills in the Questionnaire. The Questionnaire method was selected for the

present study, as it is frequently used method of data collection [13]. In the present study, questionnaire was selected to collect the maternal family back ground and nutrition status of pregnant women.

- Diet survey and Nutrient Intake:

Dietary assessment gives reliable information on dietary intake[14]. The dietary assessment of pregnant women was done at the end of first, second and third trimester, three times during pregnancy, and food intakes were obtained using 24-hour dietary recall method. Probing questions were used to help the subjects to remember different meals and drinks consumed on previous day, using standard cups and measures. Probing questions were used to help the subjects to remember all foods and drinks consumed on previous day. Questions were extended to methods of food preparation, portion sizes, as well as to approximate sizes of meals. Standard cups were used to measure the quantity of intake of the cooked food [15] The information about the quantity of raw material (raw quantity) taken for cooking as well as the cooked food by the subject was recorded in terms of household measures/number/kg to find out the quantum of raw food intake. From the information provided, the cooked and raw amount of foods consumed by each subject was then calculated. The mean intake of different nutrients consumed was then computed for a day the help of ready recknor to calculate nutritive value. The ready recknor was prepared by the investigator using Iranian food preparation and the nutritive value of Iranian national food composition[16]. Nutrient adequacy of energy, protein, calcium, iron, zinc and copper was calculated, using the ready recknor with reference to Iranian recommendation[17].

- *Anthropometric Measurements*

Anthropometry provides a simple, reliable and low-cost method of assessing maternal nutrition status which can be universally applied at the primary care was selected. Maternal anthropometry indicates the risk of intrauterine growth retardation and low birth weight[18]. Body measurements, namely height and weight, were measured by the investigator using standard methodology as described by Jelliffe [19]. The measurements were made on the participants wearing a minimum amount of clothing by using digital weighing balance with a sensitivity of 100 g. Height was measured in cm using a locally made anthrop-meter. The pregnant women were asked to maintain an upright and erect posture with her feet together and the back of her heels touching the pole of the anthrop-meter, and the horizontal headpiece was lowered onto the women's head. Body Mass Index: Body mass index in early first trimester in initial visit when pregnant women refer to health center for submitting the positive garvindex test results and apply for having prenatal care visit was calculated by using the formula: weight (kg)/ height (m)² [20]. Birth weight of newborns were taken within 24 hours after birth, using standard procedure[21]. A beam balance with an accuracy of 50 g was employed for weighing the infants. Infants were weighed with minimum clothing while the baby was restful.

Biochemical Analysis

Venous blood specimens were collected from participating pregnant women during each of the three trimesters were collected in metal-free plain tubes. Plain tubes were centrifuged (1100 g) for 15 min at 3500 rpm and the serum was separated and kept in trace elements-free tubes and stored at -40°C until analysis. Maternal serum levels of zinc,

copper, and iron were measured by an inductively couple plasma mass spectrometer (ICP/MS).

Processing of the data and statistical analysis

The data collected was subjected to statistical tests utilizing the SPSS-16.0 version (SPSS, Chicago, IL, USA). Suitable tests using Student "t" test, ANOVAs one way, Binary regression carried out to interpret the results.

Results

Family Background of Pregnant Women

Details of all selected pregnant women are presented in Table 1. The mean age of pregnant women was 26.1±5.8 years and the age range was 18-40 years. Majority (41%) of pregnant women were in age group 26-36 years, followed by the age group 20-26 years (36%). Majority of subjects (55%) had high school and diploma levels of education. Based on what the subjects and their family declared, the total income of a majority of them was Rials 3-5 million per month. The percentage of subjects with income less than Rials 3 (million/month) and above Rials 5 million/month were 27 and 25 respectivel. (See Table 1)

Anthropometric Measurements of Pregnant Women during Pregnancy

Anthropometric measurements, namely, height, weight, body mass index and upper mid arm at first, second and third trimester of pregnancy are given in Table 2. Our findings showed significant differences among weight, body mass index and upper mid arm

circumference according to different trimesters. (See Table 2)

Energy and Nutrient of Pregnant Women during Pregnancy

Energy and nutrient intakes per day of the subjects during the three trimesters are presented in Table 3. The mean energy nutrient intake per day of the subjects in the first trimester were significantly lower than in second and third trimester. Mean energy and nutrient intake by pregnant women in third trimester were slightly and not significantly lower than second trimester. It is clear from the Table 3, that in the first trimester of pregnancy energy and protein intake was inadequate. Energy and protein intakes during second and third trimester were almost adequate. Calcium, iron and zinc intakes during first, second and third trimesters were inadequate. Iron intake in dietary was inadequate but after including the iron supplements it was adequate. (See Table 3)

Profile of Serum Calcium, Iron, Zinc and Copper during Three Trimesters

The profile of selected biochemical parameters, namely, calcium, iron, zinc and copper in the serum of the pregnant women during the three trimesters of pregnancy is given in Table 4. Our findings showed that there was observable significant difference at 5% level as shown by one way ANOVA post hoc Bonferroni test during the three trimesters of pregnancy (See Table 4)

The measurements of serum calcium during the three trimesters of pregnancy showed a slightly decrease in serum calcium during second trimester of pregnancy but the mean serum calcium levels between the first and

third trimester were the same (8.9mg/dl). (See Table 4)

As it is clear from the Table 4 there was noticeable significant difference in iron and zinc levels during three trimesters of pregnancy. In comparison with the values in the first trimester, serum iron and zinc concentration kept decrease in the third trimester.

The mean levels of copper increased with the progression of pregnancy and there was significant difference at 5% level in copper levels was shown in first, second and third trimester.

Birth Weight of Neonates

The mean birth weight of neonates were 3275 ± 552 gram. Birth weight was classified according to W.H.O classification into two categories namely NBW (>2500g) and LBW (<2500g). Our findings showed a majority (89%) of them had normal birth weight and 11% of them were considered as low birth weight

Maternal Energy and Nutrient Intake in Third Trimester vs. Birth Weight

It was interesting to analyze the dietary intake of energy and nutrient in different levels intake in pregnant women with reference to variations in birth weight of neonates. The data was subjected to one-way ANOVA and the findings are presented in Table 5.

It is clear from the Table 5, with higher intake of energy resulted in heavier neonates, it means pregnant women who consumed < 75% RDA with mean intake 1794 ± 54 kcal/day gave birth to neonates with 2.6 kg, while

pregnant women with $\geq 100\%$ RDA (2698 ± 107 kcal/day) of energy gave birth to neonates with 3.6 kg. Significant difference in neonatal birth weight (400-600gr) was absurd with increasing almost 374-530 kcal/day energy intakes by subjects.

Similarly with increasing protein intake by the subjects, the birth weight of corresponding neonates increased. Pregnant women with protein intake of $< 75\%$ RDA with mean intake 38.7 ± 4.7 g/day gave birth to neonates with 2.5 kg, while pregnant women with higher intake of protein $\geq 100\%$ RDA (72.8 ± 7.4 g) gave birth to neonates with 3.4 kg. In the other word with increasing 16 -18g protein intake by pregnant women, birth weight of neonates increased 300-600 gram. (See Table 5)

It is evident from the Table 5, that the pregnant women who consumed calcium less than $< 75\%$ RDA (693 ± 207 mg/day) gave birth to lighter neonates with 3.0 kg (still in normal weight range), while the pregnant women with $\geq 100\%$ RDA (1298 ± 84 mg/day) calcium consumption gave birth to heavier babies (3.5kg).

The pregnant women with iron intake in dietary $< 75\%$ RDA (16.1 ± 3 mg/day) gave birth to neonates with 2.9 kg, while the pregnant women with higher intake of iron ($\geq 100\%$ RDA or 31.5 ± 1 mg/day) gave birth to neonates with heavier neonates (3.6 kg).

As shown in the Table 5 pregnant women who consumed zinc $< 75\%$ RDA (9.9 ± 1.3 mg/day), gave birth to neonates 2.9 kg in compared with women who consumed $\geq 100\%$ RDA (15.9 ± 0.2 mg/day) gave birth to heavier neonates (3.5 kg). Copper consumption had no significant influence on the birth weight of the babies.

3.7). Maternal Serum Calcium, Iron, Zinc and Copper Levels in Third Trimester vs. Birth Weight of Neonates

It was important to analyze the results of different categories of serum calcium, iron, zinc and copper levels of pregnant women with reference to variations in birth weight of neonates. The data was subjected to one-way ANOVA and the findings are presented in Table 6. It is clear from the Table, Pregnant women with more than 1200 μ g/dl, 80 μ g/dl serum levels of calcium and iron, gave birth to neonates with heavier birth weight 3.5 and 3.6 respectively; whereas mothers with serum levels of calcium and iron less than 900 μ g/dl and 60 μ g/dl gave birth to babies with lighter birth weight 2.9 and 2.8 respectively ($p < 0.05$). (See Table 6)

It is clear from Table 7, pregnant women with more than 70 μ g/dl serum levels of zinc, gave birth to neonates with heavier birth weight 3.5, whereas mothers with serum levels of zinc less than 60 μ g/dl gave birth to babies with lighter birth weight 2.9 ($p < 0.05$).

In the present study the association between maternal copper levels and birth weight was not significant.

3.8) Low Birth Weight and Normal Birth Weight of Neonates and Maternal Nutrient Intake

It is evident from the study that the various levels of nutritional attributes influence the birth weight of infants. Therefore, it is interesting to find out whether the low birth weight and normal birth weight infants have similar or different maternal nutritional attributes. In order to obtain an answer for this question, the data on birth weights of neonates classified as Normal and Low Birth Weight and the corresponding maternal nutritional status were illustrated in Table 7. As Table illustrates, the pregnant women who gave

birth to LBW babies had significantly lower consumption of energy, protein, calcium, iron and zinc than those who gave birth to neonates with low birth weight. It is clear from the Table 7. Pregnant women who gave birth to low birth weight had lower levels of calcium, iron and zinc levels than the pregnant women who gave birth to normal neonates. (See Table 7)

Predicting Factors for Birth Weight

The nutrient intakes such as energy, protein, calcium, iron, zinc, the maternal serum micronutrients levels such as calcium, iron, zinc affected birth weight of neonates. It was interesting to find out among these parameters, which factors can be considered as major predictable factors for birth weight of neonates. Therefore the binary logistic regression was carried out to find out the possible factors associated with birth weight (results are presented in Table 8). The findings showed calcium, protein, iron, energy intake, could be considered as "prediction factors" for birth weight of neonates. (See Table 8)

Discussion

The percentage of energy, protein and copper were adequate (85%, 80% , 86%.) in the present study while the consumption of calcium, iron and zinc were inadequate (51%,56%,50%) according the Recommended Daily Allowance [22]. In this study iron consumption in dietary was inadequate with reference to RDA, but it was adequate when iron supplementation is added. The actual consumption of supplementary nutrients was not monitored and this is one of the limitations of the study. . Similar studies in India [23]; Spain [24], USA; [25], and Iran [26], [27],

showed that energy and protein intakes were adequate while calcium, iron and zinc intakes were inadequate.

The mean serum calcium during the first, second and third trimesters were almost the same. The similar results were reported in Hungary [28], and in Argentina [29]. In pregnancy, the very high circulatory concentrations of estrogens and progesterone alter the concentration of many substances including calcium in the maternal blood [30]. Studies of calcium homeostasis responses during pregnancy have shown increase in both intestinal calcium absorption and urinary calcium excretion during pregnancy and increase rate of bone turnover during pregnancy [31, 32].

Our findings showed that there was noticeable significant difference in iron levels during three trimesters Similar variation in serum iron during pregnancy was shown in South Korea [33], and India [34]. There was significant difference in zinc and copper levels but not magnesium levels during the three trimesters of pregnancy. Regarding the zinc variations during pregnancy period, our results indicated that zinc levels of subjects kept decreasing gradually from first trimester to third trimester. Similar results were shown in other studies in Turkey [35]; Spain;[36]; and in USA [37]. The decline may be explained by a disproportionate increase in plasma volume, as well as the maternal–fetal transfer. The other reasons possibly be decrease in zinc binding [38], or low dietary bioavailability [39], or very high amounts of copper or iron in the diet that compete with zinc at absorption sites [40].

Mean serum copper levels during the three trimesters of pregnancy in our results show that copper levels rise significantly with increasing gestational periods. Similar results were shown in studies in Spain [36]; Turkey [35], and in China [41]. The increase of

copper with the progression of pregnancy could be partly related to synthesis of ceruloplasmin, a major copper binding protein, as a result of elevated levels of maternal estrogen. Another reason may be the decreased biliary copper excretion induced by hormonal changes, typical during pregnancy [42]

Results of the current study show as energy and protein intake of pregnant women are increased the birth weight of neonates also increased. Other groups of investigators in Canada[43], India [44], Iran[45] have reported comparable results with regard to protein and energy intake and birth weight.

It is interesting to observe that with increase in the amount of calcium, iron and zinc intake, the mean birth weight increased. Similar results were observed in other countries namely USA [46]; UK [47]; South Africa [48]; Iran[49].

Copper consumption had no significant influence on the birth weight of the babies. Similar results were reported in other countries like USA[50]; UK [51]; and South Africa [52].

Regarding the association between maternal serum calcium, iron and zinc and birth weight, our findings are in close agreement with studies in California [53]; UK [48]; Korea [54]; and Iran [55], which reported there were significant associations between maternal calcium, iron and zinc with birth weight of neonates. The mechanisms that operate by which poor iron status may affect birth weight and preterm births remains poorly understood [56]. A few tested hypotheses showed that iron is necessary for hemoglobin synthesis and several other important functions in the body. Iron deficiency can result not only in reduced oxygen carrying capacity due to lowered hemoglobin levels, but can also affect immunity and growth of the fetus [57].

Zinc is an important nutrient during pregnancy and plays a critical role in normal growth and development, cellular integrity and several biochemical functions. Zinc is a component of many enzymes, it takes part in normal cell division, and has a role in carbohydrate, lipid, protein, and nucleic acid synthesis. Therefore an impairment in these processes can retard fetal growth and result in LBW of the infant [37].

In the present study the association between maternal copper levels and birth weight was not significant, which is in agreement with results of other studies in Turkey [58] and Kuwait [59]

To distinguish the relationship between maternal nutrition status in low birth weight and normal birth weight of neonates, analysis of data was done in the third trimester of pregnancy. As Table 7 illustrates, the pregnant women who gave birth to LBW babies had significantly lower consumption of energy, protein, calcium, iron and zinc than those who gave birth to neonates with low birth weight. It is clear from Table 7 pregnant women who gave birth to low birth weight had low levels of calcium, iron and zinc levels than the pregnant women who gave birth to normal neonates. Similar results have been reported in Turkey [60] and Iran [61].

The findings showed calcium, protein, iron, energy intake, could be considered as "prediction factors" for birth weight of neonates. In agreement with our results other studies in Canada [62]; California [53]; and India [63] reported the same results.

It may be concluded that for the study that maternal energy, protein, calcium, iron and zinc intake along with higher maternal serum calcium, iron and zinc influenced birth weight of the neonates. The findings of the study indicated that total iron intake, calcium, protein, iron and energy intake as predictor factors for birth weight. It may be

recommended from the present study that the government and nongovernment agencies should focus on the effective implementation of program to improve the nutrient intake of pregnant women to optimize their health which will elevate the pregnancy outcome.

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Table 1: Information on Pregnant Women and Their Family Income (n=450)

Variables	Category	N	Percent
Age group(yr)	<20	81	18
	20-26	162	36
	26-36	186	41
	≥36	21	5
Education Level	≤Secondary	97	22
	High School& Diploma	249	55
	Graduation	105	23
Total income /month in million Rials †	<3	121	27
	3-5	216	48
	>5	113	25

Note: †=Rials (Iranian currency), [1 USD equal Rials.1150 and Euro equal Rials.1450]

Table 2: Anthropometric Measurements of Pregnant Women (N=450)

Parameter	Trimesters			F. Values
	1	2	3	
Height(cm)†	159.4±4.7	159.4±4.7	159.4±4.7	-
Weight(kg)	60.7±12.0 ^a	67.6±12.1 ^b	72.0±12.1 ^c	*
BMI(kg/m ²)	23.9± 3.8 ^a	25.82±4.0 ^b	28.35±3.9 ^c	*
U M A C (cm)	24.9±3.8 ^a	27.1±3.9 ^b	28.7±4.0 ^c	*

Note: †=No test was conducted as the height was the same.

Different superscripts in the same row indicate significant difference at 5% level as shown by post hoc Bonferroni test.

*Significant at 5percent level

Table 3: Mean Energy and Nutrient Intakes (per day) of Pregnant Women during Pregnancy (n=450)

Variables	Trimesters			F. Values
	First	Second	Third	
Energy(kcal/day)	1697±264 ^a (77)	2201±115 ^b (86)	2186 ± 270 ^b (85)	207.7*
RDA†(kcal)	2200	2500	2500	
Protein(g/day)	59.5±21.2 ^a (74)	77.8±13.9 ^b (83)	74.5 ± 12.7 ^b (80)	68.59*
RDA(g/day)	51.3	56.1	60.7	
Calcium(mg/day)	688±284 ^a (42)	865±290 ^b (55)	855±289 ^b (51)	47.38*
RDA(mg/day)	1200	1200	1200	
Iron(mg/day)	19.0±5.3 ^a (50)	23.1±3.4 ^b (57)	22.9±9.1 ^b (56)	41.0*
RDA(mg/day)	30	30	30	
Total Iron‡ (mg/day)	19.0±5.3 ^a	79.78±5.4 ^b	79.69±8.9 ^b	1522*
Zinc(mg/day)	9.0±2.4 ^a (48)	12.5±1.8 ^b (52)	12.3 ± 2.4 ^b (50)	166.1*
RDA(mg/day)	15	15	15	
Copper(mg/day)	1.39±0.7 ^a (82)	1.60±0.8 ^b (84)	1.58 ± 0.6 ^b (86)	5.7*
RDA(mg/day)	1.5-3	1.5-3	1.5-3	

Note:†= Recommended Daily Allowance [26, 27]

‡=Iron in dietary + Iron supplement (Ferrous Sulfate 60mg)

Figures in parenthesis indicate percent adequacy of RDA

* Significant at 5 % level

Different superscripts in the same row indicate significant difference at 5% level as shown by post hoc Bonferroni test.

Table 4: Serum Levels of Calcium, Iron, Zinc and Copper in Maternal Serum during Pregnancy (n=162)

Serum Levels	Reference values†	Trimesters			F. Values
		First	Second	Third	
Calcium (mg/dl)	8.9-10.4	8.96±0.48	8.86±0.47	8.91±0.42	NS
Iron (µg/dl)	60-160	76.0 ± 17.8 ^a	63.5 ± 15.2 ^b	70.1 ± 14.4 ^c	25.0*
Zinc (µg/dl)	70-102	79.5 ± 15 ^a	74.5 ± 16.1 ^b	65.3 ± 14.9 ^c	33.8*
Copper (µg/dl)	70-150	130.9 ± 43.5 ^a	172.0±38.94 ^b	193.2 ± 28.5 ^c	115.7*

*Significant at the 0.05 level; NS: Not Significant

Different superscripts indicate significant difference at 5% level as shown by Post hoc Bonferroni test.

†No reference values for pregnant women so values for non pregnant were considered[28]

Table 5: Birth Weight vs. Energy and Nutrient Intake of Pregnant Women in Third Trimester (n=450)

Parameter	Level of % RDA Adequacy	N	%	Mean±SD Intake	Mean Birth Weight (kg)	F test result
Energy (kcal/day)	<75	59	14	1794±54	2.6 ^a	79.6*
	75-100	331	74	2168±161	3.2 ^b	
	>100	52	12	2698±107	3.6 ^c	
Protein (g/day)	<75	71	16	38.7±4.7	2.5 ^a	54.4*
	75-100	269	60	54.5±3.9	3.1 ^b	
	>100	110	24	72.8±7.4	3.4 ^c	
Calcium (mg/day)	<75	297	66	693±207	3.0 ^a	12.2*
	75-100	84	19	1046±77	3.3 ^b	
	>100	69	15	1298±84	3.5 ^c	
Iron (mg/day)	<75	212	47	16.1±3	2.9 ^a	94.5*
	75-100	170	38	23.5±1	3.4 ^b	
	>100	68	15	31.5±1	3.6 ^c	
Zinc (mg/day)	<75	184	41	9.9±1.3	2.9 ^a	83.6*
	75-100	179	40	12.9±0.5	3.4 ^b	
	>100	87	19	15.9±0.2	3.5 ^b	
Copper (mg/day)	<75	29	6	0.61±0.09	3.4	NS
	75-100	38	9	0.91±0.08	3.3	
	>100	383	85	1.82±0.56	3.3	

Note:* P < 0.05, Different superscripts in the same row indicates significant difference at 5% level as shown by post hoc Bonferroni test.

†=Intake of energy and nutrients in pregnant women is divided in three category based on <75% RDA,75%-100% RDA,>100%RDA levels[21]

Table 6: Maternal Serum Calcium, Iron, Zinc and Copper Levels in Third Trimester of Pregnant Women vs. Birth Weight of Neonates

Parameters	Reference values†	Levels	N	%	Birth Weight (kg)	F values
Calcium (mg/dl)	9.2-11	≤8.7	21	13	2.9 ^a	12.2*
		8.8-9	87	54	3.3 ^b	
		>9.1	54	33	3.5 ^c	
Iron (µg/dl)	60-150	<60	61	38	2.8 ^a	72.7*
		60-80	48	29	3.3 ^b	
		>80	53	33	3.6 ^c	
Zinc (µg/dl)	70-110	<60	68	42	2.9 ^a	24.6*
		60-70	39	24	3.3 ^b	
		>70	55	34	3.5 ^b	
Copper (µg/dl)	80-150	<150	14	9	3.5	NS
		150-200	85	52	3.4	
		>200	63	39	3.3	

Note:* P < 0.05,

Different superscripts for each element in a column indicate significant difference at 5% level as shown by post hoc Bonferroni test.

†Reference values in non pregnant women[28]

Table 7: LBW and NBW of Neonates: Energy, Nutrient Intakes and Serum Minerals Levels of Pregnant Women in Third Trimester

Variables		LBW (n=50)	NBW (n=400)	T-test results
Intake /day	Energy(kcal)	1501±103	2202±255	*
	Protein((g)	47.1±8	64.0±9	*
	Calcium(g)	512±110	881±266	*
	Iron (mg)	20.8±8.3	22.9± 4.1	*
	Total Iron Intake (mg) ‡	77.8±6.2	79.6± 5.3	*
	Zinc(mg)	10.6±2.8	13.1±4.2	*
	Copper(mg/day)	1.81±0.53	1.65±0.21	NS
Serum Levels	Ca(mg/dl)	8.02±0.41	8.92±0.43	*
	Iron(µg/dl)	57.5±12.8	71.6±13.8	*
	Zn(µg/dl)	58.8±21.1	66.1±13.8	*
	Copper(µg/dl)	206.9±30.8	191.4±27.8	NS

Note:*Significant at 5percent level; NS: Not Significant

‡=Iron in dietary + Iron supplement (Ferrous Sulfate 60mg)

Table 8: Results of Binary Logistic Regression- Prediction Factors in Relation to Birth Weight

Variables	Binary	Standard Error	Significant
Energy Intake (kcal/day)	0.014	0.006	0.022*
Protein Intake (g/day)	0.191	0.067	0.005*
Calcium Intake(mg/day)	0.002	0.001	0.001*
Iron Intake (mg/day)	0.515	0.192	0.007*
Total Iron Intake (mg/day)‡	2.079	0.150	<0.001*
Zinc Intake (mg/day)	0.075	0.236	0.749 ^{NS}
Copper Intake (mg/day)	0.103	0.389	0.791 ^{NS}
Serum Ca(mg/dl)	0.428	0.653	0.512 ^{NS}
Serum Fe (µg/dl)	0.092	0.207	0.657 ^{NS}
Serum Zn (µg/dl)	0.010	0.033	0.758 ^{NS}
Serum Copper (µg/dl)	0.004	0.008	0.567 ^{NS}

Not: Variable(s) entered for analyzing energy, protein, calcium, iron, total iron intake, zinc, copper intake, maternal calcium, iron, zinc and copper levels.

*Significant at 5percent level; NS: Not Significant

‡= Iron in dietary + Iron supplement (Ferrous Sulfate 60mg)