Chapter III

Materials and Methods
3. MATERIALS AND METHODS

Methodology adopted in the present study entitled: “A Study on the Association between Maternal Micronutrient Status and Birth Weight of Neonates” is discussed under the following headings:

3.1. Selection of Health Centers and Hospitals

The selected urban area, the city of Khoy is located in the Western Azarbayjan, situated in North West of Iran. Khoy city was selected as the area for research work, as it is the home town of the investigator. See Fig 3 for the location of Khoy in Iran.

Fig 3: Location of Khoy city in Iran
In khoy health care centers are responsible for taking care of pregnant women during pregnancy and women are referred to these centers when they miss their menstruation and for confirming the pregnancy. Pregnant women are given prenatal care during pregnancy period in health care centers. At the end of pregnancy they are referred to hospitals for delivery. There are fifteen health centers in Khoy. Nine health centers from the fifteen health centers, namely Valiasr, Samadzadeh, Rabat, Motahhari, Salamatbakhsh, Golsanamloo, Gamariasl, Taleghani and Khorabloo along with Gamarbanihashem, the only referral obstetrics and gynecology hospital in Khoy, were selected based on their acceptance and cooperation to carry out the study.

3.2. Selection of the Subjects

A total number of 450 healthy women, aged between 17 and 45 years, after pregnancy confirmation and undergoing prenatal care during the three trimesters of pregnancy, were registered as subjects for the present study. All subjects were requested by the head midwife of the center to take part in this longitudinal study. 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. Written consent letters from all the subjects were obtained. They agreed to be the subjects during three trimesters of pregnancy period until the birth of the babies (Appendix A). Then they were referred to hospitals where their delivery would take place.

Since the investigator had registered for Ph.D in the 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 are affiliated to Urmia Medical University.

3.3. Selection and Description of the Tools Used for the Study

The required information about various aspects proposed to study was obtained using the following tools and techniques.

3.3.1. Questionnaire: A Questionnaire is a tool or device for securing answers to a set of questions from the respondent who fills in the questionnaire. The questionnaire
method was selected for the present study, as it is a frequently used method of data collection. The merits of a questionnaire are its low cost, non-bias, anonymity and application in large-scale survey (Young, 1987). In the current study, suitable questionnaires were formulated and pre-tested with a small population (pilot study) to elicit information on details like age, age at marriage, type of delivery, number of alive children, abortion, common health disorders during pregnancy, i.e., morning sickness, pica habits, heartburn and reflux, constipation, mouth and gum discomfort, swollen leg and oedema and anemic symptoms. Questions on information like educational qualification of the family and their occupations and the total monthly income of the family were included (See Performa in Appendix B).

3.3.2. Diet Survey: Diet assessment gives reliable information on dietary intake (Bamji et al., 2009). Two methods of diet survey were selected, namely, frequency of food consumption and the 24-hour recall method. The information was collected and recorded in Proforma with the help of interview which is considered as a powerful tool (Bamji et al., 2009). Information on frequency of food consumption was collected from the subjects using questionnaire which included food consumed in Iran (See Appendix C). Diet intake was 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 (Thimmayamma and Parvathi, 1990) (Appendix D).

3.3.3. Anthropometric Measurements: Anthropometry provides a simple, reliable and low-cost method of assessing maternal nutrition status which can be universally applied (Jelliffe, 1966) and this was used at the primary care. Maternal anthropometry indicates the risk of intrauterine growth retardation and low birth weight (World Health Organization, 1995). Body measurements, namely, height, weight, fundal height, mid upper arm were selected by the investigator using standard methodology as described by Jelliffe (Jelliffe, 1966).

3.3.4. Biochemical Analysis: Haemoglobin and other blood parameters are considered as good indicators of nutritional status (Bamji et al., 1996). The selected methods for haemoglobin assessment was Cyanomethemoglobin (W.H.O/ UNICEF/
UNO, 1998). Inductively Couple Plasma Mass Spectrometer (ICP/MS) (Shariati et al., 2009) was selected for serum calcium, iron, zinc, copper and magnesium analysis.

3.4. Conducting the Study

In order to accomplish the major three objectives of the study, the investigation was carried out in the following phases:

3.4.1. First Phase
3.4.1.1. Selection of Area: In this phase nine health care centers from the fifteen health centers present in Khoy, along with Gamarbanihashe hospital, were selected to conduct the study.

3.4.1.2. Selection of the Subjects: A total number of 450 healthy women with confirmed pregnancy (17-45 years) who started their prenatal care were selected based on their willingness. Written consent letters from all the subjects were obtained and they agreed to be the subjects until the birth of the babies.

3.4.1.3. Collection of Information of Family Background: Suitable questionnaires were formulated to elicit information on details like age, age at marriage, type of delivery, number of alive children, abortion, common health disorders during pregnancy, i.e, morning sickness, pica habits, heartburn and reflux, constipation, mouth and gum discomfort, swollen leg and oedema and anemic symptoms. Questions on information like educational qualification of the family and their occupations and the total monthly income of the family were included. All subjects were interviewed to elicit information on family background. This longitudinal study was carried out in the year 2009 to 2010.

3.4.1.4. Development of Ready Reckoner for Food and Nutrient Intake: The information about the quantity of the raw material (raw quantity) taken for cooking as well as the cooked food was recorded in terms of household measures/number/kg to find out the quantum of raw food intake. The mean intake of different nutrients consumed in a day was then computed for a day with the help of a ready reckoner to calculate the nutritive value. The ready recknor was developed by the investigator
using nutritive value given in food composition table from the National Nutrition and Food Technology Research Institute, Iran (NNFTRI, 1996).

3.4.1.5. Diet Survey: Diet survey was carried out using two methods: frequency of food consumption and 24-hr recall method. 24-hr recall method was done at the end of first trimester (12th week), second trimester (28th week) and third trimester (38th week). Data on frequency of consumption of food (daily, weekly, monthly) was collected by interviewing the subjects with the help of a questionnaire in the form of a checklist at the end of first trimester (12th week), second trimester (28th week) and third trimester (38th week).

3.4.1.6. Assessment of Anthropometric Measurement: Height and weight, were measured by the investigator using standard methods (Jelliffe, 1966). Measurements were taken at the end of the first (14±2 week), second (28±2 week) and third (38±2 week) trimesters. The details of the measurements are given below:

**Height:** Height was measured in cm using a locally made anthrop-meter. The pregnant woman was 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. The horizontal headpiece was lowered onto the woman’s head and the measurements were taken to the nearest 0.1 cm.

**Weight:** The measurements were taken with the participants wearing a minimum amount of clothing. The weights of pregnant women were recorded at the early first trimester on their first visit and continued in every trimester, by using digital weighing balance with a sensitivity of 100 g.

**Mid Upper Arm Circumference (MUAC):** MUAC is the circumference of the left upper arm, measured at the mid-point between the tip of the shoulder and the tip of the elbow. It was measured using fiberglass measuring tape in cm.

**Fundal Height:** Fundal height was measured as the distance between the symphysis pubis and the highest point of the uterine fundus, defined with a gentle pressure on a plan at right angle of the abdominal wall and was marked.
Abdomen Circumference: Abdomen Circumference was measured using fibreglass tape in cm at the level of the umbilicus by cross-over technique.

3.4.2. Second Phase

3.4.2.1. Biochemical Assessment (N=162): Venous blood specimens were collected from the participating pregnant women at the end of first, second, third trimester of pregnancy. New-born cord blood sample was collected before delivery of placenta. The collected blood was poured into metal-free plain tubes and was allowed to clot at room temperature. Plain tubes were centrifuged for 15 minutes at 3500 rpm and the serum was separated and kept in trace elements-free tubes and stored at $-40^\circ$C until analysis. Finally samples were kept in dried ice and were sent to Modarres University in Tehran for biochemical analysis. The analysed items consisted of haemoglobin (g/dl blood), calcium (mg/ dl), iron (µg/dl), zinc (µg/dl), copper (µg/dl) and magnesium (mg/ dl).

3.4.3. Third Phase

3.4.3.1. Body Measurement of the Neonates: The pregnant women were monitored for 3 trimesters. After delivery, the neonates were measured for weight, height, head, and chest circumferences within 24 hours after birth, using standard procedure (Jelliffe, 1966).

Height: A lightweight fibreglass infant-meter was used to measure length up to 100 cms. Infants were laid on the board of the infant-meter, which was kept on a flat table. The crown of the head was in contact with the top end of the device. The knee was extended, so that the feet were at right angles to the leg. The mobile device was then brought in contact with the feet of neonate and was allowed to be in contact with the device at the bottom end of the board. The distance between the two right angle devices was measured. The reading was recorded in centimetres with accuracy of 0.5 cm.

Birth Weight: A baby beam balance with accuracy of 50 g was employed for weighing the infants. Infants were weighed with minimum clothing while the child was restful.
**Head Circumference:** The infant’s head was steadied and the greatest circumference measured using standard method (Jelliffe, 1966).

**Chest Circumference:** The infant’s chest circumference measurement was measured at the nipple line, when the child was calm and breathing normally.

### 3.5. Processing and Statistical Analysis of the Data

The data that was elicited from different schedules were fed to spreadsheet of SPSS version 16 (SPSS Inc., Chicago, Illinois). RDA from National Nutrition and Food Technology Research Institute, Iran (NNFTRI, 1996) was used to compute nutrient adequacy for pregnant women. Computation of nutrient intake was carried out using information obtained from 24-hr recall method and ready reckoner. Body mass index in early first trimester was calculated by using the formula: weight (kg)/height (m)$^2$ (Casey et al., 1992).

All other data obtained were subjected to suitable statistical analysis. Mean, Standard Deviation (Mean± SD) and percentages were calculated wherever necessary and suitable tables and graphs were prepared. The statistical differences among the groups were analysed by Student’s t-test, and one-way ANOVA followed by post hoc test. Binary logistic regression analysis was carried out to find the predicting maternal factors in relation to birth weight.
Fig 4 Bird’s Eye View of Methodology - A Study on the Association between Maternal Micronutrient Status and Birth Weight of Neonate

Area of study: The city of Khoy, Iran, 9 health centres and one hospital
Subjects selected: 450 pregnant women registered in health centres.
Selection of Tools: Questionnaire, Anthropometric Assessment- Height, Weight and Fundal Height, Diet survey- Frequency of Food Intake and 24– hour Recall Method, Biochemical Analyses of Maternal Blood and Cord Blood – Hb, Ca. Fe, Zn, Cu, Mg

I Phase
Assessment of Family Background, Food Intake 24-hr Recall and Frequency of Food Consumption and Anthropometric Assessment of Pregnant Women – 1st, 2nd and 3rd Trimesters

II Phase
Biochemical Assessment of Maternal Blood and Cord Blood: Haemoglobin, Calcium, Iron, Zinc, Magnesium, Copper (162 pregnant women)
Anthropometric Measurements of Pregnant Women (1st, 2nd and 3rd trimesters and Neonates- Weight, Height, Head and chest Circumferences)

III Phase
Feeding the data –Excel sheets, Tabulation and statistical analysis
Using SPSS, Statistical Package Using Suitable Tests
Preparation of the Tables Graphs and Report