Zinc and vitamin A deficiency is widely prevalent during pregnancy. Deficiency of these nutrients, not only affects the health of the mother but also the outcome of pregnancy. This is because; Zinc and vitamin A deficiencies in pregnancy are associated with reproductive failure and high rates of mortality of offspring. Hence, the present research was undertaken with an objective to assess the prevalence of Zinc and vitamin A deficiencies among pregnant women and to see the impact of supplementation through Zinc and beta-carotene on pregnancy outcomes.

Materials and method for conducting this research presented as under.

3.1 Locale of the study:
The study was conducting on the pregnant women who were in their II trimester of pregnancy, residing nearby Kurukshetra University Kurukshetra (Haryana).

3.2 Sample Selection:

3.2.1 Criteria for adjudging the prevalence of Zinc and vitamin A deficiency:
1. To assess the prevalence of Zinc and vitamin A deficiency, 500 pregnant women between the 18-30 years of age were selected purposively. The subjects were selected from their health records kept in health centre. These women were regular visitors of L.N.J.P. civil hospital, Kurukshetra.
2. To adjudge the prevalence of Zinc and vitamin A deficiency among these pregnant subjects Serum Zinc and retinol levels of each subject were determined.
3. Zinc and Vitamin A levels of the above subjects were tested by using Inductively Coupled Plasma Mass Spectrometry (X Series 2 - Thermo Scientific) and High Performance Liquid Chromatography (HPLC).

3.3 Data collection:

3.3.1 General Information:

Questionnaire cum interview schedule technique was adopted for collecting the general information of each respondent. The interview schedule (Annexure- I) prepared for this purpose was classified into the following sections. Questions related to the family profile included type of family income. Information regarding education of the respondent was also gathered.

3.3.2 Reproductive information:

To adjudge the reproductive history of each respondent age at marriage, bleeding of any sort during pregnancy, number of children already having, type of delivery and birth weight of that children and number of infant deaths (below one year) were recorded. Gestational age on enrolment was determined by the date of late menstrual period (LMP) as recalled by the women.

3.4 Development of interview schedule for collecting the data:

Literature regarding antenatal women’s dietary practices, nutritional and socio economic status was reviewed in order to develop an interview schedule was prepared after consultation with eminent experts (Nutritionist and physician).

This questionnaire was first pre-tested on 30 subjects to check the reliability and validity; thereafter necessary modifications were
made according to the level of respondents and as per local considerations.

The final schedule (Annexure-I) considered of both open and close ended questions. A Performa was also framed to collect the information regarding health status, reproductive history and nutrient intake of the respondents (Annexure-I).

This personal Interview schedule Technique was applied to gather the facts as it helped to secure information through face to face interaction and thereby gain a portrait of the entire personality and to encompass the social background of the respondents.

It was also aimed at collecting personal data for quantitative purpose, besides incorporating data from persons, who were secondary source of information.

For getting the real information, subjects and their family members, specially the mother in laws were initiated for interaction and group discussions.

3.5 Experimental plan:

3.5.1 Criteria for the selection of experimental subjects for feeding trial:

From the above 105 Zinc and Vitamin A deficient pregnant women, 100 subjects who volunteered to participate in experimental feeding trial were separated and selected for feeding trial.

The details of experimental plan have been shown in Figure3.1
Fig: 3.1 Experimental Plan

500 PREGNANT WOMEN

223 Only Zinc Deficient  17 Only Vit. A Deficient  105 Zinc & Vit. A Deficient  155 Normal

100 Subject Were Selected For Feeding Trial

With Supplementation

Zinc (25) Z Group
β carotene (25) B Group
Zn+β Carotene (25) Z+B Group

Without Supplementation

Control Group (25)
Photo No. 1: Collection of important information

Photo No. 2: Collection of Anthropometric Measurements
3.5.2 Sub-grouping of the volunteered subjects for experimental feeding trial:

Above 100 subjects were sub-classified into four sub-groups (25 in each), comprising one control and three supplemental fed groups as under:

- **Group I** - Zinc supplement (N=25)
- **Group II** - β-carotene rich supplement (N=25)
- **Group III** - Zinc and β-carotene supplement (N=25)
- **Group IV** - Control (N=25)

3.6 Nutritional Status:

Nutritional status may be defined as the condition of health as it is influenced by the intake and utilization of nutrients (Caliendo, 1970).

Methods used in assessment of nutritional status of the subjects were included as per given by Both NNMB (National Nutrition Monitoring bureau and INP (India Nutrition Profile), 1979, surveys.

3.6.1 Anthropometric:

Anthropometric measurements were assessed and recorded monthly by trained midwives working in each centre. Anthropometric measurements were done jointly by the methods of Indian Council of Medical Research (ICMR, 1994) and the National Institute of Nutrition (NIN, 1991).

3.6.1.1 Measurement of Height:

Seca 217 stadiometer (for mobile height measurement) was used for the measurement of height. Measuring range was 8- 81 inches. The stadiometer can be conveniently transported to anywhere.
3.6.1.2 Measurement of Weight:

Under this weight was measured using an electronic balance (ATCO) with 100g of accuracy. The weighing balance was placed on a firm and flat ground. The subject was made to stand on the platform of the balance without footwear and with minimum clothing. The weight was recorded in the kilograms, to the nearest 100g (Jelliffe, 1966 and NIN, 2005). The average weight gain of all the selected subjects between the time of enrolment (usually 2nd trimester) and end of the pregnancy were recorded.

3.6.1.3 Measurement of Body Mass Index (BMI):

The body-mass index (BMI) is calculated by dividing weight (in kg) by the square of height (in meters). The body mass index (BMI), or Quetelet index is a heuristic proxy for human body fat based on an individual's weight and height.

\[
\text{Body Mass Index} = \frac{\text{Weight (Kg)}}{\text{Height (m)}^2}
\]

The BMI was used to classify the subjects into grades based on the classification given by ICMR (1981) and NIN (1991) as follows:

**BMI limit for Indian women is:**

<table>
<thead>
<tr>
<th>BMI Class</th>
<th>Category</th>
</tr>
</thead>
<tbody>
<tr>
<td>Less than 18.4</td>
<td>Underweight</td>
</tr>
<tr>
<td>18.5 - 22.9</td>
<td>Normal</td>
</tr>
<tr>
<td>23 - 24.9</td>
<td>Overweight</td>
</tr>
<tr>
<td>More than 25</td>
<td>Obese</td>
</tr>
</tbody>
</table>

3.6.2 Dietary status:
Dietary intakes of the subjects were taken by the method given by both NNMB (National Nutrition Monitoring bureau) and INP (India Nutrition Profile, 1979) surveys. 24-hour dietary recall method was used for 3 consecutive days. The amounts consumed were compared with the Recommended Dietary Allowance (RDA) for Indians drawn up by the Indian Council of Medical Research (ICMR) published in 1989.

3.6.3 Bio-chemical analysis of blood:

Serum Zinc and serum Vitamin A were determined to judge the bio-chemical parameters of Zinc and Vitamin A deficient antenatal females. Five ml of blood was taken in the fasting state from the subjects by expert technician from L.N.J.P. Hospital, Kurukshetra and Health Centre, Jyotisar. From this blood sample serum was separated and this separated serum was stored in the eppendorfs at -20°C in deep freezer. Samples were carried for estimation with in the box of dry carbon di oxide to maintain appropriate temperature.

Photo 3: Collection of Blood Sample

3.6.3.1 Collection and separation of the blood samples:
Five ml of fasting blood samples of each subject were collected in the beginning, after one week period. There after on third day, after delivering the baby. These blood samples were drawn by venipuncture into trace element free centrifuge tubes by the trained technician using disposable syringe (Dispovan). The blood sample of each subject were centrifuged at 2,500 r.p.m. for 15 min to separate serum from the whole blood. The serum was transferred into sterilized and labelled eppendorfs and stored in deep freezer (- 20°C) until analysis.

3.6.3.2: Analysis of Blood:

3.6.3.2.1 Serum Zinc:

Serum Zinc was estimated in Inductively Coupled Plasma Mass Spectrometry (X Series 2 - Thermo Scientific) by the method given by Kenichi et al., 2008). While carrying the samples, Samples were placed in dry ice to maintain the temperature of -20°C.

**Principle:**

An ICP-MS combines a high-temperature ICP (Inductively Coupled Plasma) source with a mass spectrometer. The ICP source converts the atoms of the elements in the sample to ions. These ions are then separated and detected by the mass spectrometer.

**Calibrator Preparation:**

For the analysis, the instrument was calibrated using single element standard of zinc prepared in the following concentration: 10 µg/l, 20 µg/l and 40 µg/l.

**Sample Preparation:**

Serum samples (100 µl) were diluted 50 folds in 1% HNO₃ to
Figure 3.2 Standard (Calibration) Curve for quantitative Estimation of Serum Zinc
Photo 4 & 5: Estimation of Serum Zinc through ICPM Spectrometry
Photo 6, 7 & 8: Estimation of Serum retinol through HPLC
make the total volume of 5 ml. 20 µg/l Gallium was used as an internal standard. 5 ml of the internal standard is added to a test tube along with sample. This mixture was then vortexed for several seconds or until mixed well and then loaded into the auto sampler tray. The calibrator and the sample preparation were done in the trace element free vials.

3.6.3.2.2 Serum Retinol:

Serum Retinol was estimated in High Performance Liquid Chromatography (HPLC) by the method of Lindsay and Kealey (1987). While carrying the samples, samples were placed in dry ice (Co₂) to maintain the temperature of -20°C.

Principle:

HPLC, is a chromatographic technique that can separate a mixture of compounds. HPLC typically utilizes different types of stationary phases, a pump that moves the mobile phase(s) and analyte through the column, and a detector to provide a characteristic retention time for the analyte.

Method:

- 100 µ lit of serum was taken and transferred into a conical glass centrifuge tube of 5 ml capacity.
- 100 µ lit of retinyle acetate (internal standard) in absolute ethanol was added in this tube.
- The contents were mixed in a vortex mixture for exactly 15 seconds, and then the tube was transferred to an icebox for minimum 5 minutes (2-8°C).
- Finally, 1 ml of n-hexane (HPLC grade) was added to the tube, and
the contents were mixed on a vortex mixture for 1 min exactly.

- The tube was centrifuged at 25°C for 5 minutes at 1,200-1,500 rpm.
- The upper clear hexane layer was carefully drawn in another test tube (5ml capacity) & put it on -20°C.
- Hexane is then evaporated gently under a stream of nitrogen gas very slowly so that retinol would not paste on the sides.
- After complete evaporation of hexane 100 µl of methanol (HPLC grade) was added, and contents were mixed on a vortex mixture for minimum of 15 seconds.
- 20 µl aliquot, thus prepared, was loaded into the vails and placed in autosampler tray of HPLC.
- Retention time was 7 minutes as Princeton Column was used.
- Recovery: 80-120%.

**Calculations:**

\[
A = \text{Retinol Conc.} = \frac{\text{ROL Area (Sample)} \times 100}{\text{Std. ROL Area}}
\]

\[
B = \text{Percentage Recovery of RAC} = \frac{\text{RAC Area (Sample)} \times 100}{\text{Std. RAC Area}}
\]

ROL = Retinol peak
RAC = Retinyl acetate peak

Actual Retinol in serum sample = \[
\frac{A \times 100}{B}
\]

Quantitation was done by HPLC. Retinol concentration was calculated by dividing the area of retinol peak (in the sample) by the
area of retinol peak obtained separately by running the standard retinol alone at a fixed concentration of 100 µg/dl and multiplying the result by 100. Correction factor (% recovery) was calculated by dividing the area of retinyl acetate peak (in the sample) by the area of retinyl acetate peak (obtained separately by the running standard retinyl acetate alone). Per cent recovery was more than 80; results of the samples yielding a lesser per cent recovery value were rejected. Values for retinol were finally adjusted by dividing the retinol value by the correction factor. Results obtained thus showed the retinol concentration in µg/dl.

3.7 **Clinical information:**

To observe the clinical symptoms of the subjects, a pre-tested questionnaire of ICMR (1989) was used with several modifications, after consultation with panel of eminent experts (Annexure I). Clinical observations of selected subjects were done under the guidance of a Doctor, L.N.J.P. civil hospital, Kurukshetra.

3.8 **Supplementation:**

Supplementation trials on the subjects were carried out in their second and third trimester. The women were informed at the beginning of the supplementation. Each woman was supplemented daily until delivery. Supplements given to the subjects were as followed.

3.8.1 Zinc syrup

3.8.2 β-carotene rich foods

3.8.3 Zinc syrup and β-carotene rich foods
Photo No. 9: β-carotene Rich Biscuits (Sweet)

Photo No. 10: β-carotene Rich Biscuits (Salty)
3.8.1 Zinc syrup:

Zinc syrup (Zinconia) was taken from medical store and given to the subjects after the consultation and under the supervision of gynaecologist, L.N.J.P. Hospital, Kurukshetra. 2.5 ml of Zinconia (Zinc gluconate) contained:

Elemental Zinc: 7.5mg

3.8.2 β-carotene rich supplement:

2.8g powdered amaranth was added in 50g wheat flour, having about 1200µg of β-carotene, which is 50% of the RDA of β-carotene. Total 4-5 sweet or salty amaranth biscuits were given to B and Z+B group daily.

3.8.2.1 Preparation of β-carotene rich food supplement:

Amaranth was used for the preparation of biscuits (Annexure-
II). The food stuff amaranth for the preparation of biscuit given to the subjects for feeding trials was firstly cleaned, dried in hot air oven, finely grounded and then incorporated in biscuits.

3.8.2.1.1 Moisture content of food: Moisture was estimated by the method of AOAC (1990).

**Principle:** this is based on principle of drying the sample to constant weight in a hot air oven.

**Apparatus:** Hot air oven
Porcelain dishes
Dessicator containing calcium chloride

**Procedure:**
- The empty dish and lid were dried in the oven for 15 minutes at 100°C and transferred to the desiccator to cool for about 15-20 minutes.
- The prepared samples were mixed thoroughly and transferred exactly 5g to the dish. The lid was replaced and the dish and contents were weighed immediately to the nearest mg.
- After removing the lid, the dish was placed in the oven avoiding contacts of the dish with the walls of the oven and dried for 8-10 hours at 100°C.
- The dish was removed from the hot air oven, the lid was replaced, cooled in desiccators and reweighed.
- Dried further for an hour to ensure that constant weight had been achieved.

**Calculation:**

\[
\text{Moisture (\%)} = \frac{\text{Loss of weight (g)}}{\text{Total weight of sample (g)}} \times 100
\]
3.8.2.1.2 β-carotene estimation:

The individual carotenoids are separated on a column of alumina and determined spectrophotometrically by the method of Ritter and Purcell (1981).

Principle:

The sample is subjected to saponification by adding ethyl alcohol and KOH. The unsaponified fraction which is obtained after boiling contains vitamin A and beta-carotene. This is extracted thrice with petroleum ether. The cooled ether extract is washed with aqueous KOH and then with distilled water to remove excess alkali. The ether extract is dried and residue obtained is dissolved in chloroform and optical density is measured in spectrophotometer.

Apparatus:

Spectrophotometer, columns, hot plate, blender.

Reagents:

- Acetone (3%) in petroleum ether.
- Alumina (aluminium oxide neutral).
- Sodium sulphate anhydrous.
- β-carotene standard: Dissolve 50 mg beta-carotene in 3% acetone in petroleum ether and dilute to 50 ml volume (1mg/ml).
- Dissolve 7g of KOH in ethanol and dilute to 250 ml with ethanol.
Figure 3.3. Standard (Calibration) Curve for quantitative Estimation of β-Carotene
Procedure:

- Preparation of chromatographic column:
  - Chromatographic column was filled with aluminium oxide which has dried in an oven.
  - Gentle filled the absorbent.
  - Covered with a 1 cm layer of anhydrous sodium sulphate.
  - Wet the column with 3% acetone in petroleum ether and don’t let it dry at any stage.

Sample preparation:

- Fresh plant materials and food stuffs:
  - 20 g finely cut materials was weighed and 30 ml acetone ether (3%) was added and allowed to stand overnight.
  - Shake it with 50 ml of water and water washings were discarded.
  - It was repeated for 2-3 times in order to make it acetone free.
  - Solvent was dried over Na$_2$SO$_4$ and dilute to 100 ml.

Chromatographic separation of beta-carotene:

- 10 ml of extract was taken and evaporated to 2 ml.
- Condensed extract was poured on to absorption column.
- Followed by 10 ml 3% acetone petroleum ether.
- Elute was collected which contained all the carotene.
- Elute was transferred to a 25 ml volumetric flask and dilute with (3%) acetone petroleum ether.

Standard curve:

- 0.1 to 1.0 ml standard solution was taken in 25 ml volumetric flask. Volume was made with 3% acetone in petroleum ether and
readings were taken at 450 nm in spectrophotometer. From the standard curve the concentration of β-carotene in the sample was calculated.

**Calculation:**

\[
\text{Beta-carotene (mg/100g)} = \frac{M \times V_1}{V_2 \times W \times 100}
\]

Where

- \(W\) = weight of the sample taken
- \(V_1\) = Volume of extract made
- \(V_2\) = Volume of extract taken from elution.
- \(M\) = Conc. of extract eluted from graph.

**3.8.3 Zinc supplement and β-carotene supplement:**

Zinc syrup and β-carotene rich biscuits were supplemented daily with in a combination.

**3.8.4 Feeding pattern of the supplements:**

- To the subjects of first group (I) Zinc syrup was given daily.
- To the subjects of second group (II) β-carotene rich foods i.e. Amaranth sweet and salty biscuits were given daily.
- To the subjects of third group (III) β-carotene supplement along with 2.5 ml of Zinc syrup were given daily.

**3.9 Outcomes Measured:**

**3.9.1 Pregnancy outcomes:**

**3.9.1.1 Type of delivery:**

Type of delivery (Caesarean and Normal) was noted under the supervision of gynaecologist and staff nurse of the hospital.
Photo No. 12  Measurement of Birth Weight of newborn.

Photo No. 13  Measurement of Head Circumference of newborn.
3.9.1.2 Term of delivery:

Preterm (< 37 weeks), full term (37-42 weeks) and post term (43-45 weeks) delivery were recorded under the supervision of gynaecologist and staff nurse according to ICMR (1981) recommendations.

3.9.2 Newborn and infant outcomes:

Anthropometric measurements of neonates (weight, length, head and chest circumferences) were taken by staff nurses within 24 h after birth, using standard procedure (Jelliffe, 1966).

3.9.2.1 Measurement of Birth Weight:

The babies delivered in the hospital were weighed naked within three hours of delivery. Birth weight of newborn was measured using
accurate and sensitive infant scale for weight measurement (Myweigh ultrascale MBSC-SS) capacity of 5kg.

Less than 2.5 kg was considered as Low Birth Weight (LBW) (ICMR, 1981).

3.9.2.2 Measurement of length:

Baby was placed on infantometer (Myweigh P.E-01) and hand was placed behind the child’s knee with thumb over the kneecap then applied gentle pressure to keep the leg strengthen. Length was recorded from infantometer in centimetres. Normal length considered was 50 cm ((ICMR, 1981).

3.9.2.3 Measurement of Head Circumference:

The infant’s head was steadied and the greatest circumference measured, by placing the fibreglass tape firmly round the frontal bones, just superior to the supra-orbital ridges, passing it round the head at the same level, on each side and laying it over the maximum occipital prominence at the back. Normal head circumference considered was 35 cm (ICMR, 1981).

3.9.2.4 Measurement of Chest Circumference:

The chest circumference was measured by placing the flexible measuring tape around the child’s chest just above the nipples. Measuring tape was placed under the child and brought it around the baby’s back to the chest. Normal chest circumference considered was 32 cm (ICMR, 1981).

3.9.2.5 Diarrheal and Cough:

Diarrheal and cough episodes of infants were noted every week under three month period.
3.10: Ethical clearance:

Research protocol was approved by Institutional Ethics Committee Kurukshetra University, Kurukshetra (Annexure III).

3.11 Determination of zinc and β-carotene content in locally available foods:

3.11.1 Zinc estimation from foods:

3.11.2 β-carotene estimation from foods:

3.11.1 Zinc estimation from foods:

Collection of food samples: Locally grown and consumed food samples were taken for the analysis of their zinc levels. Locally grown and consumed food samples were bought directly from the farmers. Vegetables and fruits were washed, cleaned and dried in the folds of what man filter paper. Cereals, legumes, dry fruits and spices and condiments were cleaned and then grounded. One g sample of each food was placed in a 100 ml volumetric flask and digested in di-acid mixture of Nitric acid and Perchloric acid (20 ml) in ratio of 4:1 according to the procedure of Johnson and Ulrich (1959). The samples were kept overnight in the digestion medium, thereafter digestion was carried out in triplicate on hot plates at 80°C until the contents were cleared. Afterwards, deionised water was added to each sample to bring the volume of digest to 50 ml.

The digested solution was analyzed for zinc content by Atomic Absorption Spectrophotometer (ChemitoAA203, version 3.30) by the method of Welz, and Sperling (1999). Samples were atomized for zinc determination at a wave length of 214.02 nm, with a slit width of 1.0 nm. using acetylene flame.
Figure 3.4. Standard (Calibration) Curve for quantitative Estimation of Zinc (mg) in digested food samples
Photo No. 15 & 16  Estimation of Zinc in food samples through AAS.
**Principal:**

Atomic absorption spectrophotometry (AAS) in technique used to measure a wide range of elements. Although it is a destructive technique, the sample size needs is very small (typically about 10 milligrams i.e one hundredth of a gram) and its removal causes little damage. The resulting solution is sprayed into the flame of the instrument and atomized. Light of a suitable wavelength for a particular element is shown through the flame, and some of this light is absorbed by the atoms of the sample. The amount of light absorbed is proportional to the concentration of the element in the solution, and hence in the original object. It can measure trace elements shown to the part per million level, as well as being able to measure elements present in minor and major amounts.

**Instrument condition for Zinc estimation:**

<table>
<thead>
<tr>
<th>Wave length</th>
<th>214.02</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slit (nm)</td>
<td>1.0</td>
</tr>
<tr>
<td>Fuel (litre/min)</td>
<td>1.8</td>
</tr>
<tr>
<td>Burner height (nm)</td>
<td>1.9</td>
</tr>
<tr>
<td>Unit of results</td>
<td>PPM</td>
</tr>
</tbody>
</table>

**Method:**

Samples were digested in di acid mixture nitric acid and Perchloric acid (4:1) according to the procedure of Johnson & Ulrich (1959).

- A known amount of sample (1 g) was taken in a 250 ml of conical flask.
- Twenty ml of di-acid mixture was added and the flask was covered
with a watch glass to prevent contamination and was kept overnight.

- The samples were digested at low temperature (40- 50°C). Before heating the watch glass was removed and a glass funnel was placed on the flask to avoid bumping and contamination. Cuprous red fumes were produced as reaction took place. After 40-50, the fumes of nitric acid were over.

- The digestion was continued till the liquid was yellow green and became colourless on cooling. The flask was removed from the hot plate when applying 1 ml of digested material was left.

- If there was carbonization, the solution became black, 1-2 ml of nitric acid was added and digested again as usually. The solution became clear in about 2-3 min if it persisted 2-3 ml of nitric acid were added and digestion was continued until solution became clear.

- After cooling, the digested material was transferred along with some glass distilled water to 50 ml volumetric flask.

**Standard stock solution:**

For preparing standard stock solution of 0.413343g ZnSO4 were diluted to 1 liter of distilled water. This solution was of 100 ppm concentration. From the above solution 1.0, 2.0, 4.0, 6.0 and 8.0 ml were transferred to different 50 ml of volumetric flask. The conc. of mineral in the solution was 1, 2, 4, 6, 8 ppm respectively. The readings of these were taken by AAS and were plotted on graph to obtain standard curve.
Preparation of blank solution:

One ml of distilled water was taken in 250 ml conical flask and 20 ml of di-acid mixture was added to it and digestion was carried out in the same way as described above.

Total mineral in food:

\[ \text{Zinc conc. in ppm} \times \text{DF} \]

Zinc concentration in 1g of food sample in mg = \[ \frac{\text{Zinc conc. in ppm} \times \text{DF}}{1000} \]

DF = dilution factor

ppm = parts per million

3.11.2 β-carotene estimation from foods:

Note: β- carotene estimation of food samples was done by the method of Ritter and Purcell (1981). (Refer section no. 3.8.2.1.2)

3.12 Data Processing and Statistical Analysis

Data were recorded on a pre designed Performa and managed in a Microsoft Excel spreadsheet. All the entries were double-checked for any possible keyboard error. The information collected during the study was condensed and a codebook was developed. The responses were then coded and transferred on to the coding sheets and tabulated. The readings and scores obtained before the start and at the termination of experiment trials were statistically analysed. The statistical analysis of data was conducted using SPSS version 15. Descriptive statistic was used for summarization of data. The statistical difference among the groups was analyzed by paired t-test and one-way ANOVA. The effect of supplementation was analyzed with multivariate analysis (1-way ANOVA) for main effects and interactions of both zinc and β-carotene supplementation. When the one-way ANOVA results were significant, the Bonferroni test was used to determine whether significant
difference exist between different variable means. Statistical
difference was considered significant if the p value was <0.05 and
highly significant if p value was <0.01. Correlations between serum
zinc levels, serum retinol levels, gestational age of new-born, birth
weight, head circumference, chest circumference and length were
analyzed in a multiple linear regression model. Multivariate logistic
modelling technique was performed to determine the set of risk factors
that predicted the mean birthweight and the risk of LBW (< 2,500g),
low head circumference (>35 cm), cesarean delivery and preterm
delivery (<37 weeks).