Materials and Methods
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The aim of the present investigation was to focus on women and study selected variables in the light of reproductive performance, inter relationship between health and nutritional status during adolescent pregnancy, and to conduct a need based target oriented nutrition counselling to the adolescent girls.

Keeping these aims in mind, the study was designed in the following phases:
1) Assessment of the prevalence of teenage pregnancy.
2) Evaluation of the previous dietary and health status as a factor to determine the present pregnancy outcome.
3) Determination of dietary, biochemical and anthropometric factors in adolescent pregnancy and the outcome.
4) Nutrition counselling to the target population.

In order to assess the prevalence of teenage pregnancy, a rapid analysis of the hospital records was conducted. Two main hospitals from each area of study that are run by the Muncipal Corporation and chiefly cater to the low economic strata were randomly selected. A total of all the deliveries that took place for a period of one year (Jan. '92 to Dec. '92) was recorded along with the age of the mothers. Teenage pregnancies were expressed as - deliveries per thousand total deliveries.

The health and nutritional status of adolescent girls was assessed by:
1) Cross sectional study on 250 subjects each randomly selected from slums of Bombay and Bangalore.
2) Longitudinal study on 200 pregnant subjects from 6 maternity homes from Bangalore.

A list of all the Corporation Maternity Homes in urban Bangalore was obtained from the office of the Medical Officer of Health, Bangalore Corporation, to select the subjects for a longitudinal study to assess the dietary, biochemical and anthropometric factors in adolescent pregnancy and the pregnancy outcome.

On the basis of exploratory visits to the 23 Corporation Maternity Homes in Bangalore, for number of new pregnancies registered in a day, it was decided to take one fourth (6) of the total number of maternity homes. These six were randomly selected as a representative sample of Bangalore Urban. Each day one hospital was visited during the outpatient timings. All the pregnant adolescent girls who came to register themselves fulfilling the criteria were selected as the adolescent pregnancy occurs less frequently than adult pregnancy. Hence, every fifth adult pregnant woman who came to register were selected.

**Criteria for the selection of the subjects were:**

1) Age of the subject (13-19 years formed the adolescent group and 20-25 years formed the adult group).

2) Registering in the first trimester of pregnancy.

3) Willingness of the subjects to cooperate throughout the period of study.

4) Availability of the subjects throughout the study period.

Home visits were made by the interviewer to collect further information for these subjects. The subjects were followed till they delivered and also during the neonatal period of the infant.
Tools used for the study

A detailed schedule was used to elicit information from the subjects. The schedule was pretested on a sub sample and necessary modifications were made wherever necessary on the basis of pretesting. The final schedule consisting of 3 parts included various parameters and the mode of collecting data are given below.

General Information

The first part of the schedule included information on type of family, socio-economic and educational status of families, food practices and beliefs during pregnancy and lactation, family planning methods adopted, individual's post obstetric information such as number of earlier pregnancies, outcome of previous pregnancies etc.

The second part of the schedule included information on previous health, nutrition and dietary status of the respondent, and the third part consisted of present gestational age, current health, nutrition and dietary status of the respondent. Parameters for assessing the nutritional status included:

Anthropometric measurements - weight, height, skinfold thickness, mid arm circumference and abdominal girth.

Dietary intake - energy, protein and iron.

Biochemical parameters - total protein, serum albumin, calcium, iron and haemoglobin.
Anthropometric Measurements

Anthropometric indices of the mothers were measured using standard techniques which are described below (Jelliffe, 1966):

Weight

Weight of mothers were recorded during the first visit (at the time of enrollment) and also during the subsequent visits up to the end of pregnancy. For this purpose, an adult personal weighing scale (Libra make) was used as this is portable and thus convenient to use in the field. The accuracy of the weights were ascertained by using standard weights (ISI).

The zero adjustments of the scale was checked prior to each measurement. The subjects were asked to wear minimum clothing. They were then asked to stand on the platform of the scale without touching anything and looking straight ahead. Weight was recorded to the nearest 0.5 Kg.

The difference in weights between the initial weight and the final weight was taken as the gain in weight during pregnancy.

Height

Heights of all the women were measured at the time of first visit. The subjects were asked to remove their foot wear before the measurements. They were made to stand with the heels, center of the back, buttocks, shoulder and back of the head touching the wall. The head was held erect and the hands were held at the sides. A thin ruler was held at the top of the head in the centre, at right angles to the wall, and this point was marked. Height was measured using a metal tape to the nearest 0.5 cm.
Triceps skinfold (mm) was measured to the nearest mm with a Lange skinfold caliper having a pressure of 10 g/sq.mm of contact surface area. The measurement was taken over the triceps muscle halfway between the elbow and the acromial process of the scapula, with the skinfold parallel to the longitudinal axis of the upper arm.

Subscapular skinfold thickness was measured just below to the angle of the right scapula parallel to the natural cleavage line of the skin with the arm at the side of the trunk.

Mid arm circumference was measured to the nearest cm. with a fibre glass tape with the right arm hanging relaxed. The measurement was taken midway between the tip of the acromion and olecranon process.

**Dietary Intakes**

Food consumption of the subjects were recorded using the 24 hr. recall method (Thimmayamma, 1987). Three day dietary records of all the pregnant mothers were collected at the time of enrollment in the study. Home visits were conducted to determine the exact size of Ragi balls/ Chapathi and the serving/portion size of different preparations, so as to get as accurate an estimate as possible, of the amount of food stuffs used. Amounts of milk, vegetable and fruits were estimated by considering the quantity brought home for family consumption. Most of the foodstuffs were purchased daily, since in most of these families, income was in the form of daily wages. Consumption of the foodstuffs was then translated into estimates of intakes in grams. From these diet records intakes of energy, iron and protein were calculated using Nutritive Value of Indian Foods (1993). In case of severe vomiting and nausea reported by the subject, the dietary record was taken at the subsequent visit.
Biochemical Parameters

Ten ml. of the intravenous blood was drawn with the help of a trained nurse in the respective maternity homes for all the subjects. The blood was analyzed for the following nutrients: Calcium, Total Protein, Albumin, Iron and Haemoglobin. Method of estimation for each nutrient was as per the standard procedure by Raghuramulu et al (1983).

Estimation Of Serum Total Proteins

Biuret method

Principle:
The - CONH groups in the protein molecule react with copper sulphate in alkaline medium to give purple colour which is then read at 540 nm.

Reagents:
1. Biuret reagent:

   Dissolve 4.25g of potassium sodium tartrate (KNaC₄H₄O₆.4H₂O), 1.5g of cupric sulphate (CuSO₄.5H₂O) and 2.5g potassium iodide in about 500 ml of distilled water. Dissolve 4g of NaOH in the solution and make up the volume to 1 l.

2. Standard:

   The standard protein solution may be either a pooled normal human serum (standardised by Kjeldahl method) or a solution of pure albumin in saline.

Procedure:

   To 0.1 ml aliquots of standard, test plasma and blank (saline or distilled water) add 5 ml of biuret reagent. Mix well and keep for 30 min. Read absorbances of test and standard against blank at 540 nm.
Calculation:

\[
\text{Total protein } \text{g/100 ml} = \frac{\text{reading of test}}{\text{reading of standard}} \times \text{concentration of standard.}
\]

**Estimation Of Serum Albumin**

**Dye binding method**

**Principle:**

The increase in absorbance of BCG at 630 nm on binding to serum albumin at pH 4.2 is directly proportional to the concentration of albumin.

**Reagents:**

1. **Buffered BCG dye solution:**

   Dissolve 8.85 g of succinic acid, 108 mg of BCG (sodium salt) and 100 mg of sodium azide in about 950 ml of water. Add 4 ml of 30% Brij-35 adjust the pH to 4.2 and make up to 1 l with water (Stable for atleast 5 months at room temperature).

2. **Standard:**

   A pooled serum sample may be used as standard after estimation of albumin by Kjeldahl method (Bovine serum albumin is unsatisfactory but human serum albumin fractions of highest purity available commercially may be used).

**Procedure:**

Take 3.0 ml buffered dye solution in a series of tubes. Add 10 micro l of serum sample vortex and read immediately (within 30 sec) the absorbance at 630
nm against blank dye solution. Analyse in duplicate and calculate the albumin content by comparison with standard run similarly.

Note:

Although the zero time absorbance method described above is a true estimate of albumin, significant errors may result if readings are taken after 60 sec. Readings must therefore be taken immediately.

Estimation Of Haemoglobin

Cyanmethaemoglobin method

Principle:

Haemoglobin is converted into cyanmethaemoglobin by the addition of KCN and ferricyanide. The colour of cyanmethaemoglobin is read in a photoelectric colorimeter at 540 nm against a standard solution. Since cyanide has the maximum affinity for Haemoglobin this method estimates the total haemoglobin.

Reagent:

Drabkin's solution:

Dissolve 0.05 g of KCN, 0.20 g of potassium ferricyanide and 1.00 g of sodium carbonate in 1 l of distilled water.

Procedure:

20 micro l of blood is transferred with the help of a haemoglobin pipette into a test tube containing ml of Drabkin's solution. The tubes are mixed and readings taken in a photoelectric colorimeter (Spectronic 20) using 54 filter. The reagent blank (Drabkin's diluent) is adjusted to zero.
Construction of standard curve:

1) Since the amount of iron present in haemoglobin per unit weight is constant, one can calculate the haemoglobin concentration of a given blood sample by determining its iron content. This blood sample can be used as a reference standard for haemoglobin estimation.

2) A standard curve can also be constructed by using the standard cyanmethaemoglobin solutions (supplied by BDH or V.P. Chest Institute, Delhi).

<table>
<thead>
<tr>
<th>Standard solution (ml)</th>
<th>Drabkin's diluent (ml)</th>
<th>Hb conc. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>5</td>
<td>2.5</td>
<td>67</td>
</tr>
<tr>
<td>5</td>
<td>5.0</td>
<td>50</td>
</tr>
<tr>
<td>5</td>
<td>7.5</td>
<td>40</td>
</tr>
<tr>
<td>5</td>
<td>10.0</td>
<td>33</td>
</tr>
</tbody>
</table>

The suppliers of the standard solutions mention the concentration of the standard on the ampoule. The corresponding blood haemoglobin in g/100 ml can be obtained by multiplying the concentration on the ampoule with the dilution factor (251).

Note:
1) Drabkin's solution should be stored in amber coloured bottle. If any precipitate is formed the reagent should be discarded.
2). Since the dilution is enormous (251 times) accurate measurement of 20 micro l of blood is absolutely essential for reproducibility. Hb pipettes must be checked for their accuracy by weighing pure mercury upto the mark.

**Estimation Of Serum Calcium**

**Colorimetric method**

**Principle:**

Calcium forms a colour complex with the O-cresolphthalein dye, which is made more specific the presence of 8 quinolinol.

**Reagents:**

1). Standard CaCb: 10 micro g Ca++/ml prepared by dissolving required amount of anhydrous CaCO₃ in dilute HCl and making upto a definite volume with water.

2). Ammonia/ammonium chloride buffer, pH 10.5: Dissolve 0.24 g NH₄ Cl in 100 ml of 5% NH₄OH solution (V/V).

3). O-Cresolphthalein solution, 0.1%: 100 mg of O-cresolphthalein dissolved in 28 ml of ammonia/ammonium chloride buffer and diluted to 100 ml with water.

4). 8-Quinolinol: 1% solution in absolute ethanol.

**Procedure:**

Different aliquots of the CaCb solution with Ca++ content varying from 2-10 micro g are taken in graduated stoppered tubes and volume in each case is made upto 5.5 ml with water. Subsequently, 1 ml of 8-quinolinol solution and 2.5 ml ammonia/ammonium chloride buffer are added and the volume adjusted to 9.0 ml.
The contents are finally treated with 1 ml of O-cresolphthalein solution. After addition of each reagent mentioned above, the contents are thoroughly mixed. Water blank in the place of CaC₂ solution is simultaneously carried throughout the procedure. Standards are read against blank in Spectronic-20 at 565 nm. Take readings after 5 min (stable upto 3 hr).

Birth Weight

Birth Weight of the infants were recorded within 24 hours of delivery using a CMS model MP 25 scale. The scale was of 10 Kg. capacity in one rotation with 50 gm. calibration. The scale was regularly checked and adjusted to zero before weightment. Weights were recorded to the nearest 50 g.

Nutrition Counselling

A target oriented Nutrition Education was given to the target population of adolescent girls. This population consisted of unmarried adolescents, married but not pregnant and adolescent primiparous. A need based nutrition lessons were planned for non formal nutrition education to be imparted to this population depending on the results obtained in the present study.

Data Analysis

All data were recorded on predesigned forms for subsequent verification and entry into a computer system. Statistical analysis were preceded by screening of all data to identify and check for detectable errors and correction. After this working files were created from the data to be used for statistical analysis. Data analysis included students - 't' tests, linear regression analysis, multiple regression, analysis of variance (ANOVA) and Odds Ratios. All statistical
tests were performed by computerized programmes, ANOVA and multiple regression was done by using SPSS-PC (Statistical Package for Social Sciences).

Multiple regression analysis was used to determine the relationship between maternal factors namely menarcheal age, gestational age, maternal age, heights, weights, parity, abdominal girth, dietary intake, biochemical parameters and birth weights.
LIMITATIONS OF THE STUDY

1) The longitudinal study was not carried out at both the places since it was felt that, factors influencing pregnancy outcome would remain the same in all population irrespective of the place of study. Only the prevalence of teenage pregnancy would vary from place to place. Hence, this aspect was looked into at both the places.

2) Infants were not followed up during their post-neonatal period till one year of age as the emphasis in the present study was on maternal nutritional status.

3) Impact of the nutrition counselling on the subjects could not be evaluated due to constraint on time as it is a time consuming process. Thus, the lessons were planned and the counselling was done. It is proposed to contact these subjects and conduct an evaluation programme.