METHODS AND MATERIALS

Selection of the sample

For the purpose of the study the rural and urban population in Chittoor district of Rayalaseema region in Andhra Pradesh was selected. This district was selected mainly because the rural area was classified as "backward and also chronically drought - stricken" and the urban area had families coming not only from different parts of Andhra Pradesh but also from Karnataka and Tamil Nadu with different educational and traditional backgrounds representing the Southern culture.

Sample size

Random sampling procedure was adopted in selecting the families as randomness or random sampling is defined as an equal chance of being selected from the universe. At first instance, Tirupati town was selected as urban area containing approximately 1.5 lakhs population. The list of streets were taken from the municipality office and the families were selected randomly. In rural area the villages which fell within a radius of 30 Km from Tirupati were selected randomly. Thus a total of six villages were selected and they were Padipeta, Gollapalli, Mallamgunta, Perumallapalli, Tanapalli and Tummalagunta. The size of the population varied from village to village. Thus, 238 families from urban area and 232 families from rural areas were selected randomly for the study.

Preparation of schedules and tools

Based on the objectives and hypothesis of the study an interview schedule was constructed, comprising of socio-economic particulars of the
respondent, literacy and occupational status of the respondent, preferences given towards the food likings of the family members, opinion towards left over food, food beliefs, food fads and fallacies, respondent's attitude towards decision making power in maintaining family's economy and purchases, knowledge about immunization and utilization of available welfare services like Integrated Child Development scheme, Rayalaseema Seva Samithi, etc.

In addition questions pertinent to dietary intake i.e., quality and quantity of food consumed, the amount of food available to the respondent from the total food purchased were included in the schedule.

Apart from these, data on health status, deficiency symptoms if any, were also recorded. Height and weight of the respondent was measured by using non stretchable tape and bathroom scale (Libra).

Pretesting of the questionnaire

The interview schedule translated into local language was pretested on 40 women, (20 from urban and 20 from rural areas) to check the completeness, correctness and validity of the questionnaire.

On the basis of responses, interview schedule was finalised. The questions that had a polarization effect, questions which were vague and confusing were altered and modified or even omitted.

Collection of data

Data were collected by oral questionnaire method using the pretested and modified questionnaire. Female head of the household was taken as the respondent for the study.
Information on general background, socio-economic status of the respondent, likes and dislikes of personal and family members, preference given to the family members in serving food especially when quantity of food runs down etc., were collected.

The food available to the respondent was assessed by the daily/weekly/monthly purchases made by the families for both perishables and non-perishables. The dietary consumption was also recorded for 3 days by 24 hr recall method. The food consumed by the respondents from the total food cooked for the family was recorded in terms of household measures and equated to previously standardised cups. The festive days and sundays were excluded while taking the food consumed by the respondents to avoid bias.

Daily activities of the respondents were noted for 24 hours over a 3 day period in a week i.e., 6 a.m. to 12 p.m. - period 1, 12 p.m. to 6 p.m. - period 2, 6 p.m to 10 p.m. - period 3 (Satyanarayana et al., 1987).

Anthropometric measurements such as height and weight of the respondents were measured.

**Weight** was measured using bathroom scale (Libra) to the nearest 100g, when subjects were in light clothig and without shoes.

**Height** was measured by means of a non-stretchable tape to the nearest 0.1 cm. Care was taken to see that the subject stood erect on level ground against a wall with their feet together and their feet, buttocks and head touching the wall while measuring the height.
Calculation of the nutritive value of the diet

The traditional recipes of the region under study were prepared in the laboratory to find out the relation between the cooked food by household measures and the raw ingredients by weight for calculating the nutrient composition of these recipes from the food tables (Gopalan, 1989). The nutrient intake of the respondents was calculated from the three day records using the above data on nutrient composition of the recipes. The food available to the respondent was calculated from the total food purchased for the family on the basis of consumption unit.

Energy expenditure of the respondents was estimated from the daily activity records by adopting the values given by Bouchard et al. (1983).

Collection of blood samples

Blood samples were drawn from a randomly selected subsample of 50 each from urban and rural areas. Eight ml of intervenous blood was drawn and 1 ml was transferred to an oxalated bulb to prevent clotting for the estimation of haemoglobin. The remaining was centrifuged for 10 min at 3000 rpm. The serum was collected in a clean tube and stored in a refrigerator till further analyses.

Methods of analysis

The blood samples collected from the respondents were analysed for haemoglobin, total serum protein, albumin/globulin ratio and serum vitamin A by using following procedures.
Estimation of haemoglobin (Cyanmethaemoglobin method, Dacie & Lewis, 1975)

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.

Reagents:

Drabkin's solution: 0.05 g of KCN, 0.2 g of potassium ferrocyanide and 1 g of sodium bicarbonate was dissolved in 1 l of distilled water.

Standard solution: Standard cyanmethaemoglobin ampoule.

Procedure: 20 µl of blood was transferred with the help of haemoglobin pipette into a test tube containing 5 ml of Drabkin's solution. After adjusting the photoelectric colorimeter at 540 nm with a blank (Drabkin's diluent) the O.D. of sample was read.

The standard solution in the ampoule contains 14.8 g of haemoglobin/100 ml. The corresponding blood haemoglobin in g/100 ml was obtained by multiplying the concentration of the ampoule by the dilution factor.

Estimation of total protein (Biuret method, Bernard, 1965)

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:

Biuret reagent: 4.25 g of potassium sodium tartarate, 1.5 g of cupric sulphate and 2.5 g of potassium iodide were dissolved in about 500 ml of distilled water. 4.5 g sodium hydroxide were added to the solution and the volume made to 1 l.

Standard: Albumin 5 g/dl ready for use. (3A089V)

Procedure: To 0.1 ml of aliquots of standard, test plasma and blank, 5 ml of biuret reagent were added and kept for 3 minutes. The absorbances of test and standard against blank were read at 540 nm.

Calculation:

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

Estimation of albumin (Dye binding method, Cooper, 1972)

Principle: Albumin in a buffered solution reacts with anionic bromocresol green with a dye binding reaction to give a proportionate green colour which is measured at 628 nm (600-650 nm). The final colour is stable for 10 min.

Buffered BCG dye solution: 8.85 g of succinic acid, 108 g of BCG (sodium salt) and 100 mg of sodium azide were dissolved in 950 ml of water. 4 ml of Birz-35 were added, pH was adjusted to 4.2 and volume made to 1 l with water.

Standard: Albumin 5 g/dl ready for use. (3A089V)
Procedure: 3 ml of buffered dye solution were taken in a series of tubes. 10 µl of serum sample and 10 µl of standard solution were added and reading was taken immediately at 630 nm as against blank dye solution.

Calculation:

\[
\text{Albumin g/100 ml} = \frac{\text{O.D. of test}}{\text{O.D. of standard}} \times \text{concentration of standard}
\]

Estimation of globulin: Globulin g/100 ml = Total protein - Albumin

Estimation of vitamin A (Carr-price method, NIN, 1983)

Principle: Vitamin A gives a blue coloured complex with antimony trichloride which has an absorption maximum at 620 nm. The intensity of the blue colour can be taken as a measure of vitamin A. Since B-carotene also gives a coloured complex with antimony trichloride, a correction has to be employed.

Reagent:

25% antimony trichloride : 25% solution of the reagent in chloroform containing 1% acetic anhydride

Procedure: An aliquot of the vitamin A solution in chloroform is taken in the spectrophotometer cuvette and the volume made up to 1 ml. The slit width of the instrument was adjusted at 620 nm with chloroform as blank. 2 ml of antimony trichloride solution is added and intensity of the blue colour read at 620 nm within 15 sec.

Standard curves were plotted with different concentrations of pure vitamin A and B-carotene. Correction for B-carotene made by estimating the actual amount of B-carotene in the solution from the OD at 460 nm.
The OD for the amount of carotene at 620 nm in the Carr-Price test was obtained from the standard curve and that value subtracted from the total OD at 620 nm (OD 620 nm observed - OD 620 nm B-carotene = OD 620 nm corrected for vitamin A in the serum).

**Statistical analysis**

Means and standard deviations were calculated for food and nutrient intakes. Z test was performed for equality of means between the food and nutrient intakes. $\chi^2$ test was also performed to see the influential factors.

Correlation coefficients were done to see if there was any correlation between the nutrient intake and various blood parameters like haemoglobin, surm total protein and serum vitamin A.