The investigation was taken up with the following objectives.

1. To record blood pressure values in the male subjects of 41-60 years of age.

2. To assess the relationship between blood pressure values, certain specific hypertensive risk factors including family history of hypertension.

3. To conduct a diet survey and estimate the nutrient intake of each subject.

4. To study the effect of intervention with Potassium and Calcium supplementation on the levels of blood pressure, Sodium, Potassium and Calcium in serum and urine.

5. To study the interrelationship and associations between biochemical parameters, Body Mass Index, Family history, dietary factors and Blood Pressure Values in the sample.
The Sample

The sample comprised of 120 men, sixty normotensives and sixty hypertensives of age 41 to 60 years. The subjects were selected after screening patients attending clinics, medical camps and hospitals of Chittoor town, Andhra Pradesh. The hypertensive subjects were not undergoing any hypertensive treatment and were known to have established moderate hypertension. The consent of the subjects was taken after explaining the objectives of the study. They were requested to extend their full co-operation. They were asked to continue with their regular diet throughout the period of the study. The experimental design of the study is given in Figure 2.

A suitable questionnaire was formulated with short, specific and direct questions to obtain information regarding aspects such as:

1. General information consisting of name address and age.

2. Information on economic status, educational level, occupation of the subjects and their family members.

3. Details regarding family history of hypertension, habit of cigarette smoking and alcohol intake.
TOTAL SAMPLE 120 MEN
AGE GROUP: 41-60 YEARS

<table>
<thead>
<tr>
<th>Normotensives</th>
<th>Border line (moderate) hypertensives</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 60 men</td>
<td>Sample 60 men</td>
</tr>
</tbody>
</table>

Pre-Analysis

- Recording of Family history
- Measure height and weight
- Diet survey for 3 days
- Recording of blood pressure
- Collection of blood and urine for sodium, potassium and calcium analysis

Supplementation period for 3 weeks

<table>
<thead>
<tr>
<th>(Age Group)</th>
<th>NC</th>
<th>NP</th>
<th>NCa</th>
</tr>
</thead>
<tbody>
<tr>
<td>41-50</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>51-60</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

- Placebo starch (500 mg)
- Potassium chloride (600 mg)
- Calcium lactate (500 mg)
- Placebo starch (500 mg)
- Potassium chloride (600 mgs)
- Calcium lactate (500 mgs)

Post Analysis

- Recording of blood pressure
- Collection of blood and urine for sodium, potassium and calcium analysis

Statistical Analysis

Figure 2: EXPERIMENTAL DESIGN OF THE STUDY
4. Specific dietary habits and
5. Diet intake for three consecutive days.

All the subjects were requested to give true facts as information in the questionnaire and they were assured that the information given would be kept strictly confidential (The Questionnaire is given in Appendix - 1).

Conducting Diet Survey

A diet survey was carried out for three consecutive days. Each subject was provided with a standard cup and spoon and were requested to measure and record the quantity of each food item they had consumed, in the questionnaire. The raw weight equivalent for the cooked weights of the various recipes were determined in the laboratory. Based on the information provided by the subjects, nutrients like calories, protein, saturated fat, poly-unsaturated fatty acids, sodium, potassium, calcium and vitamin C supplied by each food stuff consumed were calculated separately for three days by using tables of food composition (Gopalan et al, 1989). The average nutrient intake for each subject was computed.
Recording of Blood Pressure:

Blood pressure was measured using a sphygmomanometer, twice on two different visits to the patient before the study. 30 minutes before the measurement, the participants refrained from smoking or strenuous activity. They were advised to rest quietly for five minutes. The blood pressure was recorded in the upper arm of the right hand as the subject sat relaxed in a chair. The arm was placed at the heart's level while recording the pressure. Diastolic pressure was recorded when the sounds disappeared (Vth Korotkoff Phase).

Recording of Anthropometric Measurements:

The standing height and weight of the subjects were recorded using standard equipment. The obesity index was calculated using the formula weight/height$^2$ (kg/m$^2$). On the basis of the index, the subjects were graded as follows

<table>
<thead>
<tr>
<th>Grade</th>
<th>Body Mass Index (Quetelet's Index)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 0</td>
<td>20.0 to 24.9</td>
</tr>
<tr>
<td>Grade I</td>
<td>25.0 to 29.9</td>
</tr>
<tr>
<td>Grade II</td>
<td>30.0 to 39.9</td>
</tr>
<tr>
<td>Grade III</td>
<td>Above 40</td>
</tr>
</tbody>
</table>

(Deorenberg et al, 1989).
Collection of Blood and Urine

Disposable syringes were used to draw 5 ml of venous blood from each subject without the use of a tourniquet. Serum was separated by centrifugation.

Urine was collected for 24 hours in 2.5 litre dark coloured bottles with 3 ml toluene added as preservative. The total urine volume was measured and a portion was taken for analysis.

Analysis of Serum and Urine

Serum and urine from all the subjects were analysed for sodium and potassium by Flame Photometer and calcium by Cresolphthalein complexone method before and after the supplementation period.

Estimation of Sodium and Potassium

(Hawk's Physiological Chemistry, 1965 Ed Oser and Summerson).

Principle

The sample in solution was introduced in the form of a fine continuous spray into a non-luminous gas flame. The
emitted light, characteristic for the ion being analysed, was isolated and focussed on a photo electric cell and the current intensity was measured on a suitable meter.

Reagents

1. Stock solution (sodium) with 0.5 mgs of sodium per ml.

2. Working standards with a sodium concentration of 2 to 12 μg/ml.

3. Stock solution (Potassium) with 0.5 mgs of potassium per ml.

4. Working standards with a potassium concentration of 1 to 6 μg/ml.

The details of reagent preparation are given in Appendix ii.

Procedure

Serum and urine, 0.05 ml each, were taken and made upto 20 ml and 50 ml respectively with glass distilled water in suitable volumetric flasks. Aliquots were taken and introduced into the flame. The readings were noted for sodium and potassium using separate filters for both.
Figure No: 3 Standard Curve for Sodium Estimation.
Fig. 4: Standard curve for Potassium estimation

Scale: X axis 1 unit = 1 µg/ml
Y axis 1 unit = 10
Plate No:1 FLAME PHOTOMETER

Plate No:2 AUTOMATED SPECTROPHOTOMETER
A blank was run by using glass distilled water instead of the sample. Working standards at different concentrations were also read in the flame photometers in a similar way. Standard curves were drawn for sodium and potassium at different concentrations. The plotted standard curve for sodium is presented in figure 3 and for potassium in figure 3A.

Calculations:

For serum

\[ X = \frac{\text{reading of unknown}}{\text{reading of standard}} \times \frac{\text{Concentration}}{\text{of standard}} \times \text{Dilution factor} \]

= mgs of sodium or potassium per 100 ml.

To convert into m mol/litre = \[ \frac{X \times 10}{\text{atomic weight of sodium (22.98) or potassium (39.09)}} \]

For Urine:

\[ \frac{X \times 10}{\text{Atomic weight of sodium or potassium}} \times 24 \text{ hour urine volume} \]

= m mol of sodium or potassium/24 hours.

Estimation of Calcium

Principle

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

Reagents: Erba - Test Calcium kit containing a dye and base solutions. The composition comprises of Cresolphthalein complexone, 8-hydroxy quinoline, Polyvinyl pyrrolidine, 2-amino-2-methyl propanol and surfactant.

Procedure

Working standard was prepared freshly by mixing equal quantities of dye and base solutions and left for 10 minutes at room temperature.

Urine samples were heated at 56°C for 15 minutes to dissolve any precipitate. 1.0 ml of urine was diluted with 1.0 ml of glass distilled water.

To a series of test tubes, 500 µl of working reagent and 20 µl of serum or diluted urine were added. Readings as mgs of calcium per 100 ml were noted from automated spectrophotometer at 575 nm.
Calculations:

Serum calcium mmol/litre = $\frac{\text{mgs of Calcium}}{100 \text{ ml}}$ \times 4

Urine Calcium mmol/litre

= $\frac{\text{mgs/100 ml}}{4} \times \text{dilution factor} \times 24 \text{ hour urine volume}$

Supplementation Period:

After the initial survey, the subjects were divided into three equal groups among normotensives and hypertensives. Each group had 20 subjects, ten subjects of age 41-50 years and rest from 51-60 years. The participants of each group were given a placebo of 500 mg starch, 600mgs (as 'POT RELEASE', NATCO Fine Pharmaceuticals) potassium chloride or 500 mgs of calcium lactate for a period of three weeks i.e. 21 days. Blood pressure was recorded at the end of the supplementation period. Blood was withdrawn and 24 hours urine was collected for the post analysis of sodium, potassium and calcium content.

Statistical Analysis

SPSS (Statistical Package for Social Sciences) on personal computer was used for statistical analysis (Nie et al, 1975) and analysis was done at Micro Data Corporation, Hyderabad.
Discriminant function analysis was done to determine the best set of variables differentiating normotensives from hypertensives.

Multiple regression analysis was performed to study the effect of different variables on blood pressure levels.

Two way analysis of variance tested the relationship between blood pressure levels and

1) Age,
2) Family history of hypertension,
3) Alcohol intake,
4) Smoking,
5) Body Mass Index and
6) Vegetarian vs Non-vegetarian diet.

It was also done to test the relationship between various dietary components and

1) Grades of Body Mass Index,
2) Vegetarian vs Non-vegetarian diet.
Chi-square test was performed to test the relationship between hypertension and

i) Income

ii) Educational level,

iii) Family history of hypertension,

iv) Alcohol intake and

v) Habit of smoking.

Students 't' test was done to test the significant differences between Blood Pressure values and various biochemical parameters before and after supplementation.