MATERIALS AND METHODS

The present research work intends to study the body mass index and its association with blood pressure and blood glucose levels in urban and rural habitats of Chittoor district, Andhra Pradesh State of South India. This chapter is discussed under the following Heads: 1) Study area, 2) Sampling, 3) Methods and 4) Statistical analysis.

2.1. Study area

The area selected for the study is Chittoor district, in Rayalaseema region of Andhra Pradesh State. The population of Chittoor district is divided into numerous endogamous castes/sub-caste groups besides tribal groups such as the Yanadi, Yerukala, and Sugali. These populations living with different socioeconomic and cultural backgrounds in this area found to be apt for epidemiological research.

2.1.1. State of Andhra Pradesh

Andhra Pradesh State is formed on 1st November, 1956 under the States' reorganization scheme. It is the fifth largest State with an area of 2,76,754 Square Kilometers, accounting for 8.4 percent of India's territory. The State has the longest coastline (972 kilometers) among all the States in India. Andhra Pradesh is gifted with a variety of physiographic features ranging from high hills, rising and falling plains to a coastal deltaic environment.

Geographically, Andhra Pradesh is composed of most of the eastern half of the Deccan plateau and the plains to the east of the Eastern Ghats. Andhra Pradesh is divided into three regions. The northern part of the plateau is the Telangana region and the southern part is known as Rayalaseema. These two regions are separated by the River Krishna. The third region is Coastal Andhra. The plains to the east of Eastern Ghats form the Eastern coastal plains. The Eastern Ghats are discontinuous and
individual sections have local names. The Kadapa Basin formed by two arching branches of the Eastern Ghats is a mineral-rich area. The coastal plains are for the most part delta regions formed by the Godavari, Krishna, and Penner rivers. The Eastern Ghats are a major dividing line in the state's geography. The Ghats become more prominent towards the south and extreme north of the coast. The Eastern Ghat region is home to dense tropical forests, while the vegetation becomes sparse as the Ghats give way to the Deccan Plateau, where shrub vegetation is more common. Most of the coastal plains are put to intense agricultural use. The west and southwest parts of Andhra Pradesh have semi-arid conditions.

The climate of Andhra Pradesh varies considerably, depending on the geographical region. Monsoons play a major role in determining the climate of the state. Summers last from March to June. In the coastal plain, the summer temperatures are generally higher than the rest of the state, with temperature ranging between 20°C and 41°C. July to September is the season for tropical rains in Andhra Pradesh. The state receives heavy rainfall from the Southwest Monsoon during these months. About one third of the total rainfall in Andhra Pradesh is brought by the Northeast Monsoon. October and November see low-pressure systems and tropical cyclones form in the Bay of Bengal which, along with the Northeast Monsoon, bring rains to the southern and coastal regions of the state. November, December, January, and February are the winter months in Andhra Pradesh. Since the state has a long coastal belt the winters are not very cold. The range of winter temperature is generally 12°C to 30°C.

Total population of Andhra Pradesh as per 2011 census is 84,665,533 of which male and female are 42,509,881 and 42,155,652 respectively and the density of population is 308 per square kilometers. The population growth rate in this decade is 11.10 percent. Literacy rate in Andhra Pradesh has seen upward trend and is 67.66 percent as per 2011 population census. Of that, male literacy stands at 75.56 percent while female literacy is at 59.74 percent. Telugu is the first official language of the state, spoken by 84.02 percent followed by Urdu, which is the second official language of the state and is spoken by 11.76 percent of the population. Other languages spoken in
Andhra Pradesh are Hindi, Tamil, Kannada, Marathi and Oriya, Malayalam, Gujarati, Bengali, Gorkhali/ Nepali, Punjabi and Sindhi.

Rayalaseema is a geographic region in the state of Andhra Pradesh in India. It includes the southern districts of Anantapur, Chittoor, Kadapa and Kurnool. It has a total area of 67,299 Square Kilometers which is 24.46 percent of total state area with a population of 15,174,908 (2011 census) which is 17.93 percent of Andhra Pradesh state population. These Telugu-speaking districts were part of the Madras Presidency until 1953, when Telugu-speaking districts of the Presidency were carved out to form Andhra State. From 1953-56, this region was part of Andhra State. In 1956, the Telangana region was merged with Andhra State to form Andhra Pradesh State. Earlier, Bellary district was part of Rayalaseema. With the formation of states based on languages, Bellary was joined to Karnataka. The city of Bellary, which has large numbers of both Kannada and Telugu speakers, was included in Mysore after protracted debate and controversy. The present parts of the Rayalaseema district are Kurnool district, Anantapur district, Kadapa district and Chittoor district.

2.1.2. Chittoor District

The present study is carried out in the Chittoor district also known as Chittur, is a district of India's Andhra Pradesh state in the Rayalaseema region. The word Chittoor comes from Chittoor (meaning "small town") in Tamil language and also from chittadavula ooru ("dense forest" in Telugu). The district headquarters is Chittoor City. The district has a population of 4,170,468 according to 2011 census of India. Chittoor district is famous for the Tirupati, Kanipakam and Sri Kalahasti temples. It lies in the Poini river valley of southernmost Andhra Pradesh, on the NH4 Bangalore–Chennai highway. It is a major market center for mangoes, grains, sugarcane, and peanuts. There are 66 Mandals in Chittoor district. There are 14 Andhra Pradesh Legislative Assembly MLA constituencies in Chittoor district. The district has a population density of 275 inhabitants per square kilometer (710 /sq mi). Its population
growth rate over the decade 2001–2011 is 11.33%. Chittoor has a sex ratio of 1002 females for every 1000 males, and a literacy rate of 72.36%.

Chittoor is a part of Rayalaseema. The district occupies an area of 15,359 square kilometers (5,930 sq mi). The district is bounded by Anantapur District to the northwest, Cuddapah District to the north, Nellore District to the northeast, Krishnagiri District, Vellore District and Tiruvallur District of Tamil Nadu state to the south, and Kolar District of Karnataka state to the west. Chittoor district lies extreme south of the Andhra Pradesh state approximately between 12°37′ - 14°8′ north latitudes and 78°3′ - 79°55′ east longitudes. Thirty percent of the total land area is covered by forests in the district. Mango and tamarind groves surround the city of Chittoor, and cattle are raised in the district. The soils in the district constitute red loamy 57%, red sandy 34% and the remaining 9% is covered by black clay black loamy, black sandy and red clay. Chittoor is around 180KM from Bangalore, around 160KM from Chennai, and around 590KM from Hyderabad, state capital and around 1800KM from National capital Delhi.

The important rivers in the district are Ponnai and Swarnamukhi rivers which originate in Eastern Ghats. Other rivers include Kusasthali, Beema, Bahuda, Pincha, Kalyani, Araniyar and Pedderu. The temperature in the western parts of the district like Punganur, Madanapalle, Horsley Hills are relatively lower than the eastern parts of the Chittoor District. This is because of the higher altitude of the western parts compared to the eastern parts. The summer temperatures touch 46°C in the eastern parts whereas in the western parts it ranges around 36° to 38°C. Similarly the winter temperatures of the western parts are relatively low ranging around 12°C to 14°C and in eastern parts it is 16°C to 18°C. Chittoor District receives an annual rainfall of 918.1 mm. The South West Monsoon and North East Monsoon are the major sources of rainfall for the district. On average the district receives 438.0 mm of rainfall through the South West Monsoon (From June to September) and 396.0 mm from North East Monsoon (From October to December). The rainfall received by the district in the years 2002 and 2003 are 984.2 mm and 934 mm respectively.
Fig 5: Map showing the study area and villages selected for the study

1. Gajulamandyam
2. Vedurlachervu
3. Rayalacheru
4. Kupuchandrapuram
5. Vadamalapuram
6. Kayam
7. Thandlam
8. Thondamanadu
9. PanagPallu
10. Damalchervu
11. Vallivedu
12. Busipalle
13. Palakuru
14. Bangarupalem
15. Nallangadu
16. Polakala
17. Errepalle
18. Nallasiddannapalle
19. Varadarajulapalli
20. Gundlakattamanchi
2.2. Sampling

The materials selected for the present study belongs to heterogenous population inhabiting with different geographic, socioeconomic and cultural milieu of Chittoor District. Data collection took place in between August 2011 to January 2013. Pilot study conducted for befriending the samples of the study and explaining the purpose of the study. And when they are more receptive then the primary data collection is started. Respondents are encouraged to speak freely.

The materials drawn for the present study are 1520 healthy adults, of which 802 are from urban centres (males: 421 and females: 381) and 718 are from rural villages (males: 382 and females: 336) of Chittoor District, Andhra Pradesh State, India. The data for urban sample is collected from Tirupati and Chittoor towns of Chittoor District and the data for rural sample is collected from 20 randomly selected villages of Chittoor District. A simple random sampling technique is employed in selecting the subjects. The study is approved by the Departmental Ethics Committee of Sri Venkateswara University, Tirupati. The objectives of the study are explained to all of the subjects before their consent is given. Written informed consent is obtained from all the participants. The age range of the sample is between 30 and 65 years.

The district population size according to 2011 census is 41, 70,468. House hold list are procured from the District Revenue Department and subject’s households are selected randomly from each of the village/wards. The inclusion criteria for the survey are apparently healthy unrelated adult individuals aged 30 years and above who are willing to provide written informed consent. The exclusion criteria include subjects with any gross abnormalities, bedridden patients or patients suffering from any chronic diseases and pregnant/lactating women. A structured and standardized questionnaire is used to collect the data on the demography, socioeconomic status, physical activity, smoking and alcohol habits and lifestyles via face-to-face in depth interviews.
2.3. Methods

For the purpose of comparison, the subjects are divided into different age groups like 20-29 years, 30-39 years, 40-49 years, 50-59 years, 60-69 years and >70 years. Socioeconomic status (SES) is classified into five groups (i.e. I–V) based on scores of education, occupation, housing condition, ownership of durables and per capita income of the family (Singh et al., 1997). SES-I is the highest and SES-V is the lowest class. Physical activity is assessed from occupational and spare time activities and subjects are graded as sedentary, mild, moderate or heavy, based on scores of activities according to Indian criteria (Singh et al., 1997).

Data on subject’s anthropometric measurements like height, weight, waist and hip circumferences are taken. Subject’s blood pressure is recorded as per the standard procedure.

2.3.1. Anthropometric Parameters

The physical assessment included height, weight and circumferences of waist and hip as per the procedure specified by Lohman et al (1988). The instruments are calibrated prior to take the measurements.

2.3.1.1. Height or Stature

It measures the vertical distance from vertex to floor. The height is measured in centimeters using anthropometer rod. The subject is asked to stand on a horizontal platform with the heels together, stretching upward to the fullest extent, aided by the measures on the mastoid processes and by encouraging the subject to 'stand tall, take a deep breath and relax'. The subject’s back is as straight as possible with the head in Frankfort horizontal (F.H) plane while taking the measurement, which is achieved by rounding or relaxing the shoulders and manipulating the posture.
2.3.1.2. Body Weight

The weight is recorded in kilograms to the nearest 0.5 kg with the subject standing bare-foot and with minimum clothing on a weighing machine without any support.

2.3.1.3. Waist Circumference

The waist circumference is taken as the minimum circumference between the umbilicus and xiphoid process and measured to the nearest to 0.1 cm using measuring tape.

2.3.1.4. Hip Circumference

The hip circumference is measured as the maximum circumference around the buttocks posteriorly and the symphysis pubis anteriorly and measured to the nearest to 0.1 cm using measuring tape.

2.3.1.5. Derived Measurements

2.3.1.5.1. Body mass index (BMI)

Body mass index is most frequently used to define individuals in to underweight, normal weight, overweight and obesity, which is a single number that evaluates an individual's weight in relation to height (weight in kilograms/height in metres square; kg/m$^2$). It is commonly employed index in epidemiological studies, to predict the obesity related morbidity and mortality in adults. Overweight/obesity is defined using the revised criteria as specified by WHO (2004).
<table>
<thead>
<tr>
<th>Classification</th>
<th>BMI (kg/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Underweight</td>
<td>&lt; 18.5</td>
</tr>
<tr>
<td>Normal weight</td>
<td>18.5 - 24.9</td>
</tr>
<tr>
<td>Overweight</td>
<td>25.0 - 29.9</td>
</tr>
<tr>
<td>Obese class I</td>
<td>30.0 - 34.9</td>
</tr>
<tr>
<td>Obese class II</td>
<td>35.0 - 39.9</td>
</tr>
<tr>
<td>Obese class III</td>
<td>&gt; 40.0</td>
</tr>
</tbody>
</table>

### 2.3.1.5.2. Waist Hip Ratio (WHR)

Waist hip ratio is calculated as waist circumference/hip circumference. WHR (Abdominal obesity) is diagnosed when WHR is >0.9 in males and >0.8 in females as per the National Cholesterol Education Programme (NCEP, 2002).

### 2.3.2. Physiological Parameters

#### 2.3.2.1. Blood Pressure

Blood pressure is measured at the study site by measuring the appearance and disappearance of Korotkoff’s sounds to the nearest 2 mmHg on a seated subject, with a random zero mudler sphygmomanometer as specified by Rose et al. (1982).

Hypertension is diagnosed when the systolic blood pressure is >140 mmHg and/or the diastolic blood pressure is >90 mmHg, as per the guidelines prescribed by the seventh joint national committee on detection, evaluation and treatment of high blood pressure (JNC, 2003).

#### 2.3.2.2. Blood Collection

A fasting blood sample is collected in the morning for biochemical investigation after an overnight fast of at least 10 hours. Two ml of blood sample is drawn from each subject by anticubital vein puncture with the help of a disposable syringe and needle. It is transferred into sterile, clean pre-labelled tube containing disodium ethylenediamine tetra-acetate (EDTA) as anticoagulant (0.5 mg/ ml) and immediately placed on ice in a thermos flask. These tubes are then brought to the laboratory in ice box to the Department of Anthropology, Sri Venkateswara University, Tirupati. The serum is
separated after centrifugation at 2500 rpm for 20 minutes into pre-labeled sterile tubes and separated serum is used for biochemical analysis. Biochemical analyses include estimation of fasting blood glucose only.

2.3.2.2.1. Estimation of Blood Glucose

Blood glucose is determined by the glucose-oxidase method with an intra- and inter-assay coefficient of variation of <1% as per the procedure of Trinder (1964). Glucose is oxidized by the enzyme Glucose oxidase (GOD) to give D- gluconic acid and hydrogen peroxide. Hydrogen peroxide in the presence of the enzyme Peroxidase (POD) oxidizes phenol which combines with 4-Aminoantipyrine to produce a red colored quinoneimine dye. The intensity of the color developed is proportional to glucose concentration in the sample. To 10 microlitres of the test sample, 1000 microlitres of the enzyme reagent is added, mixed well and incubated at 37°C for 10 minutes. Measures of the absorbance of test and standard against blank on a spectrophotometer at 505 nm.

2.3.4.2. Calculation

\[
\text{Glucose in mg\%} = \frac{\text{Absorbance of Test Sample}}{\text{Absorbance of Standard}} \times 100
\]

Hyperglycaemia is defined as blood glucose >120 mg\% (Diabetes Care, 2005; WHO, 2007).

2.4. Statistical Analysis

Statistical analysis is carried out via SPSS – 16.0 and alpha levels are set at p<0.05. Continuous variables are provided with descriptive statistics and discontinuous variables with percentages. Due to less number of subjects grouped as Obese-II, these subjects are further dropped in all other statistical analysis. Mean values between groups are compared with students “t” test and within the age groups, socioeconomic status and physical activity with one way analysis of variance (ANOVA). Chi square test is applied to see the association of overweight/obesity with age, socioeconomic
status and physical activity. Bivariate relationships among the variables are tested by Pearson correlation coefficients. Age and sex adjusted binominal logistic regression model is used to see the effect of socioeconomic and lifestyle factors towards the prevalence of overweight and obesity. Further a simple linear regression model is fitted to see the variation exerted by the body mass index on blood pressure and blood glucose levels after adjusting for age and sex.

2.4.1. Statistical Treatment

The following statistical measures are employed for the analyses of the data.

2.4.1.1. Descriptive statistics

i) Arithmetic Mean

This is one of the measures of location which is a most widely used measure representing the entire data by one value.

\[ A.M. = \frac{\sum fd}{N} \times CI \]

Where,
- \( A \) = Assumed or arbitrary mean
- \( fd \) = Summation of class frequency (f) deviation from the assumed mean measured in classes (d)
- \( CI \) = Class Interval

ii) Standard deviation (S.D.)

It is a measure of dispersion and is the square of variance.

\[ S.D.(\sigma) = \sqrt{\left(\frac{\sum fd^2}{N}\right) - \left(\frac{\sum fd}{N}\right)^2} \times CI \]

Where,
- \( f \) = Class frequency
- \( d \) = deviation from the assumed mean measured in classes
- \( N \) = Total number
- \( CI \) = Class Interval
2.4.1.2. Tests of significance

Different tests are employed to evaluate the magnitude of difference between the observed and expected values of variables under comparison.

i) Chi – square ($\chi^2$) test

To test the significance of difference between the observed ($O_i$) and expected ($e_i$) numbers for a given set of variables ($k$) distributed in rxc contingency tables, the following formula is applied.

$$\chi^2 = \sum_{i=1}^{k} \frac{(O_i - e_i)^2}{e_i}$$

An appropriate correlation (0.5) has been made whenever necessary for continuity when the exact distribution of $\chi^2$ in a rxc (eg.2x2) table is discrete. The following is the formula.

$$\chi^2 = \frac{\left[(O_i - e_i) - 0.5\right]^2}{e_i}$$

The degree of freedom (df) is noted as number of categories in rows – 1 x number of categories in columns-1.

ii) t-test

The t test is applied to know the significance difference, if any, between the mean values of quantitative variables of various components.

$$t = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{S.E_{1}^2 + S.E_{2}^2}} \text{ with df (n}_1 + n_2 - 2)$$

Where,

$\bar{x}_1$ = Mean of the variables of first sample

$\bar{x}_2$ = Mean of the variable of second sample
$S.E_1 =$ Standard error of first sample
$S.E_2 =$ Standard error of second sample
$n_1 =$ Size of the first sample.
$n_2 =$ Size of the second sample

In case of dermatoglyphics the variables $x_1$ on the right (R) and left (L) hands of an individual are not independent; hence paired t-test is applied to see the significance of difference between the palms.

\[
    t = \frac{\bar{d}}{sd} \text{ with } (n-1)d.f
\]

Where,
\[
    \bar{d} = \frac{\sum d_i}{n}
\]
\[
    d_i = X_{iR} - X_{iL}, \text{ and}
\]
\[
    sd = \sqrt{\frac{\sum d_i^2 - \left(\sum d_i\right)^2/n}{(n-1)n}}
\]

iii) F – test (ANOVA)

Analysis of variance (ANOVA) is performed to know the extent of variability so as to understand whether the variance between the groups is more than that within the group. The procedural steps are tabulated below.

**ANOVA TABLE**

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>Sum of squares (SS)</th>
<th>df</th>
<th>Mean squares (MS)</th>
<th>F-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between the groups</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>$\sum_{i=1}^{h} k \left( \bar{x}_1, \bar{x}_2 \right)^2$</td>
<td>$h-1$</td>
<td>$\sum_{i=1}^{h} k \left( \bar{x}_i, \bar{x} \right)^2 / h-1$</td>
<td>Between MS/Within MS</td>
</tr>
<tr>
<td>Within the group</td>
<td></td>
<td>$N-h$</td>
<td>$\sum_{i=1}^{h} \sum_{j=1}^{k} k \left( \bar{x}_{ij}, \bar{x}_i \right)^2$</td>
<td>$N-h$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>$N-1$</td>
<td>$\sum_{i=1}^{h} \sum_{j=1}^{k} k \left( \bar{x}_{ij}, \bar{x}_i \right)^2$</td>
<td></td>
</tr>
</tbody>
</table>