ANTHROPOMETRIC AND BIOCHEMICAL ANALYSIS OF NORMAL AND OBESE SUBJECTS ON AN INDIAN POPULATION

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ABSTRACT: Obesity is a very serious health problem worldwide. Obesity depends on several physical and bio-chemical parameters that are discussed in this work, i.e., BMI, cholesterol level that includes Total cholesterol (TC), High density lipoprotein concentration (HDL-c), Low density lipoprotein (LDL-c), Triglycerides level (TG), Glucose level, Postprandial glucose level. The present study throws light on the efforts being made to find out the prevalence of obesity over different anthropometrical and biochemical parameters. Anthropometrical parameters were considered in the school going children from families financially poor or with bound, while the biochemical parameters were performed on subjects from age group from 16 to 70 years. Many questions were asked in the form of a questionnaire related to their lifestyle. Anthropometrical data from school going children showed 11% obesity and overweight. Biochemical data showed 20.27% persons were having a TC level, 46.08% having HDL-c, 19.35% having LDL-c, 29.05% having TG, 36.48% having fasting glucose level and 37.18% having a postprandial glucose level higher than the threshold values. Henceforth, the results explained the prevalence and epidemic conditions of obesity in developing countries like India.

INTRODUCTION: Obesity is a very serious global health issue. In developing countries like India there is large number of people suffering from it. Obesity, abdominal obesity and comorbidities are ever more common among urban Indians ¹. Regional fat distribution, particularly abdominal obesity, is considered significant for expansion of insulin resistance, the metabolic and coronary heart disease (Misra and Vikram, 2003).

Subcutaneous adipose tissue (SCAT) shares more than 80% of total body fat and 10-20% shared by visceral or intra-abdominal adipose tissue (IAAT) in adults ². Asian Indians show exclusive features of obesity like excess body fat, abdominal adiposity, augmented Scat, IAAT, and deposition of fat in ectopic sites (liver, muscle, etc.) ³, that may be accountable for high propensity to expand insulin resistance and dysmetabolic state.

As far as young people are concerned, it is still a matter of concern that need debates an discussion for solution. Weight beyond 125% of median weight for height is obesity. Only the weight is not a good index of fitness because it does not consider height. The most accepted definition is given by who and IOTF in terms of BMI.
This is the measure derived from dividing body weight (kilograms) by the square of height (meters). The BMI of <18.5 is considered to be underweight, 18.5-24.9 as normal weight, 25.0 to 29.9 as overweight and 30 or above as obesity. Body fat is not directly measured by BMI but it has been investigated that BMI is associated with more direct measures of body fat, such as skinfold, bioelectrical impedance, thickness measurements, densitometry, dual energy x-ray absorptiometry (DXA) and other methods.

For children and teens, BMI is calculated based on age and sex and is frequently called BMI-for-age. After BMI is calculated for children and teens, the BMI number is plotted on the Centers for Disease Control (CDC) BMI-for-age growth charts (for either girls or boys) to find a percentile ranking. The BMI is not used by itself while evaluating probable obesity in children and teens. Percentiles are the most commonly used indicator to evaluate the size and growth patterns of individual children in the United States. The percentile shows the relative position of the child’s BMI number among children of the same sex and age. The growth charts show the weight status categories used with children and teens (underweight, healthy weight, overweight, and obese). BMI tables for adults are not accurate for children and teens. Less than 5th percentile is considered to be underweight, 5th percentile to 85th percentile as healthy weight, 85th to less than 95th percentile as overweight and equal to greater than 95th percentile as obesity.

Obesity depends on several other physical and biochemical parameters besides BMI. Some of the parameters which are discussed in this work are Waist-hip ratio, Cholesterol level that includes Total Cholesterol (TC), High Density Lipoprotein concentration (HDL-c), Low Density lipoprotein concentration (LDL-c), Triglyceride level (TG), Glucose level, Postprandial glucose level. Waist-hip is the ratio of the circumference of the waist to that of the hips. It is calculated by dividing the waist measurement to hip measurement. Lipid profile includes TC, HDL-c, LDL-c and TG. Total blood cholesterol is a measure of LDL cholesterol, HDL cholesterol, and other lipid components. Triglycerides are the forms in which most fat exist in food and the body.

A high triglyceride level has been linked to higher risk of coronary artery disease and is associated with obesity and regional adipose tissue distribution to plasma lipoprotein-lipid composition in premenopausal women. HDL is considered as the good cholesterol (Centers for Disease Control and Prevention, 2017) and its higher level reduces the risk as it protects against heart disease by taking the bad cholesterol out of the blood and keeping it from building up in the arteries and reduce macrophage accumulation. The studies suggest some pathway for removal of cholesterol. The LDL cholesterol is considered as bad cholesterol as it can build up on the walls of arteries and increase the chances of getting heart disease. Lower the level of LDL, the lower is the risk associated with obesity.

Blood sugar concentration or blood glucose level is also an indicator of obesity. It is the amount of glucose (sugar) present in the blood. The body naturally tightly regulates blood glucose levels as a part of metabolic homeostasis.

In the present study, effort is being made to find out the prevalence of obesity over different anthropometrical and biochemical parameters. Anthropometrical parameters were considered in the school going children of financially weak or very average family while the biochemical parameters were performed on people above 40 years in age.

MATERIALS AND METHODS: We performed a cross-sectional, epidemiological study using stratified cluster sampling design in two private schools of Vellore district (Tamil Nadu), India with students from very average or poor financial backgrounds. Two schools were randomly selected for the study named Williams Higher Secondary School, Kangeyalore having students from STD I to XII and Holy Trinity Royal School, Kangeyalore having students from Class Nursery to V. All students were willing to participate in the study from both schools. All subjects were assessed for demographic and socio-economic profiles and their family history. All the subjects were fully informed about the purpose of the study and a written consent was obtained from each of them. Approval for the study was obtained from the institutional ethics committee of Vellore Institute of Technology, Vellore.
Anthro-Pometric Measurements: Body weight (to nearby 0.1 kg) and height (to nearby 0.1 cm) were measured while subjects were wearing light clothing and stood straight with naked foot and eyes heading for straight in front. Body Mass Index (BMI) was calculated as weight (kg) / height \( m^2 \) . Waist circumference (WC) and Hip Circumference (HC) were measured with tailor tape. The mean of three readings for every measurement was taken for the calculation of Waist-Hip Ratio (WHR). Many other questions were also asked like parent’s/family annual income, food habit, lifestyle (wake up and sleeping time, total sleeping duration, internet browsing duration, indoor and outdoor playing duration and duration of watching Television) in the form of questionnaire.

Biochemical Study of Normal Weight, Overweight and Obese Subjects:
Sample Size: For biochemical analysis, same 217 subjects (normal weight, overweight and obese) were used for the study, which was made from an Indian population.

Blood Sample Collections: This work was also permitted by Vellore Institute of Technology, (Vellore, Tamil Nadu, India) Institutional Ethics Committee to study the human subjects (Ref. no.VIT/IECH/012/Sept.12.2015). Two medical expert lab technicians were recruited for the collection of blood samples from A.K diagnostic Lab (Giddi, Ranchi, Jharkhand, India).

The participants also gave written consent form for this work. Here also BMI was considered as the inclusion criteria for recruiting and categorizing the subjects. For normal weight (BMI<25 Kg/m\(^2\)), for overweight (BMI>25 Kg/m\(^2\)) and for obese (BMI>30 Kg/m\(^2\)) (Obesity: preventing and managing the global epidemic. Report of a WHO consultation on obesity, 2000) \(^{16}\). The underweight and subjects with other health complications were not used for this study. In this study, peripheral blood (1 ml) was collected in EDTA (anticoagulant) treated sample vials from different age (16-68 years) and sex (male and female) of normal weight, overweight and obese subjects. Plasma was extracted from this 1ml of blood sample from whole blood for biochemical analysis. The plasma isolation from blood sample was accomplished at Dr. Kunal Mukhopadhyay’s Lab, Birla Institute of Technology (BIT), Mesra, Ranchi, and Jharkhand, India.

Plasma Extraction from Blood: The plasma extraction from the blood sample was accomplished as described by Henry, 1979 (Clinical Diagnosis and Management by Laboratory Methods) \(^{17}\) and Haeberle et al., (2006) protocol \(^{18}\).

Procedure: First of all, 1ml of freshly collected whole blood sample was taken in the sample tube. Then the sample tube was centrifuge with a speed of 2000 rpm for 15 min at 4 °C in the refrigerated centrifuge. As a result supernatant (plasma, yellowish color) was collected in a fresh sample tube. The plasma samples were further stored at –20 °C for the lipid and glucose profile analysis.

Biochemical Profiles Determination: All the recruited subjects (normal weight, overweight and obese) were tested and analyzed by using enzymatic kits (Autospan, Span Diagnostic Ltd, India) and methods described by Cox and Garcia-Palmieri (1990) \(^{19}\) for different biochemical parameters such as, TC, HDL-c, LDL-c, TG and postprandial blood glucose. The LDL-c was analyzed as described by Friedewald equation \(^{20,21}\). The postprandial glucose was determined as described by Kaplan (1984) protocol \(^{22}\).

Estimation of Total Cholesterol (TC):
Procedure: The TC estimation was performed by using an enzymatic kit (Autospan, Span Diagnostic Ltd, India) and Cox and Garcia-Palmieri (1990) protocol \(^{19}\). Three sample tubes were taken and marked as blank, standard and test, accordingly. Further, 10\(\mu\)l of plasma sample and 1ml of cholesterol reagent (reagent 1) were added in the tube marked with test. Then, 10\(\mu\)l of cholesterol standard (reagent 2) and 1ml of cholesterol reagent (reagent 1) were added in the tube marked with standard. Afterward, 1ml of cholesterol reagent (reagent 1) was only added in the tube marked with blank. Finally, all the components were mixed well and kept for the incubation at 37 °C for 10 min or at room temperature (15–30 °C) for 30 min. Finally, the absorbance was measured at 505nm in spectrophotometer. The final concentration of TC
was anticipated in mg/dL, which was calculated with absorbance of test divided by absorbance of standard and multiplied with 200.

**Estimation of High Density Lipoprotein (HDL-c):**

**Procedure:**
The HDL-c estimation was executed by using an enzymatic kit (Autospan, Span Diagnostic Ltd, India) and Cox and Garcia-Palmieri (1990) protocol 19, in two steps. At the first step, there is separation of HDL-c. For this, 200µl of plasma was taken and mix it well with reagent 3 (precipitating reagent, PEG 6000) and incubated at room temperature (15-30 °C) for 10 min. After incubation, centrifuge the mixture with a speed of 2000rpm for 15 min at room temperature and separate the supernatant which contains HDL-c. Further, this supernatant will be used in the second step.

In second Step, three sample tubes were taken and marked as blank, standard and test accordingly. Then, 100µl of supernatant that was obtained in first step and 1 ml of cholesterol reagent (reagent 1) were added in the tube marked with test. After that, 100µl of HDL-c standard (reagent 4) and 1ml of cholesterol reagent (reagent 1) were added in the tube marked with the standard. Afterward, 1ml of cholesterol reagent (reagent 1) was only added in the tube marked with blank. Furthermore, all the components were mixed well and kept for the incubation at 37 °C for 10 min. Finally, the absorbance was measured in spectro photometer at 505nm. The final concentration of HDL-c was estimated in mg/dL, which was calculated with absorbance of test divided by absorbance of standard and multiplied with 100.

**Estimation of Tryglycerides (TG):**

**Procedure:** The TG estimation was also executed by using an enzymatic kit *i.e.*, Autospan, Span Diagnostic Ltd, India and Cox and Garcia-Palmieri (1990) protocol 19. Three sample tubes were taken and marked them as blank, standard and test, accordingly. Furthermore 10µl of plasma sample and 1ml of TG mono reagent (reagent 1) were added in the tube that was marked with test. Afterward, 10µl of TG standard (reagent 2) and 1 ml of TG mono reagent (reagent 1) were added in the tube marked with standard. Afterward, 1ml of TG mono reagent (reagent 1) was added only in the tube marked with blank. Moreover, all the components were mixed well and kept for the incubation at 37 °C for 10 min. Finally, the absorbance was measured in spectrophotometer at 505 nm. The final concentration of TG was estimated in mg/dL, which was calculated by dividing absorbance of test to absorbance of standard and multiplied with 200.

**Estimation of Low Density Lipoproteins (LDL-c)**

**Procedure:** The LDL-c concentration was estimated in mg/dL and calculated as described by Friedewald’s equation 20, 21.

\[
\text{LDL-c (mg/dl)} = \text{TC} - \frac{\text{TG}}{5} - \text{HDL-c}
\]

**Estimation of Glucose:**

**Procedure:** In this case, the blood samples were taken from all the subjects in morning before meals for estimation of fasting glucose level and then after 2h of meal for the estimation of postprandial glucose. The estimation of fasting and postprandial glucose was carried out by using an enzymatic kit *i.e.*, Autospan, Span Diagnostic Ltd, India and Kaplan, 1984, protocol 22. Three sample tubes were taken and marked them as blank, standard and test accordingly. Furthermore 20µl of plasma sample and 1.5 ml of working glucose reagent were added in the tube marked with test. Then, 20µl of glucose standard (reagent 3) and 1.5 ml of working glucose reagent were added in the tube marked with standard. Afterward, 1.5ml of working glucose reagent was added only in the tube marked with blank. Furthermore, all the components were mixed well and kept for the incubation at 37 °C for 10 min. After incubation, 1.5 ml of purified water was added in each sample tube and mixed well. Finally, the absorbance was measured with spectrophotometer at 505 nm. The final concentration of fasting and postprandial glucose was estimated in mg/dL, which was calculated with absorbance of test divided by absorbance of standard and multiplied by 100.

**Definitions:** Overweight and obesity were defined as BMI ≥ 23–24.9 kg/m2 and BMI ≥ 25 kg/m², respectively (Misra et al., 2009). Waist circumference > 90 cm for males and >80 cm for females was considered an indicator of abdominal obesity 3. Impaired fasting glucose and T2DM were diagnosed according to the diagnostic criteria of the
American Diabetes Association (Diabetes Care, 2003) 23. The modified criteria (three out of five) of National Cholesterol Education Program, Adult Treatment Panel III (NCEP ATP III) were used to define the metabolic syndrome; waist circumference, males > 90 cm, females >80 cm, fasting blood glucose >100 mg/dl, postprandial glucose level >120, serum TG >150 mg/dl, HDL-C; males <40 mg/dl, and females <50 mg/dl and LDL-c ≤ 130mg/dl 23, 24, 25.

Statistical Methods: Data were recorded on a pre-designed proforma. Before entering the data on an excel spreadsheet, the proforma were reviewed for any incomplete information. All the entries were double-checked for any potential keyboard mistake. For the variables, subsequent estimated normal distribution, mean and standard deviation (SD) was computed, while for non-normally distributed variables, summary statistics were computed by median and range.

RESULTS:

Anthropometric Results: Anthropometric studies were carried out on school going children from age group 5-18 years. Two schools, Williams Higher Secondary School, Kangeynalore (Total students: 366, No. of boys: 249 and No. of girls: 117) and Holi Trinity Royal School, Kangeynalore (Total students: 56, No. of boys: 36, No. of girls: 20) were selected for this study. The total number of students was 424 (No. of boys: 285 and No. of girls: 137). As per BMI is concerned out of these 424 students, it was found that 230 (54.24%) were normal weight, 149 (35.14%) under weight, 36 (8.49%) were overweight and 9 (2.12%) were obese. The total number of boys was 287 out of which 152 (52.96%) were of normal weight, 104 (36.23%) were of underweight, 25 (8.71%) overweight and 6 (2.09%) were obese. The total number of girls was 137 out of which 78 (56.93%) were of normal weight, 45 (32.48%) were of underweight, 11 (8.02%) of overweight and 3 (2.18%) were obese.

Many other questions were asked in the form of a questionnaire. The result shows that about 62.56% of students were watching Television for more than 2 h. 18.30% students were using the internet for more than 1 hour. Indoor game playing students percentage also seems to be high, about 72.13% than the outdoor game playing students i.e. 45.62%. The percentage of students consuming fast/junk food was approximately 37.70%. And, the students who were actively participating in physical exercise were about 40%.

Biochemical Analysis: The total sample size for biochemical study was 217. The age group for this study was above 16 years old. The sample from 137 males and 80 females from mixed population of India. It was observed that out of total 217 samples, 27 males (19.7%) and 17 females (21.25%) were found to have the TC level higher than the threshold. Similarly, 70 males (51.0%) and 30 females (37.5%) were found to have high HDL-c, 32 males (23.35%) and 10 females (12.5%) have higher level of LDL-c, 43 males (34.30%) and 22 females (27.5%) were affected high level of TGL, 19 males (40.42%) and 8 females (29.62%) were affected by the higher glucose level at fasting condition and 49 males (37.98%) and 25 females (35.71%) were affected with higher level of glucose at post meals Table 1.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Total</th>
<th>Male</th>
<th>Female</th>
<th>p – value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC</td>
<td>44 (20.2%) n=217</td>
<td>27 (19.7%) n=137</td>
<td>17 (21.2%) n=80</td>
<td>0.473</td>
</tr>
<tr>
<td>HDL-c</td>
<td>100 (46.0%) n=217</td>
<td>70 (51.0%) n=137</td>
<td>30 (46.0%) n=80</td>
<td>0.105</td>
</tr>
<tr>
<td>LDL-c</td>
<td>42 (19.3%) n=217</td>
<td>32 (23.3%) n=137</td>
<td>10 (12.5%) n=80</td>
<td>0.111</td>
</tr>
<tr>
<td>TGL</td>
<td>65(29.9%) n=217</td>
<td>43 (34.3%) n=137</td>
<td>22 (27.5%) n=80</td>
<td>0.476</td>
</tr>
<tr>
<td>Fasting Glucose</td>
<td>27 (36.4%) n=74</td>
<td>19 (40.4%) n=47</td>
<td>8 (29.6%) n=27</td>
<td>0.820</td>
</tr>
<tr>
<td>Postprandial Glucose</td>
<td>74 (37.1%) n=199</td>
<td>49 (37.98%) n=129</td>
<td>25 (35.7%) n=70</td>
<td>0.971</td>
</tr>
</tbody>
</table>

FIG. 1: DISTRIBUTION OF OBESITY

Table 1: Prevalence of Obesity
DISCUSSIONS: The present work shows the prevalence of obesity in school going children from financially poor background families. Anthropometric data from school going children showed combined approx 11% obesity and overweight (Boys- 10.80% and Girls-10.20%). BMI and waist hip ratio were considered as the major parameters for measurement of obesity. The mean of BMI of total subjects was 15.91 with a standard deviation of 3.83 and the mean of waist hip ratio was found to be 0.88 with standard deviation of 0.05 Table 2.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Total no. of subjects</th>
<th>Total subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total no. of subjects</td>
<td>n = 423</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>15.91 ± 3.83</td>
<td></td>
</tr>
<tr>
<td>Waist Hip Ratio</td>
<td>0.88 ± 0.05</td>
<td></td>
</tr>
</tbody>
</table>

The study was performed on very economically poor families with an annual income of less than INR 1.5 lakhs. This is also reflected when we consider the case of underweight category which comprises 35.14% (Boys- 36.23% and Girls-32.84%) of the total subjects. Healthy weight was found to be 54.24% (Boys-52.96% and Girls-56.93%). The other questions asked in the form of the questioner were about the lifestyle and food habits and it is suggested that sedentary lifestyle and consumption of fast food is very common. 72.13% subjects were playing indoor game. Fast food/junk food consumption was also found among 37.07%, even after belonging from the very poor financial background.

Television watching (62.56%) and internet use (18.30% subjects using the internet more than 1h) were also seen very prominent among the subjects Table 3.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Percentage of Students (Subjects)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Watching television &gt; 2 hours</td>
<td>62.56%</td>
</tr>
<tr>
<td>Using internet &gt; 1 hours</td>
<td>18.30%</td>
</tr>
<tr>
<td>Indoor playing &gt; 1 hours</td>
<td>72.13%</td>
</tr>
<tr>
<td>Outdoor playing &gt; 1 hours</td>
<td>45.62%</td>
</tr>
<tr>
<td>Physical exercise</td>
<td>39.89%</td>
</tr>
<tr>
<td>Consumption of junk/fast food</td>
<td>37.70%</td>
</tr>
</tbody>
</table>

The other part of the current study was biochemical one. The age group selected for this study was above 16 years for the mixed population of Giddi (Jharkhand) part of India. Lipid and glucose profile were analyzed for this study. Lipoproteins are complex molecules in the form of conjugated proteins for their main function as transport vehicles for lipid in blood plasma. They deliver the lipid components like cholesterol, triglycerides, etc., to various tissues for utilization. Glucose is produced in the body after the food metabolism and leads to the generation of ATP. The glucose and lipid profiles within the limit are essential for the body to perform normal metabolic activities, but the increased level will lead to obesity related health complications. Amongst the various lipid profiles, the percentage of increased level of HDL-c was found to be the highest i.e. 46.08% (male-51% and female-37.5%) followed by Triglycerides with 29.95% (male-34.30% and female-27.5%).

Increased level of TC was found to be 20.27% (male-19.7% and female-21.25%) followed by LDL-c i.e., 19.35% (male-23.25% and female-12.5%).

As far as glucose profile is concerned, the increased level of the postprandial glucose level was prominent with a percentage of 37.18% (male-37.98% and female-37.70%) followed by fasting glucose level i.e., 36.48% (male-40.42% and female-29.62%). The high percentage of increased level of postprandial glucose level also shows that the prevalence of diabetes was very common. It also reflects the eating behavior of the population. The sample were basically from Tamil Nadu based population of South India and Jharkhand based population of north India, where rice is very common in food and it is a fact that rice contains high amount of sugar. The overall increased level of lipid and glucose profile is also an indicator of the prevalence of obesity.

The p-value was not coming with significant value and it may be due to random sampling and marginal difference in age groups. The different biochemical parameters were studied over different categories. The different parameters over male and female, obese/overweight and non obese are depicted in Table 4 and 5, respectively. The biochemical parameters were observed high in obese subjects than non obese subjects in both males and females.
This study can be linked with the further molecular and computational biology work for establishment of association of genetics in the development of obesity along with all anthropometrical and biochemical factors. A number of computational studies has been performed to examine the association of obesity with genetical parameters. four nsSNPs from FTO gene i.e., rs139000284, rs139577103, rs368490949, rs373076420 and four nsSNPs from NEGR1 gene i.e., rs145524630, rs267598710, rs373419972, rs375352213 were found to be frequent and functionally important by all the different computational tools (PolyPhen and PANTHER in case of FTO and PolyPhen, PANTHER, PROVEAN, SIFT, Mut Pred and M-CAP in case of NEGR1) [27, 28, 29]. Furthermore a number of genetic variants were also identified from gene SLC6A14 [30]. Similarly a number of other genes were tested for its linkage with development of obesity and other biochemical and anthropometrical parameters. All these experiments needs to verify on human being through in-vivo studies.

**CONCLUSION:** Obesity is an epidemic state of health, natural consequence of over nutrition and a sedentary lifestyle. Genetics is also playing an important role in the development of obesity. The combined percentage of obese and overweight by approximately 11% in children who belong to the financially poor background showing the prevalence of obesity but not limited to only over nutrition. Many children were found at the line of normal health, overweight and obesity.
By the time when they grow up may resulted in more epidemic state. Biochemical studies which show the increased level of lipid and glucose profiles are also indicate the prevalence of obesity. Earlier, it was thought that obesity is mainly found epidemic in developed countries, but now a days it is epidemic worldwide and developing countries like India is also at great risk.

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