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ASSOCIATION OF INSULIN RESISTANCE WITH ALANINE AMINOTRANSFERASE (ALT) LEVELS AS A MARKER OF HEPATIC VISCERAL OBESITY IN OVERWEIGHT ADOLESCENTS.

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ABSTARCT

Insulin resistance (IR) is well known to be an established predictor of cluster of disorders such as Type 2 Diabetes mellitus(T2DM), hypertension, dyslipidemia and atherosclerosis. In non-alcoholic fatty liver disease (NAFLD), there is a documented well known fact of liver biochemical abnormality including increased alanine amino-transferase (ALT) levels. Hence, in this study we tried to ascertain whether there could be any association between insulin resistance and ALT level as a marker of visceral obesity in overweight adolescents. ALT levels were significantly higher among overweight adolescents than healthy controls. There is a close association observed in overweight adolescents with reference to the presence of cardiometabolic risk factors, IR and increased ALT levels. Markers of hepatic visceral obesity such as ALT and insulin resistance (HOMA-IR) are the important parameters for prediction of visceral obesity associated with liver in overweight adolescents.

KEYWORDS: adolescents, insulin resistance, cardiometabolic risk factors, obesity, alanine aminotransferase, aspartate aminotransferase

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INTRODUCTION

As the number of overweight adolescents has increased in India as well as globally, risks for overweight-related metabolic and endocrine disorders, including hyperinsulinemia, hypertriacylglycerolemia, and hypercholesterolemia have also increased [1]. All of these metabolic and endocrinal cluster have led to the early development and enhanced incidence of T2DM, hypertension and cardiovascular diseases [2,3]. The cardiometabolic risk factors encompasses a wide gamut of factors that together constitute an increased risk of cardiovascular disease and is associated with insulin resistance (IR) and T2DM [4]. The National Cholesterol Education Program (NCEP), Adult Treatment Panel III has mentioned the criteria for cardiometabolic risk factors and these individuals can be labeled as having metabolic syndrome if they possess three or more parameters (positive) elevated blood pressure, low levels of high-density lipoprotein cholesterol (HDL-c), high triacylglycerol (TG), high fasting glucose level and abdominal obesity [5].The risk factors for cardiometabolic abnormalities appear to track from adolescents [6]. Thus, primary prevention and modification of life style during adolescence might not only alleviate chronic disease burden early in life but also be helpful in lowering the proportion of adults who will develop the disease including visceral fat deposition in different organs such as liver [7]. Obesity can be assessed by various parameters. Out of these physiological and anthropometric measurements are easy, cheaper as well as can be used for field based large population survey. Visceral obesity is more specific indicator of obesity than generalized as well as central obesity. Generalised obesity is assessed by body mass index (BMI). Central obesity is assessed by waist circumference. But, fat deposition to particular organ in abdominal area which is called as visceral obesity is better marker of obesity than central as well as generalized obesity. Although abnormal biochemical liver parameters, like ALT indicating hepatic visceral obesity do not essentially constitute cardiometabolic risk factors and metabolic syndrome definition, it is widely considered that the hepatic manifestation and visceral fat deposition in various organ of the metabolic syndrome are also significant [8,9]. Our primary aim was to study association of visceral obesity with cardiometabolic risk factors in obese adolescents.

MATERIALS AND METHODS

This study included overweight adolescents drawn from school located in the semi urban area of Puducherry, a union territory of Indian Governments. Ethical permission was obtained from Institutional Human Ethical Committee (IHEC) prior to the starting study. The following inclusion criteria were applied: simple overweight, both sexes, age range 11-18 years. Assent were obtained from the adolescents for inclusion in this study and written informed consent from parents. Following cases were excluded such as cases with known disease to cause fatty deposition in liver like diabetes mellitus and hepatitis and any other cause with syndromatic obesity. The total numbers of enrolled overweight adolescents were one hundred and fourteen (fifty seven males and fifty seven females). Their ages ranged between 11 and 18 years and the median age was 14. The total number of enrolled healthy control (BMI<75th percentile) adolescents were one hundred and fourteen (fifty seven males and fifty seven females). Their age ranged between 11 to 18 years and the median age was 14.5. All the cases as well as healthy controls were subjected to the following:

Anthropometricassessment

These measures included weight (Wt) and height (Ht). Body mass index (BMI) was calculated as body weight (in kilogram) divided by height square (in meters). All were plotted on growth charts. Adolescents were defined as overweight if their BMI was equal to or above

85th percentile as per the criteria [10,11]. Adolescents abdominal obesity was evaluated by waist circumference (WC) measurements to the age- and gender-specific population distribution. Equal or more than 90th percentile for age and gender for adolescents were considered as having abdominal obesity [12].

General obesity

Height was measured in erect posture with bare foot on floor using vertical scale nearest to 0.1 cm. The weight of subjects was measured using weighing scale, while the participants were without shoes and minimally clothed. Body mass index (BMI) was calculated using the formula BMI=weight(kg)/height(m^2). The cut off value for obesity was more than 95th percentile in adolescents and overweight ≥85th percentile.

Central obesity

Waist-Hip ratio (WHR) was calculated to assess central obesity. Waist circumference (in cm) was measured at a point mid-way between the lower rib and iliac crest with the measuring tape centrally positioned at the level of umbilicus. Waist circumference is the average of two measurements one taken after inspiration and other taken following expiration in standing position. Hip circumference was measured (in cm) at trochanter major of the head of femur. WHR was calculated using the following formula to assess central obesity. WHR=Waist(cm)/Hip(cm).

Blood pressure measurement

Blood pressure was measured using sphygmomanometer on two different occasions. Average systolic and diastolic pressure more than 90th percentile was considered as hypertension.

Biochemical parameters

All overweight as well as healthy adolescents underwent the following biochemical tests (following not less than twelve hours fasting period): Total cholesterol (normal range 100–200 mg/dl), high-density lipoprotein cholesterol (HDL-c) (normal desirable range 30–70 mg/dl), low-density lipoprotein cholesterol (LDL-c) (normal value <130 mg/dl), triacylglycerol (normal range 35–150 mg/dl) and fasting blood sugar (FBS) (normal desirable range <65-mg/dl). All biochemical measurement was carried out using a fully automated-chemistry analyzer unless stated otherwise. Fasting serum insulin measurement was performed by chemiluminescence. Insulin resistance was calculated using the following equation: The homeostasis model assessment method(HOMA-IR)

HOMA-IR = fasting insulin (μU/ml) × fasting glucose (mmol/l)/22.5.

IR was defined as HOMA-IR ≥ 3.16 [13].

Liver biochemical profile

The following tests were conducted using a fully automated-chemistry analyzer : Total serum bilirubin (normal range: 0.2–1 mg/dl), direct serum bilirubin (normal range 0.1–0.3 mg/dl), ALT (normal range 5–41 U/l), aspartate aminotransferase (AST) (normal range 5–37 U/l), alkaline phosphatase (AP) (normal range 180–240 U/l) were estimated by methods as per IFCC guidelines in fully automated analyzer.

Metabolic syndrome (MS)

Adolescents were labeled as having MS by the presence of three or more of the following modified criteria: (1) TG levels ≥ 130 mg/dl, (2) HDL-c ≤ 40 mg/dl, (3) fasting blood glucose levels ≥ 100 mg/dl, (4) WC ≥ 90th percentile for age and gender, and BMI>95th percentile of age and gender and (5) blood pressure ≥ 90th percentile specific for age and gender [14].

Ultrasonography abdomen

USG abdomen done to confirm the finding of fat deposition in liver (fatty liver) in oberweight adolescents. Overweight adolescents with increased ALT levels were having changes of fat deposition in liver (fatty liver), as sign of visceral obesity.

Statistical methods

Statistical Package for Social Science (SPSS) program version 16.0 was used. Data were summarized as mean and standard deviation (SD). Differences in Biochemical characteristics

among overweight adolescents were tested by unpaired Student's t test. A two-tailed *P* value <0.05 was considered statistically significant. Correlation between ALT levels and cardiometabolic risk factors was calculated by Pearson's correlation.

RESULTS

Demographic, physical and biochemical characteristics of the 114 overweight adolescents are depicted in (Table1). BMI was greater than or equal to 85th percentile. Systolic and diastolic blood pressures were normal in all healthy controls as well as overweight adolescents. Overweight adolescents had significantly higher level of ALT as compared to healthy controls; otherwise, serum albumin and total serum bilirubin were within normal limits**.** fourty one overweight adolescents (35.9%) had three or more criteria of cardiometabolic risk factors. More specifically, 30% had hypertriacylglycerolemia, 13.3% had high LDL levels and 10% low HDL-c and 16.6% high fasting blood glucose (Table 2). Insulin resistance (IR) was present in fourty five overweight adolescents (39.4%). Adolescents with metabolic syndrome had significantly higher WC, insulin fasting and insulin resistance (HOMA-IR) ($p<0.01$) and glucose and mean arterial blood pressure compared to healthy adolescents (*P*<0.05). IR was significantly higher among overweight adolescents ($p<0.001$). ALT level is significantly high in overweight adolescents as compared to healthy controls. Details pertaining to parameters as referred to be overweight adolescents are shown in Table 2.

Correlation analysis of ALT with insulin and insulin resistance (HOMA-IR)

Pearson's correlation of ALT levels with insulin shows positive correlation r= 0.77($p<0.01$). ALT showing positive correlation with insulin resistance (HOMA-IR) r=0.86 ($p<0.01$).

DISCUSSION

Among the overweight adolescents presenting with cardiometabolic risk factors 36.7% met three or more criteria for metabolic syndrome. Overweight adolescents had significantly raised ALT levels as compared to healthy control, a marker of visceral obesity more specifically in liver. Many previous studies, reported the prevalence of metabolic syndrome among overweight adolescents to between 12.4 and 54.2%, reaching approximately 50% in some populations. This fact also accounts for the higher percentage of hypertriacylglycerolemia (30%) seen in our overweight adolescents, in comparison to 26% in obese children as reported by previous studies. Overweight adolescents had significantly higher ALT levels as compared to those with healthy adolescents (mean ALT 40 ± 9.5 vs. 25± 7.4; *P*<0.001). This assumes relevance because not many reports take account of the visceral obesity component while evaluation of cardiometabolic risk factors in overweight adolescents. Overweight and obesity are strictly related to the development of fatty liver changes other than alcoholic cause [15,16]. Generally, there is more tendency of fat deposition in liver, in adolescents with cardiometabolic risk factors specifically, insulin resistance and hyperinsulinemia [17]. This difference may be explained by genetic and/or environmental factors, similar to what has been hypothesized for adults. Moreover, it is to be noted that of our overweight adolescents with increased ALT levels were overweight and having insulin resistance (HOMA-IR) and increased insulin level. In our study, we got significant correlation between ALT and HOMA-IR (insulin resistance) and insulin level with reference to overweight subjects in adolescents. Thus, in our study presence of three or more than three cardiometabolic risk factors, specifically insulin resistance is more related to biochemical changes in parameters of liver than a mere increase in weight(overweight). In the present study, fasting serum insulin was significantly higher in overweight adolescents with liver biochemical abnormality. The high

rates of obese, insulin-resistant adolescents with non-alcoholic fatty liver disease (NAFLD) meeting the criteria for three or more cardiometabolic risk factors suggests that a large number of these adolescents will go on to developing diabetes in future (T2DM). In the present study, 100% of overweight adolescents with increased ALT levels had an elevated WC, a marker of abdominal obesity. This is a significant finding since in recent years WC (physiological marker of abdominal obesity) has replaced BMI (physiological marker of generalized obesity) as an objective measure of central obesity [18]. General obesity is indicative of overall fat deposition all over the body. BMI though simple to measure, does not give an accurate idea about fat distribution in different tissues. Principally, BMI does not distinguish between increased mass in the form of fat, lean tissue or bone and it may lead to misclassification and misclassification of clinical data [19]. In the present era, researchers are more focusing on visceral obesity (obesity localized to any organ or tissues) than general or abdominal obesity. In our study, alterations in ALT level, a known marker of fatty liver indicates the presence of visceral obesity in overweight adolescents- a fact that hitherto has not been addressed strongly by the researcher. Obesity in prolonged period causes fat accumulation in different organs like liver. This is called as visceral obesity. In liver, fat accumulation causes fatty liver. Fatty liver is also caused by alcohol. Liver biochemical parameters alteration in those who do not consume alcohol comes under non alcoholic fatty liver disease (NAFLD).

Generally, obesity is causing fat accumulation in liver causing alterations in liver enzymes such as ALT and AST abnormalities, all is contributed mainly by decreased synthesis of apolipoproteins and microsomal transfer protein gene polymorphism, ultimately causing decreased transport of triacylglycerol out of liver [20]. In our study group of overweight adolescents alteration in ALT levels could be because of fat accumulation in liver and not because of any liver injury or damage, which is confirmed by USG abdomen. However, our study should be extended by incorporating biochemical parameters such as CETP (cholesterol ester transfer protein) and Apo B-100(apolipprotein). The biochemical study findings are confirmed by sonographic (USG) investigation of liver for confirmation whether there is any liver pathology in all overweight adolescents with increased ALT levels. In conclusion, insulin resistance and hyperinsulinemia are main key factors lead to fat accumulation in liver. There is a close association among overweight, presence of three or more cardiometabolic risk factors, insulin resistance(HOMA-IR) and increased ALT levels in overweight adolescents. Hence, overweight adolescents with clinical or biochemical abnormalities of the liver are prone to suffer from metabolic syndrome(MS), IR and non-alcoholic fatty liver changes.

Prevention of obesity and early intervention might thus be needed to reverse these liver abnormalities to reduce morbidity among overweight adolescents. Because of metabolic stress in overweight in adolescents, deposition of visceral fat in organs including liver causing abnormal hepatic parameters, visceral fat deposition is also considered important in the investigation of overweight adolescents. Primary prevention of obesity will help in prevention of liver pathology such as non-alcoholic fatty liver changes, that may eventually lead to organ fat accumulation (visceral obesity) associated with metabolic syndrome/T2DM [21]. It is also advised that supplements with B6 (Pyridoxine) in particular or B-complex vitamins in general, vitamin E and peripheral insulin sensitizers like metformin would help to reverse the hepatic visceral obesity, in such vulnerable overweight adolescents with insulin resistance and altered ALT level [22].(Prophylaxis) Markers of heoatic visceral obesity like ALT, insulin resistance (HOMA-IR) and impaired fasting glucose are the important parameters for prediction of presence of liver fat deposition in overweight adolescents. Prevention of overweight which will ultimately lead to obesit, and visceral obesity like deposition of fat in organ such as liver can be reversed by early and prompt

prevention. Daily routine exercise and dietary control can promptly prevent fatty changes occurring in liver in insulin resistant obese adolescents.

Table1
Demographic, physical and Biochemical characteristic of overweight adolescents

Characteristics	Subjects
Sex (Male:female)	1:1
Median Age	14.5
Overweight adolescents	114
Waist circumference (cm)	79.92±5.95
Body mass index (Kg/m2)	27.73±1.87
ALT(IU/L)	40±9.5(95% CI)

Table 2
Parameters among overweight adolescents and healthy controls.

Variable	Normal weight healthy adolescents	Overweight Adolescents	p value
Sex(M/F)	57/57	57/57	NS
Median Age(years)	14	14.5	NS
Overweight/obese BMI(kg/m2) (mean±SD)	19.62±2.44	27.33±1.87**	P<0.001
Waist circumference(inch) (mean±SD)	26.67±1.78	31.47±2.34**	P<0.001
ALT((IU) (Mean+SD)	25±7.4	40±9.5**	P<0.01

***** p value less than 0.05- highly significant***

CONCLUSION

Biochemical markers such as ALT and IR (HOMA-IR) are the important parameters for prediction of presence of hepatic visceral obesity and fatty liver changes in overweight adolescents.

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