A STUDY OF SDLDL-C AND INSULIN RESISTANCE IN APPARENTLY HEALTHY SOUTH INDIAN OBESE YOUNG ADULTS

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ABSTRACT

Background: *Obesity is now occurring in pandemic proportions and has earned a new name as "Globesity" for it. Unlike earlier times developing countries too are facing the challenge of obesity and the modern epidemics have a common root cause tapering to obesity. Obesity during adolescent and young adulthood usually persists to adulthood in almost 50% cases and gives rise to early onset of type 2 diabetes mellitus, cardiovascular disorders and metabolic syndrome for which insulin resistance being the common link to all.*
Objective: *The present study was taken up to study the prevalence of insulin resistance in apparently healthy young adult obese population and study its correlation with different cardiovascular risk factors like lipid profile and sdLDL-C.*
Material and Methods: *In a randomized control study 106 apparently healthy young adults in the age group of 21-34 years were chosen from the community out of which 45 were obese and 61were age and gender matched non-obese controls. They were divided in obese and non-obese groups based on cut-off BMI of 25Kg/m^2. Along with physical parameters fasting plasma glucose, lipid profile and routine biochemical parameters were assayed by standard kit methods and plasma insulin was measured by sandwich ELISA method. Different lipid ratios, atherogenic index and insulin resistance were calculated. Atherogenic index was calculated and insulin resistance was measured by HOMA-IR model and QUICKI index. Small dense LDL-C (sdLDL-C) was quantified by modified Tsutomu –Hirano method.*
Results: *In the obese group BMI, waist circumference (WC) and waist-hip ratio (WHR) elevated significantly (p=0.0001) and TG, VLDL-C and sdLDL-C as well as atherogenic index elevated significantly (p<0.001).Significant Hyperinsulinaemia (p<0.0001) was found in the obese group and 50% of obese cases had hyperinsulinaemia. Insulin resistance calculated by HOMA-IR and QUICKI index was statistically significant (p<0.0001) in obese. Linear regression analysis showed sdLDL-C (R^2=0.08, p=0.05 at 95% C.I.), hyperinsulinaemia (R^2=0.089, p=0.054 at 95% C.I.) and insulin resistance (R^2=0.099, p=0.03 at 95% C.I.) significantly dependent on WC and atherogenic index was significantly dependent on TG (R^2=0.0036, p=0.05 at 95% C.I.) rather than any other lipid factors. On ROC analysis either method of insulin resistance showed equal efficacy (AUC for HOMA-IR= 80.3% and QUICKI = 80.14%; C.I. 95%) and atherogenic index turned out to be a better predictor than sdLDL-C (AUC for Atherogenic index= 76.14% and QUICKI = 71.46%).*
Conclusions: *For Indian subpopulation WC and WHR should also be evaluated along with BMI. Insulin resistance should be identified early and interventional measures should be taken in terms of low carbohydrate protein-rich diet, physical exercise and insulin receptor sensitizers for a short-term. sdLDL-C rises earlier than total cholesterol and hence can be accepted as CVS risk predictor. Not many studies have been done in India on young adult health which is the group that can be targeted for early prevention of the modern epidemics like DM, Metabolic syndrome, CVS disorders and cancers.*

Key words: *Hyperinsulinaemia, insulin resistance, Small dense-low density lipoprotein, Type-2 diabetes mellitus, metabolic syndrome.*

INTRODUCTION

The looming health crisis around the world due to excessive weight gain has earned the epidemic of obesity a new name "Globesity". Obesity which was thought to be the problem of developed countries is now equally shared by the developing countries and both malnutrition and obesity occur within the same countries. In India too obesity has reached epidemic proportions and as other countries, childhood obesity and obesity in youth is becoming more prevalent with little gender difference. As per 2007 National Family Health Survey data Tamilnadu ranked 4th in obesity amongst all states of India [1]. Approximately 50% of obese adolescents with a body mass index at or above the 95th percentile become obese adults [2]. There has been an escalation of

cardiovascular risk factors like dyslipidaemia, hypertension and metabolic syndrome in urban Asian Indian adult population that traces its pathophysiology in childhood and adolescent obesity [3]. More than 30 million overweight children are living in developing countries and 10 million in developed countries [2]. The worldwide prevalence of childhood overweight and obesity increased from 4.2% (95% CI: 3.2%, 5.2%) in 1990 to 6.7% (95% CI: 5.6%, 7.7%) in 2010. This trend is expected to reach 9.1% (95% CI: 7.3%, 10.9%), or 60 million, in 2020 [4]. Young adults, the age group that is defined differently ranging from 16-35 years, are a transition from adolescent to adulthood and health challenges faced during the period is a gateway to many disease processes in the adulthood and hence need special attention.

South Asians have a different tendency for adiposity and have at least 3 to 5% higher body fat for the same BMI as compared to Caucasians. The fat is typically located 'centrally' (i.e. waist, trunk) and around visceral organs which is metabolically more dangerous than peripheral fat [5]. Overall prevalence of obesity has reached 6.8% and for overweight 33.5% in India [6]. Obesity plays a central role in the insulin resistance syndrome, which includes hyperinsulinaemia, hypertension, hyperlipidaemia, type 2 diabetes mellitus, and an increased risk of atherosclerotic cardiovascular disease [3]. The typical dyslipidaemia of obesity consists of increased triglycerides (TG) and FFA, decreased HDL-C with HDL dysfunction and normal or slightly increased LDL-C with increased small dense LDL. A lower amount of cholesterol in LDL generates smaller, denser particles (sometimes referred to as LDL subclass B) known as sdLDL-C. LDL-C and sdLDL-C are susceptible for oxidation and generate oLDL (oxidised LDL). The damage caused by oLDL leads to a cascade of immune response which over time produces foam cells and eventually atheroma [7].

Hyperinsulinemia and insulin resistance are pervasive features of obesity, increasing with weight gain and diminishing with weight loss. Insulin resistance is more strongly linked to intra-abdominal fat than to fat in other depots. The molecular link between obesity and insulin resistance in tissues such as fat, muscle, and liver has been sought for many years. Major factors under investigation include: (1) insulin itself, by inducing receptor down regulation; (2) free fatty acids, known to be increased and capable of impairing insulin action; (3) intracellular lipid accumulation; and (4) various circulating peptides produced by adipocytes, including the cytokines TNF-α and interleukin (IL)-6 , and the "adipokines" Adiponectin and resistin, which are produced by adipocytes, have altered expression in obese adipocytes, and are capable of modifying insulin action. Insulin resistance is associated with hyperglycemia, dyslipidaemia with high TG and low HDL-C, central obesity and hypertension greater than 130/80. Upto 92% Type 2 DM people show insulin resistance and insulin resistance can precede diagnosis of type 2 DM by upto 12 years. But the risk of atherosclerosis is apparently comparable in non-diabetic, insulin-resistant individuals and in those with type 2 diabetes [8].

The present study was taken up to study the prevalence of insulin resistance in apparently healthy young adult obese population and study its correlation with different cardiovascular risk factors like lipid profile and sdLDL-C.

MATERIAL AND METHOD

This study was done in the department of Biochemistry, Vinayaka Mission's Medical College and Hospital Karaikal taking 106 apparently healthy young adults in the age range 21-35 years from the community out of which 56 were obese (25 Kg/m^2); 34 males and 22 females and 50 age and age and gender matched controls; 22males and 28 females. Persons with known endocrinal diseases such as hypothyroidism, PCOS, Cushing's syndrome, DM, any cardiovascular diseases or any chronic illnesses, undergoing treatment for infertility, taking contraceptive pills, any other steroids or any other treatment, smokers and alcoholics and pregnant woman or women breast-feeding were excluded from the study.

A fasting blood sample of 7 ml was collected out of which 1.5 ml blood was collected in EDTA tube for plasma extraction

and rest was allowed to clot. Fasting plasma glucose, Urea, Creatinine and Lipid profile were analysed. **SdLDL-C (small dense LDL-C)** was quantitated by a modified method of Tsutomu Hirano et al [9]. In this method Lipoproteins of density < 1.044 g/ml which includes Very low density lipoprotein (VLDL), Intermediate density lipoprotein (IDL) and large buoyant low density lipoprotein (lbLDL) is precipitated first by a precipitating agent consisting of manganese chloride ($MnCl_2$) and heparin sodium salt and the total cholesterol content in the supernatant is assayed from which value of HDL-C is deducted to obtain the value of small dense Low density lipoprotein-cholesterol (sdLDL-C).

Plasma Insulin was assayed by sandwich ELISA method. Statistical analyses were done by Microsoft Excel and Graph pad prism software. Continuous variables were compared by Student's T-test. Linear regression was applied to identify and characterize different risk factors and individual prognostication. Chi-square test was also applied to study the risk factor association. ROC analysis was done to compare the performance of different indices and predictive value of risk factors.

RESULTS AND DISCUSSION

The study was done with 106 subjects chosen from community after taking their informed consent and obtaining institutional ethical clearance.

Table 1: Comparison of physical parameters of controls and cases

Physical parameters	Controls (n=50) Mean± SD	Cases (n= 56) Mean± SD	t value	p value
Age (years)	21.97± 2.97	22.86±3.32	0.16	0.87
Pulse rate	72.21± 5.72	71.95± 5.46	0.82	0.42
SBP (mmHg)	115± 13.2	122.8±12.2	1.98	0.05
DBP (mmHg)	78±10.2	79.5± 11.2	0.2	0.85
BMI (Kg/m^2)	21.29± 2.39	30.11 ± 3.26	6.37	0.0001
WC (in)	31.88 ± 2.66	37.7 ± 2.88	2.78	0.0065
WHR (females)	0.8 ± 0.07	0.98 ± 0.06	3.49	0.0011
WHR (males)	0.88 ± 0.06	0.99 ± 0.07	9.4	0.0001

Table-1 shows the comparison of the physical factors among the obese and non-obese controls. SBP was significantly higher in obese group. BMI, waist circumference (WC), waist-hip ratio (WHR) was significantly higher in the obese group. In Indian subpopulation there is a tendency of adipose tissue deposition around the visceral organs and hence while evaluating for obesity WC and WHR also should be taken into consideration.

Table-2 Chi-square table showing association of obesity with F/H

F/H of Obesity	Obese	Non-obese	
Yes	30	23	OR=1.35
No	26	27	P=0.25

Table-2 shows that family history of obesity was not significantly associated with the present obese group that were studied. Though genetic defects and different syndromes are associated with early-onset morbid obesity, they are extremely rare. Since in the present study group none of the subjects were found to be morbidly obese and hence family history may not play an important role in development of obesity. However, in the causation of obesity environmental and dietary factors weigh more than genetic factors [10].

Table-3 Comparison of Lipid profile, lipid ratios between controls and cases

Biochemical parameters (mg/dl)	Controls (n=50) Mean± SD	Cases (n= 56) Mean± SD	t value	p value
TC	146.6 ± 31.24	141.51 ± 31.08	0.66	0.5
TG	106.75 ± 34.3	147.25 ± 40.43	6.43	0.0001
HDL-C	51.2± 11.9	48.58± 13.47	0.3	0.76
VLDL-C	21.35± 6.86	29.43 ± 7.9	4.65	0.0001
LDL-C	74.05± 32.23	73± 33.28	0.08	0.94
sdLDL-C	38.21 ± 14.11	55.75 ± 26.3	2.5	0.01
NHDL-C	95.4 ± 31.87	92.94± 27.2	0.67	0.5
LDL/HDL-C	1.58 ± 0.86	1.62 ± 0.96	0.29	0.77
TC/LDL-C	2.18 ± 0.83	2.46 ± 0.79	0.08	0.94
NHDL/ LDL-C	1.34 ± 0.23	1.6 ± 0.45	0.01	0.99
Atherogenic index	0.31± 0.19	0.48± 0.16	2.38	0.0001

Table-3 shows TG, VLDL-C and sdLDL-C to be significantly higher in obese group. Atherogenic index was also was significantly high. Researchers have shown that obesity increases cardiovascular risk through risk factors such as increasing fasting triglycerides, high LDL-C, low HDL-C, elevated blood glucose and insulin level in blood and high blood pressure [11]. In the present study however NHDL-C or no other lipid ratios were significantly altered in the obese group. A significant rise in atherogenic index (p=0.0001) shows the impending cardiovascular risk in the obese group. Obesity leads to dyslipidaemia which leads to atherogenic changes over time, one of the factor being inhibition of clearance of LDL-C and small dense LDL-C (sdLDL-C) through LDL scavenging pathway that leads to oxidation of LDL-C and deposition for atheromatous plaque formation. sdLDL-C gets deposited in the arterial wall and escapes the scavenging pathway due to its size and over time its ApoB is modified and serves as a ligand for the scavenger receptors of monocyte and macrophage. Cholesterol accumulates and gives rise to foam cells and in the long run atherosclerosis. In Framingham offspring study sdLDL-C was found to be elevated in CHD patients [12]. In this study since most of the subjects are in early young adulthood yet subtle changes of lipid profile and atherogenicity has been observed, interventional measures should be started in forms of dietary modification and physical exercises.

Table-4 Comparison of plasma insulin and insulin resistance in the study groups

Parameters	Controls (n=50) Mean± SD	Cases (n= 56) Mean± SD	t value	p value
Insulin (µIU/ml)	11.58 ± 7.45	18.39± 7.8	2.87	0.005
IR (HOMA-IR)	2.09± 1.03	3.63±1.68	9.08	0.0001
IR (QUICKI)	0.32± 0.02	0.35±0.03	3.86	0.0002

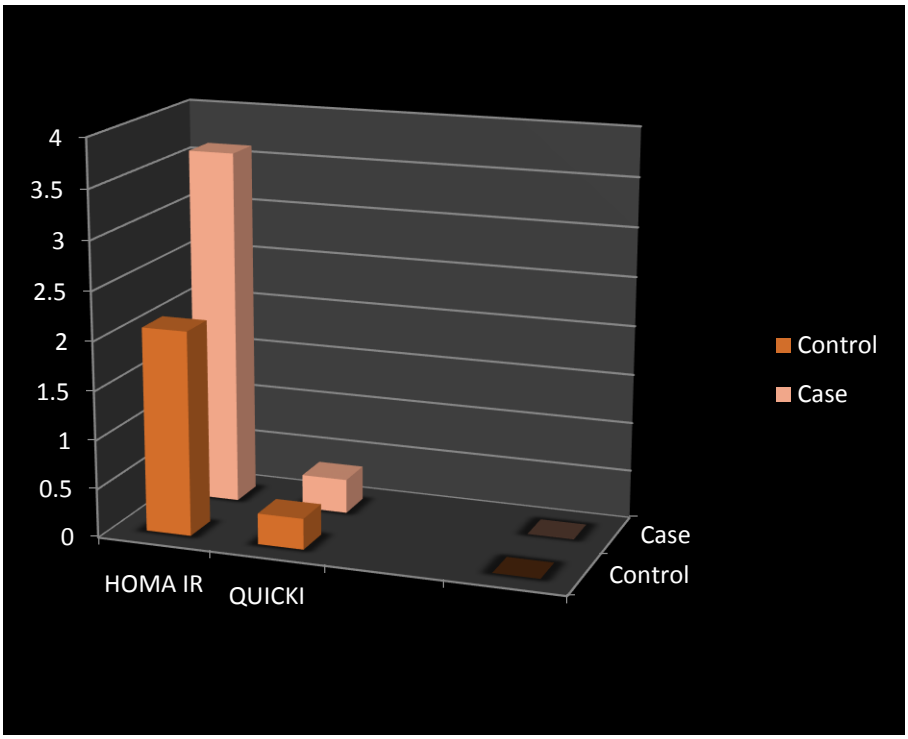

Fig-1 Comparison of IR by HOMA-IR and QUICKI

Obese group had statistically significant hyperinsulinaemia and insulin resistance (Table-4) and almost 50% of the obese had moderate to severe hyperinsulinaemia (Table-5). Different researchers have found that with mild to moderate exercise insulin level decreases and insulin resistance improves. Abdominal adipose tissue is resistant to insulin action for lipolysis and leads to increased FFA level in blood. That leads to TG accumulation and hepatic insulin resistance [13]. With

Table-5 Level of plasma insulin in obese group

Plasma insulin	**n= 56**	**percentage**
Normal (<10 IU/ml)	30	53.5%
Mild hyperinsulinaemia (10-14 IU/ml)	12	21.4%
Mod/severe hyperinsulinaemia (>14 IU/ml)	14	25%

Table-6 shows linear regression of waist circumference as independent variable with other parameters out of which sdLDL-C, insulin and insulin resistance by both HOMA-IR and QUICKI index were found to be significantly dependent on waist circumference. Anoop Misra et al have found that WC cut-off point should be lowered for Indian ethnic population as they develop higher morbidity at lower WC [14].

Table-6 Comparison of WC as independent variables with TC, TG, LDL-C, atherogenic index, insulin and insulin resistance by linear regression

Dependent variables	R^2	p
TC	0.023	0.32
TG	6.81	0.99
LDL-C	0.05	0.15
sdLDL-C	0.08	0.05
Atherogenic index	0.16	0.7
Insulin	**0.09**	**0.04**
HOMA-IR	**0.099**	**0.03**
IR by QUICKI index	**0.099**	**0.04**

Atherogenic index was statistically significantly dependent on TG but however, the linear regression graph shows positive linearity with insulin, insulin resistance and atherogenic index (Fig.2).

Fig. 2 Linear regression model taking TG as independent variable

CONCLUSIONS

It was found that in early young adulthood though there is no overt pathology, there has been undercurrent biochemical and hormonal changes. TG and sdLDL-C being the earliest alteration in lipid profile a dietary shift from carbohydrate-rich one to protein predominant one and physical exercise are highly recommended. While evaluating and treating obesity, insulin and insulin resistance should be taken into consideration as through a complex interplay of events insulin resistance leads to development of dyslipidaemia and CHD and a short-term treatment with insulin receptor sensitizers may be included in the therapy.

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