Significance of hs-CRP and Oxidative Stress as Early Novel Markers of Subclinical Atherosclerosis in Young Healthy Obese Males

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Abstract:
This study was conducted to assess the significance of early novel markers of sub-clinical atherosclerosis in the young healthy obese males, thereby predicting the future risk of cardiovascular diseases and other obesity related disorders. The control group was comprised of 60 healthy adult non-obese males between 20-70 yrs and 60 age-matched healthy adult obese males comprised the study group. Study group was further divided into 20-29 yrs, 30-39 yrs, 40-49 yrs and 50-59 yrs age groups. Anthropometric measurements (Body Mass Index, Waist to Hip ratio) and biochemical estimations (blood glucose, high sensitive C-reactive protein, lipid profile, oxidative stress markers like serum malondialdehyde and total antioxidant capacity) were carried out on all groups. There were significant increases in Body Mass Index, Waist-to-Hip ratio, malondialdehyde and hs-CRP levels in obese group compared to non-obese controls with advancing age and significant decrease in total antioxidant capacity in obese individuals compared to non-obese controls with advancing age. Negative correlations were observed between total antioxidant capacity and waist-to-hip ratio and between malondialdehyde and total antioxidant capacity in all age groups. Positive correlations were observed between malondialdehyde and waist-to-hip ratio in all age groups. Thus, hs-CRP and precise oxidative stress markers might be useful as early novel markers of subclinical atherosclerosis, for identifying subjects at higher risk for obesity related co-morbidities and therefore would benefit from preventive interventions.
Key words: *obesity; waist-to-hip ratio; body mass index; hs-CRP; MDA; total antioxidant capacity.*

Introduction:

Obesity is associated with a state of chronic low grade inflammation which brings about alterations in adipose tissue metabolism and endocrine function leading to an increased release of hormones and proinflammatory molecules that contribute to associated complications of obesity, including metabolic syndrome, type-2 Diabetes mellitus and cardiovascular disorders (CVD) [1]. Oxidative stress is an imbalance between injurious oxidant and protective antioxidant events in which the former predominate. An overwhelming body of evidence indicates that oxidative events including modification of proteins and DNA, alteration in gene expression, promotion of inflammation, and deterioration in endothelial function in the vessel wall either trigger or exacerbate the atherosclerotic process in obese individuals [2]. There are very limited studies on the relationship of oxidative stress markers and markers of preclinical atherosclerosis in young healthy obese individuals [3,4]. Hence, this study was conducted to assess the significance of early novel markers of sub-clinical atherosclerosis in the young healthy obese group before the manifestation of dyslipidemia, thereby predicting the future risk for cardiovascular diseases, metabolic syndrome and other obesity related disorders.

Materials and Methods:

This study was conducted at the Endocrinology Department, M.S. Ramaiah Memorial Hospital, Bangalore, and at Sri Siddhartha Medical College and Research centre, Tumkur on 60 healthy adult non-obese males between 20-70 yrs with BMI <23Kg/ m^2 (controls) and 60 age-matched healthy adult obese males with BMI >23 Kg/m^2 (study group). Patients of both groups were normotensive and normoglycemic. The cut-off value of BMI between non-obese and obese group was taken as 23 Kg / m^2 based on the WHO recommendations for Asians [5]. Both controls and the study group were selected after obtaining informed consent from them. In both the groups, those with diabetes mellitus, other endocrinal disorders, smoking, hypertension, cardiac diseases, bronchial asthma, acute or chronic inflammatory diseases, autoimmune diseases and on other medications like steroids, antipsychotic drugs were excluded from the study.

The following anthropometric parameters were recorded for non-obese and obese group- Weight, Height, Waist

circumference, Hip circumference, BMI (body weight in Kg/height in m^2) and Waist to hip ratio (waist circumference / hip circumference).

Under strict aseptic precautions, 5 ml of venous blood sample from antecubital vein were collected after overnight fasting from non-obese and obese subjects, in appropriate vacutainers. Blood samples were taken again 2 hrs after breakfast to measure post prandial blood sugar. The sample was then centrifuged and serum was separated for the determination of the fasting blood glucose, post prandial blood glucose, lipid profile and high-sensitivity C - reactive protein. Estimation of serum High-sensitivity C-Reactive protein (hs-CRP) was carried out by turbidimetry latex-high sensitivity kit (Biosystems S.A. Costa Brava, Barcelona [Spain]) method [6]. Blood glucose was assayed by the glucose oxidase kit (Vital Diagnostics Pvt. Ltd, Mumbai) method [7], serum cholesterol by the end point enzymatic kit (Bio systems, S.A Barcelona [Spain]) method [8], HDL-cholesterol by phosphotungstate Precipitation kit (Bayer Diagnostics, Baroda) method [8] and serum triacylglycerol by glycerol phosphate oxidase kit (vital diagnostics Pvt Ltd, Mumbai) method [9] . LDL and VLDL were calculated from the estimated values of cholesterol, triglyceride and HDL-C, using the equation of Frieldwald et al[9] as given below.

[LDL- Cholesterol] = [Total Cholesterol] – [HDL-C] – [Triglyceride]/5. (The factor [Triglyceride]/5, is an estimation of VLDL-Cholesterol). Lipid peroxidation was measured by serum MDA estimation according to the colorimetric method of Satoh.k [10]. Total antioxidant capacity was measured by ferric reducing antioxidant power assay (FRAP assay) according to the method of Benzie.F.F. and J.J.Strain [11].

Statistical Analysis:

Values were expressed as Mean ± SD. Statistical comparisons were carried out by student't' test and ANOVA tests. Correlations were done by calculating Pearson's correlation coefficient. All statistical analysis were done at 5% level of significance using SPSS software, version 16.

Results:

The BMI and W/H ratio were significantly elevated in obese group compared to the control group. FBS, PPBS and lipid profile were in the normal range. There was a significant increase in FBS in the obese group when compared to healthy non-obese group. There was significant increase in total cholesterol, triacylglycerol, LDL and VLDL levels in obese group compared to the healthy non-obese group but there was no significant change in HDL levels. There was a significant increase in Hs-CRP in the obese group compared to healthy non-obese controls. There was significant increase in MDA levels and significant decrease in TAC levels in obese group compared to healthy non-obese controls [table 1].

Among 20-29 yrs age group, there were significant increase in BMI, W/H, MDA and Hs-CRP levels in obese persons and significant decrease in TAC in obese individuals compared to non-obese controls [table 2]. A significant negative correlation was observed between TAC and W/H and between MDA and TAC [table 4].

Among 30-39 yrs age group, there were significant increase in BMI, W/H, MDA and Hs-CRP levels in obese persons and significant decrease in TAC in obese individuals compared to non-obese controls [table 2]. Significant negative correlation was observed between TAC and W/H [table 4].

Among 40-49 yrs age group, there was significant increase in BMI, W/H, MDA and Hs-CRP levels in obese and significant decrease in TAC in obese individuals compared to non-obese controls [table 2]. Significant negative correlations were observed between TAC

and W/H and between MDA and TAC [table 4].

Table 1: Comparison of anthropometric and biochemical parameters between obese and control Group

	Group	N	Mean	'p' value
BMI	**Obese**	60	31.3±4.5	<0.05
	Non Obese	60	20.2±1.9	
W/H	**Obese**	60	0.9±0.07	<0.05
	Non Obese	60	0.86±0.05	
FBS	**Obese**	60	92.4±13.5	<0.05
	Non Obese	60	82.6±10.2	
PPBS	**Obese**	60	108.2±14.6	>0.05
	Non Obese	60	105.9±12.6	
TC	**Obese**	60	181.7±19.4	<0.05
	Non Obese	60	170±19.4	
TGL	**Obese**	60	156.4±27.3	<0.05
	Non Obese	60	147.4±.03	
LDL	**Obese**	60	108.9±17.2	<0.05
	Non Obese	60	99±18.3	
HDL	**Obese**	60	41.5±3.9	>0.05
	Non Obese	60	41.4±2.1	
HsCRP	**Obese**	60	8.9±2.9	<0.05
	Non Obese	60	3.1±1.6	
MDA	**Obese**	60	2.4 ± 1.1	<0.05
	Non Obese	60	1.1± 0.9	
TAC	**Obese**	60	928.9±32.9	<0.05
	Non Obese	60	1353.1±31.6	

Among 50-59 yrs age group, there was significant increase in BMI, MDA and W/H ratio in obese persons and significant decrease in TAC in obese individuals compared to non-obese controls [table2]. Significant negative correlations were observed between TAC and W/H and between MDA and TAC. Significant positive correlation was observed between MDA and W/H [table 4].

Significant positive correlation was observed between hs-CRP and W/H among all the age groups [table 4].

Inter-group comparisons among obese individuals showed BMI, W/H ratio, TAC and Hs-CRP levels decreasing progressively with increasing age. MDA levels were increasing progressively with increasing age [table 3].

Discussion:

Obesity is considered as a low-grade inflammatory condition with elevated proinflammatory molecules like TNF-α, IL-6 and CRP, derived from the infiltration of macrophages into the adipose tissue [12].

BMI is a measure of relative weight and is largely independent of height. BMI doesn't reflect obesity, but rather mass, and is not a measure of central adiposity and cardiovascular risk. The waist-to-hip ratio (WHR) is independently associated with prevalent atherosclerosis and is a better discriminator of subclinical disease than other common measures of obesity, such as body-mass index (BMI) or waist circumference alone [13]. WHR is an indexed value to lower body girth and provides a more precise assessment of relative central adiposity (visceral obesity) across the body sizes compared with waist circumference. Visceral obesity is associated with metabolic abnormalities that increase the risk of type 2 diabetes mellitus and atherosclerosis in the coronaries and the aorta. Normal reference value for WHR is <0.85 in females and <0.95 in males [14]. In the present study, WHR showed significant increase in obese

Table 2: Age-related intra-group comparison of anthropometric and biochemical parameters between obese individuals and non-obese controls

	Group	20-29 YRS			30-39 YRS			40-49 YRS			50-59 YRS		
		N	Mean ±S.D	p	N	Mean ±S.D	p	N	Mean ±S.D	P	N	Mean± S.D	P
BMI	Obese	9	32.6± 5.7	<0.05	29	30.8± 4.09	<0.05	14	31.7± 5.0	<0.05	8	30.8 ±4.0	<0.05
	Non Obese	25	20.3± 1.9		28	19.8± 1.9		4	22.1± 1.3		3	19.9 ±2.9	
W/H	Obese	9	0.87± 0.06	<0.05	29	0.89± 0.08	<0.05	14	0.93± 0.06	<0.05	8	1.1± 0.05	<0.05
	Non Obese	25	0.75± 0.05		28	0.8± 0.05		4	0.8± 0.05		3	0.85 ± 0.07	
Hs CRP	Obese	9	10.4± 1.9	<0.05	29	8.9± 2.9	<0.05	14	8.9± 2.9	<0.05	8	.9± 0.06	>0.05
	Non Obese	25	3.19± 1.6		28	3.2± 1.8		4	2.1± 0.7		3	2.8± 0.05	
MDA	Obese	9	1.4±1. 9	<0.05	29	1.9± 0.7	<0.05	14	2.3± 0.9	<0.05	8	2.6± 0.6	<0.05
	Non Obese	25	0.78 ± 0.12		28	0.83± 0.11		4	0.99 ± 0.11		3	1.2 ± 0.11	
TAC	Obese	9	1050± 40.2	<0.05	29	1038± 32.9	<0.05	14	996± 36.4	<0.05	8	913 ± 36.8	>0.05
	Non Obese	25	1215.1 ± 55.5		28	1103.6 ± 86.5		4	1024.5 ± 88.4		3	935 ± 38.2	

group when compared with non- obese group.

Obesity alters metabolic and endocrine functions of adipose tissue and leads to an increased release of fatty acids, hormones and pro-inflammatory molecules that contributes to obesity associated complications[15]. The fasting and postprandial blood glucose levels were within normal limits in obese. However fasting blood glucose levels was higher in obese persons when compared to non-obese controls. In obesity, there is altered glucose metabolism, which may be due to significantly reduction in the insulin sensitivity and impaired fasting glucose levels in obese[16]. This shows that obese are predisposed to develop metabolic syndrome. The lipid profile, which included Total Cholesterol, triacylglycerol, HDL-C, LDL and VLDL, were within normal limits showing that obese subjects in this study were not dyslipidemic. However Total Cholesterol, triacylglycerol, LDL and VLDL were increased in obese compared to non-obese. Hypertriglyceridemia in obesity is due to

Table 3: Age related inter-group comparison of anthropometric and biochemical parameters in obese patients

	20-29 yrs N = 9	**30-39 yrs N = 29**	**40-49 yrs N = 14**	**50-59 yrs N = 8**	**P value**
	MEAN ± SD	**MEAN ± SD**	**MEAN ± SD**	**MEAN ± SD**	**Comparison**
BMI	32.6±5.7	**30.7**±4.1	31.7±5.0	30.8±**4.07**	>0.05
WAIHIP	0.87± 0.06	0.89±0.08	0.93± 0.06	1.1± 0.05	<0.05
FBS	96.7±12.4	89.8±12.6	93.2±13.4	95.5±**18.2**	>0.05
PPBS	110.2±11.6	106.7±15.7	109.7±13.5	**108.7**±17.7	**>0.05**
TC	189.4±10.9	180.1±20.5	177.5±21.1	186.1±**19.2**	>0.05
TGL	162±18.9	157±27.1	151.8±35.4	156.1±**23**	>0.05
LDL	100.2±12.3	101±13.0	109.5±13.2	115±18.9	<0.05
HDL	39.8±3.3	41.2±2.7	44.1±5.9	40.1±**2.3**	<0.05
MDA	0.78 ± 0.12	0.9 ± 0.11	0.93 ± 0.14	1.2 ± 0.11	<0.05
TAC	1050 ± 40.2	1038 ± 32.9	996±36.4	913 ± 36.8	<0.05
HSCRP	10.4±1.9	8.9±2.9	8.9±2.9	**7.6±3.6**	**>0.05**

the dysregulation of fatty acid synthesis, esterification and lipoprotein hydrolysis, leading to dyslipidemia and contributes greatly to the metabolic syndrome [17]. This shows that obese individuals with higher BMI tend to have altered lipid profile levels.

As the degree of obesity increases there is greater infiltration of macrophages into the adipose tissue and these macrophages are important sources of inflammation in the adipose tissue [12]. The nature of the atherosclerotic process can be described as an ongoing inflammatory process where fatty streaks, formed by lipoprotein

Table 4: Age related correlations between anthropometric and biochemical parameters in obese patients

4A. 20-29 Yrs age, obese group

PARAMETERS	R VALUE	p VALUE
TAC and W/H	-0.01	<0.05(significant)
MDA and W/H	0.24	>0.05
MDA and TAC	-0.01	<0.05(significant)
CRP and W/H	0.01	<0.05(significant)

4B. 30-39 Yrs age, obese group

PARAMETERS	R VALUE	p VALUE
TAC and W/H	-0.01	<0.05(significant)
MDA and W/H	0.09	>0.05
MDA and TAC	- 0.12	>0.05
CRP and W/H	0.01	<0.05(significant)

4C. 40-49 Yrs age, obese group

PARAMETERS	R VALUE	p VALUE
TAC and W/H	-0.01	<0.05(significant)
MDA and W/H	0.17	>0.05
MDA and TAC	- 0.01	<0.05(significant)
CRP and W/H	0.01	<0.05(significant)

4C. 50-59 Yrs age, obese group

PARAMETERS	R VALUE	p VALUE
TAC and W/H	-0.01	<0.05(significant)
MDA and W/H	0.01	<0.05(significant)
MDA and TAC	- 0.01	<0.05(significant)
CRP and W/H	0.01	<0.05(significant)

oxidation, are one of the earliest changes seen. CRP release from the liver is promoted by IL-1, IL-6 and TNF-α as an acute-phase reactant in response to inflammation. CRP may directly promote atherosclerosis and endothelial inflammation by attenuating the release of NO, a key molecule in the endothelium that plays a pivotal role in the maintenance of vascular tone. The levels of serum CRP usually amount to several hundred milligrams/l. In contrast, a cut-off limit level >3 mg/l indicates low-grade inflammation, which in turn might be a prognostic marker for further cardiovascular events [18]. In the present study, high Hs-CRP levels in obese subjects in the age group 20-29 yrs than non-obese controls of the same age group, with normal lipid profile, with significant positive correlation with WHR, may suggest the ongoing inflammation contributing to the pathogenesis of atherosclerosis, from the initiation of fatty streak of acute coronary syndromes [16,18].

MDA is a three carbon, low molecular weight aldehyde that can be produced from free radical attack on polyunsaturated fatty acids of biological membranes. The determination of MDA is used for monitoring lipid peroxidation in biological samples[19]. FRAP assay is considered as a useful indicator of the system's ability to regulate the damage due to ROS and thus, a novel method of assessing total antioxidant capacity as the individual antioxidant components may not fully reflect the protective efficiency of blood, probably because of interactions that occur

in vivo among different antioxidant compounds [20] . Although there is the strong etiologic link between increased vascular oxidative stress and subsequent development of atherosclerosis in experimental studies, clinical confirmation of this phenomenon has been impeded by the absence of reliable measures of oxidative stress in humans, especially those that will be predictive of early stages of atherosclerosis [21,22]. In the present study, increase in MDA levels and decrease in TAC levels suggest oxidative stress which correlates positively with obesity (WHR).

Although studies have shown positive correlation between adiposity and inflammatory markers like CRP, TNF-ά, IL-6 in obese subjects in middle-aged and elderly subjects with or without metabolic syndrome, the present study is the first of its kind which have focussed on predicting future risk of CVD, through hs-CRP estimation and precise oxidative stress markers in apparently healthy young adults in India.

Conclusion:

The present study suggests that obesity is associated with pro-inflammatory changes as evident by increase in hs-CRP levels in young obese healthy adults. Increase in malondialdehyde levels and decrease in total antioxidant capacity suggest oxidative stress which correlates positively with obesity (waist-to-hip ratio). Thus, hs-CRP and precise oxidative stress markers might be useful as early novel markers of subclinical atherosclerosis, for identifying subjects at higher risk for obesity related co-morbidities and therefore would benefit from preventive interventions.

Study limitations:

The observed correlation between the marker of oxidative stress and atherosclerosis may imply either that oxidative stress is a precipitating factor for development of atherosclerosis, or that atherosclerosis causes elevation of these markers. Moreover, we were unable to study the relationship between the markers of oxidative stress and temporal progression of atherosclerosis or occurrence of future cardiovascular events. These issues need to be further addressed in other populations, including those with more advanced disease.

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