

Serum high sensitivity C-reactive protein levels in metabolically healthy obese individuals versus lean type 2 Diabetes Mellitus patients

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Abstract

Background: State of low-grade systemic inflammation is associated with obesity as well as T2DM. But not all obese develop T2DM and not all T2DM patients are obese. This paradox signifies that obesity is not sole determinant of its metabolic complications. The subset of obese individuals who are normotensive, normoglycemic having favorable lipid profile but excess adiposity is known as “Metabolically healthy obese”. C-reactive protein is an acute phase reactant protein synthesized by hepatocytes in response to several cytokines.

Material and Methods: Study aimed to evaluate and compare levels of serum hsCRP in MHO (group I) and lean T2DM (group II) and controls (group III). In this case control study, we enrolled 50 lean patients of T2DM and 50 age and sex-matched MHO individuals. Demographic data, anthropometric measurements, BMI were recorded. After overnight fast, blood samples were collected and assayed for blood glucose, serum cholesterol, triglycerides, LDL, HDL, TG/HDL and hsCRP.

Results: BMI was significantly higher in-group I ($p=0.031$) in comparison with group II and III. Blood sugar, TC, TG, LDL and hsCRP showed significant rise ($p=0.039, 0.024, 0.028, 0.0008$ respectively) and HDL ($p=0.027$) was significantly decreased in group II while these values were normal in group I and III. Diabetic dyslipidemia was found in 64% (32 out of 50) of the cases. Significant positive correlation of hsCRP was observed with TG, LDL and TG/HDL ($r=0.68, 0.69, 0.8, 0.81$) while inverse correlation was seen with serum HDL ($r=-0.74$) in T2DM group.

Conclusion: Our study findings demonstrate adverse metabolic health of lean T2DM is more dangerous, proatherogenic than that of MHO individuals. Despite of low or normal body weight, T2DM group has unfavorable inflammatory status in comparison with metabolically healthy obesity.

Key words: hsCRP, metabolically healthy obesity, T2DM, inflammation, lipid profile.

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Introduction

Exponential rise in the prevalence of Type 2 Diabetes Mellitus (T2DM) is imposing a major health-care burden globally. There is upsurge in the cases of T2DM with lifestyle transition in Asia, especially India and China being the leading countries contributing to epidemics of T2DM. Even though obesity is a well-known risk factor for the development and progression of T2DM, interestingly 60-80% patients are non-obese.^[1] Hence concept of lean diabetes including underweight or normal weight diabetes mellitus patients has emerged. In India, clinical profile and pattern of T2DM is different from Western counterparts.^[2] One of the multicenter Indian studies indicated approximately 11-25% prevalence of lean T2DM. This proportion depends on the type of population, geographical region and ethnicity. Clinical profile and complications related to DM are different in lean and obese T2DM patients.^[3]

T2DM is a state associated with insulin resistance, which could stimulate hepatic synthesis of CRP, by attenuating effect of insulin on formation of acute phase reactant proteins.^[4] Exact pathogenic mechanism of lean DM is not clear, smaller beta mass may be result of adverse intrauterine or early postnatal environment with malnutrition.^[5]

Obesity is a complex multifactorial disease caused by chronic surplus energy when energy intake exceeds energy expenditure resulting in accumulation of adipose tissues. It is a risk factor for various non-communicable diseases like coronary heart diseases (CHD), stroke, T2DM, metabolic syndrome, certain types of cancers, and sleep apnea disease. But not all obese individuals develop such chronic diseases and metabolic disturbances like dyslipidemia and dysglycemia. This subset of obese individuals who are normotensive, normoglycemic having favorable lipid profile but excess adiposity is known as “Metabolically healthy obese” (MHO). Among obese population, as much as 20-30% obese individuals may be of MHO phenotype.^[6] One Asian Indian study reported 13.3% prevalence of MHO type of phenotype.^[7]

State of low-grade systemic inflammation is associated with obesity as well as T2DM. But not all obese develop T2DM and not all T2DM patients are obese. This paradox signifies that obesity is not the sole

determinant of its metabolic complications.^[8] There is a unique subset of MHO people free from obesity related metabolic disturbances and are protected from the complications of obesity. Because of favorable metabolic profile, MHO individuals do not display clustering of cardiovascular and metabolic risk factors.^[9] So we hypothesized that MHO individuals may have favorable inflammatory status in comparison with lean T2DM patients.

C-reactive protein is an acute phase reactant protein synthesized by hepatocytes in response to several cytokines. It is a pentamer of identical subunits and nonspecific but stable and extremely sensitive marker of systemic inflammation.^[10] Data is obscure about association of serum hsCRP with MHO individuals who are resistant to develop adiposity-associated cardio-metabolic abnormalities and lean T2DM who are at high risk of cardio-metabolic disorders.

Present study was aimed to evaluate and compare levels of serum hsCRP in MHO and lean T2DM individuals. We also intended to explore effect of BMI on inflammatory status in obese without T2DM versus lean T2DM individuals.

Material and Methods

Present study was conducted in the department of Biochemistry, Government Medical College Aurangabad in collaboration of department of Medicine. Study was carried out as per the guidelines of Institutional Ethics Committee. In this Out Patient Department based case control study, we enrolled 50 lean patients of T2DM and 50 age and sex-matched MHO individuals. Age group of controls and cases was 18 to 50 years. MHO subjects were selected by the criterion: obese (BMI < 25) but normotensive, non-diabetics, having fasting triglycerides (TG) and high-density lipoprotein cholesterol (HDL) within normal limit. They were not using any medications like antihypertensive, lipid lowering, anti-inflammatory and hypoglycemic agents. Patients of lean and normal weight T2DM were selected on the basis of BMI less than 25 kg/m². Individuals with complications of T2DM, smokers, hypertension, acute and chronic inflammatory diseases, other endocrinology disorders, malignancy and major illness were excluded from the study group. Also patients on steroids, statins and NSAIDs were excluded. Informed consents were obtained from the participants and confidentiality of data assured.

After thorough physical examination, in detail medical history, treatment modalities, demographic data and anthropometric measures were recorded. Anthropometric measurements including body weight (Kg), height (m) and waist circumference (cm) were recorded. Body Mass Index (BMI) was calculated as Body weight (Kg)/ Height (m)². Eligible participants

were invited to give blood samples after overnight fast and 5 ml venous blood samples were drawn in fluoride and plain bulbs. In clinical laboratory of Biochemistry department, blood samples after separation of serum were assayed for biochemical parameters on fully automatic chemistry analyzer XL 640 ERBA TRANSASIA. Blood glucose levels were estimated by Glucose oxidase-peroxidase method. Serum total cholesterol (TC) was estimated by Cholesterol oxidase peroxidase method, TG by glycerol-3 phosphate oxidase-3,5-dichloro-2-hydroxybenzenesulfonic acid method, and HDL by direct method-polyethylene glycolpretreated enzymes using commercial kits from Erba kits. LDL was calculated by –Formula as: LDL= TC-(TG/5 + HDL) We calculated TG/HDL ratio, which is an indirect marker of insulin resistance. Serum hsCRP was estimated by chemiluminescence immunoassay using master-kit from Acculite CLIA microwells with Assay kits from Monobind INC, Lake forest, CA 92630 USA.

Statistical Analysis: Data analysis was carried out using SPSS 11.0 statistical analysis software. Continuous variables were calculated by descriptive statistics and reported as mean +/- standard deviation. We compared these variables across studied groups by one-way analysis of variance (ANOVA). Pearson's correlation analysis was used to calculate correlation coefficients to study association between serum hsCRP and various metabolic parameters.

Results

Demographic, clinical and biochemical characteristics of study population are depicted in the (Table 1). Age, systolic, diastolic blood pressures did not show any significant differences in 3 groups. BMI was significantly higher in-group I in comparison with group II and III while group II and III did not differ from each other. Biochemical parameters blood sugar, TC, TG, LDL and hsCRP differed significantly in-group I and II. Biochemical parameters blood sugar, TC, TG, LDL and hsCRP showed significant rise and HDL was significantly decreased in lean and normal weight T2DM subjects while these values were normal in MHO and control groups. Diabetic dyslipidemia was found in 64% (32 out of 50) of the cases.

Correlation analysis was performed (Table 2) to study correlation of serum hsCRP with TG/HDL ratio, which is an indirect marker of insulin resistance in T2DM group. Significant positive correlation of hsCRP was observed with BMI, TG, LDL and TG/HDL while inverse correlation was seen with serum HDL in T2DM group.

Table 1: Demographic, clinical and biochemical variables of study participants

Variables Mean+/-S.D.	Group I (MHO) n= 50	Group II Lean normal weight T2DM n=50	Group III Controls n=50
Age (years)	46.5+/- 5.7	44.9 +/- 6.8	43.9+/-4.2
Sex (Male:Female)	28:22	30:20	24:26
BMI (kg/m ²)	31.2+/-3.2*	21.4+/-3.8	21.1+/-2.6
Fasting blood sugar (mg/dl)	81.5+/-12.3	116.8+/-22*	75.3+/-11.6
Serum TC (mg/dl)	137.8+/-22.8	194.3+/-42*	128.8+/-19.6
Serum TG (mg/dl)	93.8+/- 17.5	198.9+/-37*	87.98+/-17.89
Serum LDL (mg/dl)	111.8+/-16.8	145.9+/-32.8**	98.89+/-21
Serum HDL (mg/dl)	49.8+/-8.9	35.8+/-10.8*	52.76+/-9.8
Serum hsCRP (mg/dl)	3.3+/-1.56*	5.3+/-2.8**	1.23+/-0.76
TG/HDL ratio	1.88+/-0.3	5.55+/-1.78**	1.66+/-0.26

statistically significant p value < 0.05, ** highly significant p< 0.001

Table 2: Pearson Correlation Analysis (r Values) of hsCRP with biochemical variables in lean T2DM group

	TC	TG	LDL	HDL	TG/HDL
hsCRP r=	0.64	0.69	0.8	-0.74	0.81
p=	0.039	0.024	0.028	0.27	0.0007

Discussion

Present study compared serum hsCRP levels in controls, MHO and lean T2DM groups and assessed its correlation with BMI and metabolic health profile. We observed favorable metabolic and inflammatory profile among MHO individuals in spite of significantly high BMI in comparison-deranged profile in lean and normal weight T2DM patients with normal BMI. We did not find statistically significant difference in metabolic profile of MHO in comparison with controls. But BMI was significantly high in MHO group. Metabolic and cardiovascular risk profile of MHO is intermediate between T2DM patients and healthy normal weight controls. Our findings concur with certain previously published data. Fronczyk and associates studied CRP concentration in obese DM patients and its association with complications. They reported strong positive correlation between CRP and BMI in obese diabetics.^[11]

Lean T2DM is a variant of classical T2DM with good insulin C-peptide reserve without autoimmune destruction of beta cells.^[1] In T2DM patients, hyperglycemia associated with deranged metabolic profile and adipocytes activate immune cells to release cytokines, which modulates inflammatory reactions. Non-diabetic obesity is also associated with sub minimal inflammation suggesting role of adipocytes in initiating and sustaining state of low-grade inflammatory state.^[11] State of subclinical inflammation has also been proposed as one of the mechanism for pathogenesis of T2DM.^[12]

One study from India observed 3.5% lean (BMI<18.5%) and 63% ideal body weight individuals in a cohort of 10,000 T2DM patients with male preponderance.^[13] Deepa M et al recently compared

metabolic profile of lean persons with dysglycemia versus centrally obese people without dysglycemia in a cohort of 2350 subjects from CURES epidemiological study. They found that dysglycemia was associated with a worse cardio-metabolic profile in the form of deranged lipoprotein pattern with raised inflammatory markers than central obesity alone.^[14] But in the group of lean subjects with dysglycemia, more smokers were found. In our study, we excluded smokers from our all three groups as smoking itself results in insulin resistance and inflammatory status. Al-Hamodi et al compared altered adipokines and inflammatory markers in obese and non-obese T2DM individuals. They reported significantly raised serum hsCRP in both groups in comparison with controls. But T2DM subjects with normal weight had higher serum hsCRP in comparison with obese subjects.^[12] Accumulation of fat in adipocytes leads to its hypertrophy resulting in release of proinflammatory cytokines. This promotes infiltration of immune cells and exacerbation of inflammatory reactions.^[8]

Karelis AD et al investigated inflammatory status of postmenopausal obese women. Out of 88 obese women, 22 were MHO with significantly lower levels of visceral fats, fasting insulin, TG, CRP, α -1 antitrypsin and higher levels of HDL (p<0.05).^[15] Durwad et al studied all-cause mortality risk of MHO individuals in NHANES III and identified 40 MHO individuals out of 1160 obese by different definitions of metabolic health. MHO cases had superior levels of risk factors in comparison with unhealthy obese while MHO inferior compared to healthy lean groups. But MHOs were not having significantly increased risk of all-cause mortality over a follow up of approximately 15 years, though their clinical risk profile was worse than that of

metabolically healthy lean group.^[16] Recently Appleton et al reported more than 67% of MHO individuals stable for over 10 years of duration.^[17]

Obesity is a heterogeneous multifactorial disorder with various subtypes. Identifying MHO and metabolically obese but normal weight subtypes is important for primary and secondary prevention therapeutic strategies.^[9] Researchers are focusing on potential mechanisms of protection from chronic diseases and mortality in MHO. One of the proposed mechanism is that ability of MHOs may be better to handle excess calories by storing as subcutaneous fat or use as energy.^[18] Also some studies showed smaller visceral adipocytes among MHO individuals than metabolically unhealthy individuals.^[19,20] Favorable inflammatory milieu reflected by low levels of circulating inflammatory markers, raised anti-inflammatory natural killer cells and cytotoxic T lymphocytes and decreased activation of certain proinflammatory pathways in visceral adipose tissue in MHO may also be possible explanation for lower risk of metabolic complications.^[15,20,21,22] Even though MHO individuals display healthy cardio-metabolic and inflammatory profile, they are at risk for other obesity related complications like osteoarthritis, sleep apnea syndrome, and low quality of life. In the same manner even if lean or normal weight T2DM subjects have normal BMI, waist circumference and body fat content, there is Obesity paradox. One of meta-analysis involving 5 longitudinal cohorts of 2625 participants reports higher mortality in normal weight group than overweight or obese group.^[23]

Obesity, a major determinant of T2DM is associated with metabolic aberrations impairing insulin sensitivity, which also changes secretion of cytokines leading to proinflammatory condition.^[24] Both obesity and T2DM are associated with chronic systemic low-grade inflammation leading to cardiovascular complications. Serum hsCRP is not only just inflammatory marker, but it is well-established independent powerful cardiovascular risk predictor. It is widely used for cardiac risk stratification. CRP exerts proatherogenic inflammatory effect in many cells.^[25]

Asia is a major site experiencing rapidly emerging epidemics of obesity and T2DM with their complications. Indians exhibit high risk of T2DM even at low BMI.^[26] Asian Indian phenotype including insulin resistance, higher visceral adiposity, raised proinflammatory markers despite of generalized obesity measured by BMI drives individuals to high cardio-metabolic risk with greater propensity.^[27] Both obesity and T2DM have been identified as independent predictors of CVD. India is harboring the highest number of T2DM subjects. Hence management of cardio-metabolic health rather than BMI alone by lifestyle modifications will be the most cost-effective intervention in high-risk population.

Limitations of our study

This is a smaller scalar case control study. In future longitudinal studies are warranted to evaluate role of weight reduction in MHO on metabolic profile. Also role of administration of anti-inflammatory therapeutic agents in lean T2DM to improve outcome needs to be explored.

Conclusion

Our study findings demonstrate adverse metabolic health of lean T2DM which would be more dangerous, proatherogenic than that of MHO individuals. Despite of low or normal body weight, T2DM group has unfavorable inflammatory status in comparison with metabolically healthy obesity. This emphasizes need to promote the control of adverse metabolic risk factors along with weight reduction. Identification of metabolic phenotypes plays important role in preventive strategies. Clinicians should not overlook metabolic abnormalities in normal weight individuals. Metabolic health is more important determinant of development of cardio-metabolic disorders.

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Conflict of interests: None

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