Effect of Obesity and Associated Disorders like Diabetes, Dyslipidemia & Hypertension on Levels of Serum Complement Component C3 in Indian Ethnic Population

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Abstract

Background: *Obesity is a well recognised as a state of chronic low grade inflammation and the main source of complement factors is the adipose tissue which is associated with Insulin Resistance, altered glucose and lipid metabolism, all of which promote the development of metabolic & cardiovascular disorders.* ***Objective:*** *Till date no study has been conducted in Indian ethnic population exploring the relationship of serum complement C3 with obesity and disorders like diabetes, dylipidemia and hypertension so our objective was to study the effect obesity & associated disorders on serum C3 levels.* ***Material & Methods:*** *The present study included 290 subjects (121 men & 169 women) out of which 203 (70 %) were overweight 61 (21 %) were obese class I and 26 (9 %) were Class II obese according to International Diabetes Federation (IDF) - Modified ATP III criteria. Biochemical parameters like Serum C3, Fasting sugar levels, serum Insulin levels and lipid profile were measured. Homeostasis model of assessment for insulin resistance (HOMA-IR) was calculated. Statistical analysis was done by Medcalc.v11.5.0.0.software.* ***Results:*** *Mean C3 levels in total no. patients were 148.61± 38.82 mg/dl. As BMI increased, there was significant increase in serum levels of C3. When the distribution of variables were studied in both sexes, no statistically significant differences were found for all variables except Age, BMI, blood pressure & C3 levels. Serum C3 also correlated significantly with BMI ($r = 0.812$, $P< 0.0001$), insulin resistance ($r= 0.262$, $P <0.001$), Triglyceride ($r = 0.338$, $P < 0.001$) & LDL ($r= 0.431$, $P < 0.001$). As associated disorders with obesity increased, there was significant increase in levels of C3 than only obese patients with no other associated disorders. (ANOVA, $P< 0.001$).* ***Conclusion:*** *In this study, association of serum C3 with increase in BMI was established & also relationship of C3 with Insulin levels, Insulin resistance and cardiovascular risk factors was found. Our study concluded that obesity associated with dyslipidemia, diabetes & hypertension have a significant effect in increasing the levels of serum C3 concentration.*

Keywords: *Inflammation, Obesity, Diabetes, Complement component 3, Insulin resistance*

Introduction

Obesity is major problem worldwide and prevalence has increased, not only in developed but also in developing countries. Abdominal obesity is a potent risk factor for the development of Type 2 diabetes mellitus, Metabolic syndrome and Cardiovascular disease (CVD). Adipose tissue, particularly white adipose tissue, is an endocrine organ that releases adipocytokines into the blood stream. [1] These cytokines includes Interleukin-6 (IL-6), Tumor necrosis factor-alpha (TNF-a), Leptin, serum complement (C3) and Acylation-stimulating protein (ASP).[2] Such cytokines have a role in developing insulin resistance by causing the phosphorylation and proteosomal degradation of insulin receptor substrates or by indirectly interfering with the insulin receptor substrate interaction. Some of them plays an important role in obesity associated insulin resistance and cardiovascular complications.[3]

The complement system is a complex protein network and initially was considered as part of the innate immune system. Previously, the major source of complement was considered as the liver but in recent years, various non-hepatic sources of complement, like adipose tissue and endothelial cells, have been identified. Complement can be activated by several pathways – the classical, the lectin and the alternative – all of which converge on complement C3, the central component of the complement system.[4]

Various Clinical studies shows that elevated plasma C3 levels are associated with Type 2 diabetes, and also correlate with measures of obesity, dyslipidemia and insulin resistance. Functional role for the complement system in the pathogenesis of Type 2 diabetes and insulin resistance is supported by a variety of in vitro and in vivo studies suggesting pleiotropic effects of complement components on adipocytes, endothelial, and inflammatory cell function (3).[5] Increased C3 concentrations is also associated with cardiovascular risk factors [6], insulin resistance & Obesity [7], waist circumference and Triacylglycerol concentrations [8]. All these findings suggest that circulating C3 could be a risk factor for the development of Obesity and Type 2 diabetes[7] and also risk indicator for cardiovascular disease.[9] Moreover, C3 concentrations are also useful as biomarkers to identify subjects with metabolic syndrome.[10]

Thus the purpose of the study was to study the effect of different grades of obesity and associated dylipidemia, diabetes & Hypertension on Serum complement C3 levels and to study its relationship with insulin resistance & different cardiovascular risk factors.

Material & methods

Subjects

The cross-sectional study included 290 subjects attending the daily Outpatient Clinic run by Dept. of Medicine, Dhiraj General Hospital, Gujarat, India during the time period Jan 2014 to Feb 2015. Subjects with BMI more than >23 kg/m^2 were included in the study. Further these patients are divided in 3 subgroups according to International Diabetes Federation (IDF) - Modified ATP III criteria: 1. Overweight (BMI ≥ 23.00 – 24. 99 kg/m^2) 2. Obese class I (BMI ≥ 25.00 – 29.99 kg/m^2) 3. Obese Class II (BMI ≥ 30.00 kg/m^2). All the subjects detailed history of Diabetes, Hypertension or Cardiovascular disease was taken. Subjects with a systolic blood pressure (SBP) of ≥ 140 mm Hg or diastolic of ≥ 90 mm Hg, or taking any hypotensive agent, were considered hypertensive. Subjects with fasting Glucose Levels ≥126 mg/dl on at least two occasions were considered diabetic. [11] When total cholesterol (TC) or triglycerides (TG) exceeded 200 mg/ dl, or when the subjects were on lipid-lowering drugs, dylsipidemia was diagnosed.

Subjects weight measurement was done on a calibrated weighing machine. Height was measured with bare feet on calibrated fixed scale. The BMI was calculated by dividing the weight in kilograms by the square of the height in metres. Waist Circumference (WC) was measured at the level between the lowest rib margin & iliac crest & Hip Circumference (HC) was measured at the widest points of two trochanters using a measuring tape. Blood pressure was measured by using a mercury sphygmomanometer, maintaining the subject in the seated position, twice consecutively.

Blood samples analysis

Fasting blood samples were collected by venipuncture and collected in Fluoride and Plain vacuette. Samples were allowed to clot for 10 min and centrifuged for 15 min at 3000 r.p.m. Serum was separated and anlaysis of Serum Complement component C3, fasting blood sugar levels, Glycosylated haemoglobin, Insulin levels, Lipid profile was carried out. Serum C3 was analysed on semi-automated analyser ERBA-CHEM 5 plus and Serum Insulin levels were measured on TOSOH AIA-360 system analyser (AIA-IRI pack). The insulin resistance was calculated from the Homeostasis Model Assessment (HOMA). [12] Triglyceride levels (TG) were analysed by GPO-PAP method, Total cholesterol (TC) by CHOD-PAP method and high-density lipoproteins (HDLs) by colorimetric enzymatic assay on fully automated ERBA EM200 analyser. The low-density lipoproteins (LDLs) were calculated using Friedewald's

formula[13]. Blood Glucose was determined by glucose-oxidase method on fully automated ERBA EM200 analyser.

Statistical analysis

Medcalc.v11.5.0.0. software was used for statistical analysis. For comparison between two groups, Student's t-test was used. To study the differences between more than two groups ANOVA test was done. For Correlation between the groups Spearman's rank correlation coefficient was used. $P < 0.05$ was considered significant.

Results

The study included total 290 subjects of which 203 (70%) subjects were overweight, 61 subjects (21%) were Class –I obese and 26 (9%) were Class-II obese. Analysis of anthropometric and biochemical parameters is shown in Table 1. It was found that females were more overweight & obese than males. There was significant difference in all the anthropometric and biochemical parameters in Overweight & class-II obese groups except Blood pressure and but no significant differences were found between Overweight & class-I obese except age, BMI, Waist to hip ratio & C3. There was significant increase in levels of Complement C3 in class-I obese & Class-II obese than overweight subjects. (Table-1)

When gender based distribution was studied for anthropometric variables, in total 290 patients (121 men and 169 women) statistically significant differences were found between men and women except age, Blood pressure & BMI. Only significant difference was found between HDL-c levels between men and women among the study subjects. Also no significant difference was found between serum C3 levels in both sexes. There was significant correlation between, serum C3 and all variables, and significant positive correlation found between C3 and BMI (Fig 1), waist to hip ratio, Fasting blood sugar levels (FBS), serum insulin levels and HOMA-IR, Serum Triglyceride levels, LDL and Negative correlation was found between serum HDL levels. (Table-2)

Table 1: Anthropometric & biochemical variables in different groups

	Overweight	Class-I obese	Class-II obese	P-value
n (%)	*203 (70%)*	61 (21%)	26 (9%)	-
Age (years)	46.45 ± 15.19	51.11 ± 14.22	55.19 ± 14.75**	a. *P=0.03 b **P = 0.0008
Gender (M/F)	88/115	23/38	10/16	-
BMI (kg/m^2)	24.41 ± 1.75	26.58 ± 5.32*	30.65 ± 5.65	a. ** P <0.0001 b. ** P< 0.0001
Waist-Hip ratio	0.86 ± 0.05	0.90 ± 0.12	0.92 ± 0.16	a. **P = 0.0001 b. **P <0.0001
Systolic blood pressure	133.21 ± 12.52	131.52 ± 11.31	130.75± 13.55	a. P=0.39^{ns} b. P=0.22^{ns}
Diastolic blood pressure	83.43 ± 6.87	82.28 ± 8.28	82.16 ± 8.43	a. P=0.08 ^{ns} b. P= 0.12^{ns}
FBS	111.32 ± 32.17	113.31± 33.13	125.35± 33.23	a. P=0.79^{ns} b. * P= 0.02
Total Cholesterol	192.15 ±36.21	194.34 ± 44.15	213.48± 48.71	a. P=0.73^{ns} b. **P=0.008
Triglyceride	184.65 ±111.21	189.51 ± 118.36	228.85± 123.40	a. P=0.51^{ns} b. *P=0.02
HDL-c	32.18 ± 12.39	28.41 ± 11.51	26.32 ± 11.56	a. P=0.42^{ns} b. *P=0.03

LDL-c	123.23 ± 33.15	126.46 ± 38.18	149.52 ± 44.77	a. P=0.50^{ns} b. **P<0.0001
Insulin	15.73 ± 6.92	17.46 ± 7.24	19.23 ± 7.65	a. P=0.20 ^{ns} b. **P=0.008
HOMA-IR	3.76 ± 2.13	3.78 ± 2.46	4.19 ± 3.58	a. P=0.83 ^{ns} b. *P= 0.02
C3	131.69 ± 26.32	143.63 ± 31.28	172. 45 ± 57.45	a. **P=0.003 b. **P=0.0001

a. - Class –I obese compared to Overweight, b. - Class-II obese compared to overweight, *P<0.05,** P< 0.001 compared to Overweight

Table 2: Correlation between C3 and anthropometric and biochemical variables

	r	P- value
Age (yrs)	0.116	0.51
BMI (kg/m2)	0.812	<0.0001
Waist/hip ratio	0.262	<0.001
Systolic Blood pressure	0.138	0.44
Diastolic blood pressure	0.109	0.62
FBS	0.966	<0.0001
HbA1C	0.943	<0.001
S.Insulin	0.909	<0.001
HOMA-IR	0.262	<0.001
Total cholesterol	0.874	<0.001
Triglyceride	0.338	<0.001
HDL-c	-0.281	0.02
LDL-c	0.431	<0.0001
VLDL-c	0.194	=0.02

r = Spearman's rank correlation coefficient, P<0.05 & P<0.001 considered statistically significant

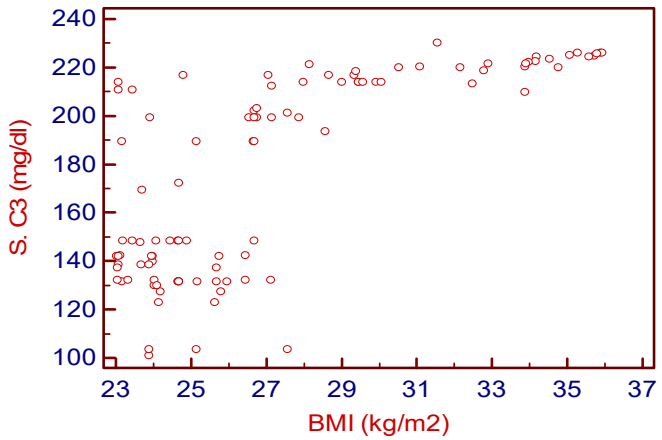

Fig 1: Correlation between S. C3 and BMI (r = 0.812, P< 0.001)

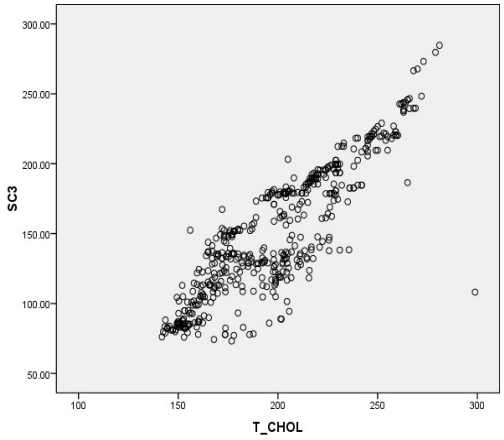

Fig 3: Correlation between S. C3 & T.Chol (r= 0.874) (p<0.001)

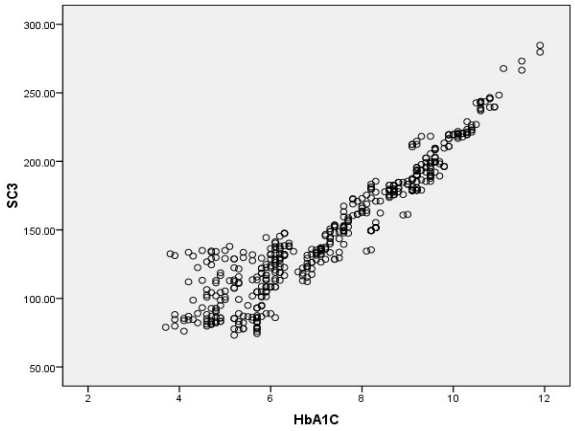

Fig 4: Correlation between S. C3 & HbA1C (r=0.943) (p<0.001)

When serum C3 levels were studied in patients with only obesity and obese patients with one or more associated disorders like Hypertension, Diabetes & Dyslipidemia, it was found that there was significant increase in C3 levels in patients with only obesity and in patients with number of associated disorders (ANOVA P<0.001) (Table 3).

Table 3: Levels of C3 in obese patients & obese patients with one or more associated disorders like diabetes, hypertension & dyslipidemia

	Obese Patients (n= 82)	**Obese +1 (n= 147)**	**Obese + 2 (n= 45)**	**Obese + 3 (n= 16)**	**ANOVA**
S. C3 (mg/dl)	125.83 ± 25.67 (109.76 – 150.23)	129.84 ± 27.12 (124.41 – 125.13)	138.36 ± 24.72 (128.31 – 146.51)	141.36 ± 25.77 (130.25 – 149.67)	P <0.001

Values are Mean ± S.D. and 95 % Confidence Interval. Differences between the groups were analysed by ANOVA (P<0.001).

Discussion

In the present study, it was found that serum C3 levels significantly increased as the BMI increases in overweight & obese population. Serum C3 levels increased significantly in Class-II obese compared to Overweight patients which shows obesity has a effect in increasing serum C3 levels. Moreover, there was significant association Serum C3 levels and serum fasting insulin levels and Insulin resistance. A significant increase in serum C3 levels was found as the associated disorders such as Hypertension, Dyslipidemia and Type-2 diabetes with obese patients increased.

Recently, there has been an increasing consequences of overweight and obesity in adolescents. Adipose tissue secrete a variety of autocrine and paracrine factors, such as TNF-α, IL-6, complement factors & Leptin which are involved in regulation of glucose & body weight homeostatis and is nowadays considered metabolically active.[14] Adipocytes synthesize and secrete C3, Factor B and adipsin.[15] These factors interact and results in the generation of C3a, which is transformed into C3adesarg which is also called acylation stimulating protein (ASP), and is secreted & synthesized by the adipocytes. [16] Also a positive correlation has been found between C3 and ASP. [17]

Previous studies had shown that C3 is significantly increased in patients with conditions such as obesity, Type II diabetes and dyslipidaemia.[17] Fasting C3 concentrations have been significantly raised in Pima Indians, and in familial combined hyperlipidemia (FCHL) [18] There are various evidences where there seems to be an increase in C3 in situations of ischemic cardiopathy and insulin resistance [17] and also C3 were found present on the arteriosclerotic plaque.[19] These findings indicate that C3 could be used as very important factor, which supports the hypothesis that insulin resistance could give rise to atherosclerotic process. Study by Muscari et al.[6] in 1068 subjects (29.8% with BMI > 28 kg/m^2), found that there is significant increase in C3 levels in different BMI tertiles. Studies by Halkes et al. [8], Onat et al[9], Ylitalo et al.[18] shows that anthropometric measurements, such as BMI, waist circumference and waist-to-hip ratio are considered predictors of high C3 concentrations. Koistinen et al.[20], have shown higher C3 expression in obese subjects than lean men.

In the present study, we found that there was significant increase in serum C3 in both men and women as BMI increased and also there was significant relationship between the studied parameters. These results are in accordance with study done by Hernandez-Mijares et al[15] who found increase in C3 in obese subjects but there are no studies in literature regarding C3 levels in Overweight, Class-I obese and class-II obese in Indian subjects. Our study showed a significant correlation between C3 & glycemia, insulin levels & insulin resistance which are in accordance with study done by Bhavita et al[24]. Study done by Muscari et al.[6] found a significant correlation between the glycemic levels (baseline and at 2 h after overload) and insulin resistance. Inflammatory cytokines, like TNF-alpha & interleukin 6 (IL6) inhibit tyrosine phosphorylation of the insulin receptor substrate-1 & causes insulin resistance in majority of obese subjects. TNF-alpha produces 30–40% of the circulating levels of IL6, which is the main source of production of C-reactive protein in the liver [21]. The increase in production of cytokine causes a low-grade chronic inflammation and activates the complement system, which contributes to the metabolic complications observed in obesity.[22]

Also in our study we found positive correlations between C3 concentrations and fating blood sugar levels, Glycoylated levels & Cardiovascular risk factors, such as Total Cholesterol, Triglyceride, HDL-c, LDL-c & VLDL-c which is consistent with others studies by Onat et al, Oostrom et al, Hernandez-Mijares et al & Bhavita et al. [9, 10, 15, 24]. The relationship between C3 and triacylglycerol can be explained by, ASP which is a hormone produced by adipocytes because of interaction of C3, factor B, and adipsin, stimulates glucose transport through membranes and increase the synthesis of Triacylglycerols in adipocytes.[23] Also in our study we found that there was increase in C3 levels with increase in associated disorders, which is consistent with the study done by Hernandez-Mijares et al & Bhavita et al.[15,24] Thus in this study we found that this disorders like diabetes, hypertension &

dyslipidemia have effect in increasing serum C3 concentration in addition to obesity which increases cardiovascular risk in patients with obesity.

Conclusion

Thus from this study, we conclude that serum concentration of C3 increases with increasing BMI in both men & women and the association between levels of C3 and increased obesity results from synthesis of these proteins by adipose tissue. There is also relationship between Serum C3 levels, Insulin resistance and Cardiovasucular risk factors in Indian population. Our study found that disorders such as Dyslipidemia, Diabetes & Hypertension have a role in increasing the levels of serum C3 in Obese patients.

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