Original Article

COMPLEMENT C 3 LEVELS IN METABOLIC SYNDROME

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ABSTRACT

Background: The metabolic syndrome consists of constellation of central obesity, hyperglycemia, hypertension, hypertriglyceridemia and low HDL cholesterol. Elevated serum Complement C3 levels independently predicted incident type 2 diabetes and increased cardiovascular risk in prospective studies in apparently healthy men and women.

The present study was done to determine the association of serum Complement C3 in Metabolic Syndrome as well as to determine its relation with individual components of metabolic syndrome & number of components metabolic syndrome.

Materials and Methods Our study included 50 subjects with Metabolic Syndrome as per IDF criteria for South Asians and 50 age and sex matched controls. Detailed history, anthropometric assessment, blood pressure recording, blood investigation including complete hemogram, urea, creatinine, Fasting blood sugar, Fasting lipid profile and complement C3 levels was done.

Results: In our study there were 23 male and 27 female subjects with metabolic syndrome and 22 male and 28 female subjects in the control group. Significant difference ($p<0.001$) was noted between the two groups with respect to height, weight, waist circumference, BMI, blood pressure, FBS, total cholesterol, LDL, HDL, VLDL, triglycerides, total count.

Complement C3 levels were significantly elevated in subjects with metabolic syndrome compared to the control population (139.95 ± 9.59, 102.12 ± 6.65; $p<0.001$). Significant difference was also noted in women with metabolic syndrome compared to men with metabolic syndrome ($p<0.001$).Complement C3 correlated significantly with waist circumference, BMI and total count. C3 also correlated with TC, LDL, HDL, triglycerides but was not significant. As the number of metabolic syndrome components increased, there was significant increase in complement C3 levels ($p<0.05$). Triglycerides and VLDL also increased with increase in number of metabolic syndrome components.

Conclusion: The results of the present study conclude that subjects with metabolic syndrome have significantly high levels of Complement C3 compared to healthy subjects. Also women have higher Complement C3 compared to men with metabolic syndrome and thus are at higher risk of cardiovascular disease. Complement C3 may be used as a marker in subjects with metabolic syndrome to assess the cardiovascular risk.

Key words: Metabolic Syndrome, Complement C3, Waist circumference, Obesity, Diabetes, Hypertension, Dyslipidemia, Cardiovascular disease.

INTRODUCTION

The Metabolic Syndrome is a highly prevalent entity characterised by clustering of risk factors of metabolic origin that are accompanied by increased risks of cardiovascular disease and type - 2 Diabetes Mellitus. These risk factors are atherogenic dyslipidaemia, elevated blood pressure, impaired glucose tolerance, a prothrombotic and a proinflammatory states[1].

The global burden of Metabolic Syndrome is 20-25%[2]

and in India the prevalence varies from region to region is about 19-25%[3, 4]. In the US, almost 50 million adults have metabolic syndrome[5].

People with metabolic syndrome are twice as likely to die from, and three times as likely to develop, myocardial infarction (MI) or stroke compared to people without metabolic syndrome[6]. They also have a five-fold greater risk of developing type 2 diabetes (if not already present) [7]. Metabolic syndrome is increasingly being recognised as a risk factor for cardiovascular

Complement C3 and C4 are the major plasma proteins of the immune system complement pathways. It is an acute phase reactant and a cytokine produced by the liver as well as secreted by the adipose tissue and is a marker for chronic low grade inflammation. Complement C3 is highly associated with dyslipidemia, obesity, hypertension, atherosclerosis and A recent prospective population-based study identified C3 as a risk factor for the development of type 2 diabetes mellitus[13]. C3 has been positively associated with insulin resistance, obesity, fasting and postprandial triacylglycerol concentrations, hypertension, and cardiovascular disease[14]. More recently, a significant relation has been seen between the concentrations of complement c3 and the number of metabolic syndrome components[15].

However, the association between complement C3 and metabolic syndrome has been less well explored in India. This study aims to find out the association between complement C3 concentration as an inflammatory marker in relation to metabolic syndrome & the individual components. insulin resistance [10-12]. disease (CVD) [8].

MATERIAL AND METHODS

Source of Data

Patients attending outpatient department, diabetic clinic or as inpatients in the Department of General Medicine, Victoria hospital and Bowring and Lady Curzon hospital, BMCRI, Bangalore between the period of November 2012 to September 2014 were included in the study.

Prior approval for the study and protocol was obtained from the Institution ethical committee.

After explaining the need of the investigations and explaining about the study, all the cases and controls were included in the study. Informed written consent was obtained from the subject before actual study was performed.

Study design: Case control study

Sample size: 50 subjects with Metabolic syndrome as defined by IDF criteria for South Asians and 50 age and sex matched controls were enrolled into the study.

Inclusion criteria: Patients aged 18 years and above, who fulfilled the criteria of metabolic syndrome defined by IDF were enrolled into the study.

IDF criteria for metabolic syndrome

Waist circumference ⩾90 cm in men or ⩾80 cm in women (south Asians) plus Two or more of the following:

- Fasting triglycerides > 150 mg/dl or specific medication
- HDL cholesterol <40 mg/dl(men) or <50mg/dl(women) or specific medication

Method of collection of data

Subjects presenting to OPD, diabetic clinic and in-patients of our hospital were included in the study if they were cleared off the exclusion criteria. Informed consent was taken. A pre- structured proforma was used to collect the baseline data. Blood pressure was measured using a mercury sphygmomanometer to the nearest 2 mm Hg in sitting position. 2 separate readings were taken, one in the beginning and other at the end of the examination. Average of two readings was taken. Patients satisfying the inclusion criteria underwent relevant investigations which included complete hemogram, urea, creatinine, fasting blood sugars, fasting lipid profile, complement C3 levels. Height was measured with a non-stretchable standardized measuring scale with an accuracy of up to 0.5 cm and without footwear. Weight was measured using a standard ISO certified weighing scale with minimum clothes and

mid-way between the last palpable rib and the top part of the iliac crest. Before reading measurements it is estimated that the tape is snug but does not compress the skin and is parallel to floor. Measurement was at the end of normal expiration. Blood samples for fasting blood glucose were taken after eight hours of overnight fast. Blood samples for lipid profile were taken after 12 hours overnight fast.

For Complement C3 Assay, the method is immunoturbidimetry enhanced by polyethylene glycol (PEG). Specific antiserum is added in excess to buffered samples. The increase in absorbance caused by immunoturbidimetry is recorded when the reaction has reached its endpoint. The change in absorbance at 340 nm is proportional to the amount of C3 in solution. Normal value ranges from 90-180 mg/dl.

Statistical methods

Descriptive and inferential statistical analysis has been carried out in the present study. Results on continuous measurements are presented on Mean±SD (Min-Max) and results on categorical measurements are presented in Number (%). Significance is assessed at 5 % level of significance. The following assumptions on data is made, Assumptions: 1.Dependent variables should be normally distributed, 2.Samples drawn from the population should be random, Cases of the samples should be independent Student t test (two tailed, independent) has been used to find the significance of study parameters on continuous scale between two groups Inter group analysis) on metric parameters. Leven1s test for homogeneity of variance has been performed to assess the homogeneity of variance. Analysis of variance (ANOVA) has been used to find the significance of study parameters between three or more groups of patients. Chi-square test has been used to find the significance of study parameters on categorical scale between two or more groups was performed.

ROC curve analysis has been used to find the cutoff to predict the cases. The level of significance was set at p value of <0.05. Statistical analysis was done using SPSS v. 18

RESULTS AND ANALYSIS

Study Design: A Comparative two group case control study with 50 Cases of Metabolic syndrome and age and sex matched controls is undertaken to study the levels of complement C3 in the two groups also its relation to individual components of Metabolic syndrome.

In the study, 50 patients of Metabolic syndrome and 50 patients without Metabolic syndrome were present. Most common age group in both groups was 35-45 category which comprised 38% of the subjects. Mean age in the cases was 50.76 ± 10.36 years and in the controls was

50.78 ± 10.58. The youngest patient The metabolic syndrome group consisted of 23 males (46%) and 27 females (54%) and the controls had 22 males (44%) and 28 females (56%). However there was no significant difference in the two groups with respect to sex (p=1). Male female ratio in cases and controls were 1:1.173 and 1:1.272 respectivelywas 35 years old and oldest patient was 70 years old.

Majority of subjects in the metabolic syndrome group were obese (56.5% males, 51.85% females) whereas most of the subjects in control group had normal BMI (81.8% males, 96.4% females). Mean BMI in cases was 29.78±4.06 kg/m^2 and in control group was 20.78±2.07 kg/m^2

According to IDF, for the diagnosis of MetS, WC cut off for south Asians is > 90 cm in males, > 80 cm in females. Among the male subjects with MetS, 43.48% had WC in the range of 90-99 cm, 47.83% had WC in range of 100-109 cm. Majority of the subjects (86.37%) in control group had WC in the normal range (70-89 cm).

Mean WC in males in cases and controls were 100.87±7.162 cm and 84.91±5.108 cm respectively. Among the female subjects in cases, majority had WC in the range of 90-99 cm (29.63%), followed by 25.92% in the range 100-109 cm. 85.71% of subjects in control group had WC in the normal range (70-79 cm). Mean WC in females in cases and controls were 102.15±12.005 cm and

76.46±3.124 cm respectively.

In the patients with metabolic syndrome, 88% were diabetics whereas there were none had diabetes in the control group. 50% of the cases had diabetes for duration between 1 to 5 years, 25% less than 1 year.

35 (70%) subjects with MetS had hypertension whereas none in the control group had hypertension. Majority of subjects had hypertension for duration of 1 to 5 years (42.85%) followed by 5 to 10 years (34.28%).

Complement C3 levels are increased in subjects with both diabetes and hypertension in subjects with MetS compared to subjects who have either diabetes or hypertension alone, however the difference was not statistically significant (p=0.278)

IDF criteria uses a blood pressure of >130/85 mm Hg in the diagnosis of MetS. 66% of the cases had BP > 130/85 mm Hg. Among the controls 10% had BP >130/85 mm Hg. Thus 2 patients who had hypertension were well controlled.

Among the cases 8% had normal fasting glucose, 26% had impaired fasting glucose and 66% were diabetics (FBS>126). 98% of subjects in control group had normal blood sugars, 2% had impaired fasting glucose and none were diabetics. Thus 2 subjects who weren't diabetics had impaired fasting glucose.

Among male cases, HDL was low (<40 mg/dl)in 60.87% subjects , whereas only 9.1% in control group had low HDL. There was a statistically significant difference among the 2 groups (p < 0.01)

Among female cases, HDL was low (<50 mg/dl) in 85.19% of the subjects whereas only 32.14% of subjects in control group had low HDL. There was a statistically significant difference among the 2 groups (p < 0.01)

The two groups were matched with respect to age and sex. Significant differences (p<0.001) in the two groups were noted in height, weight, waist circumference, BMI, SBP, DBP, FBS, TC, Triglycerides, HDL, LDL, VLDL.

Complement C3

	Cases		Controls	
	Male (n=23)	Female (n=27)	Male (n=22)	Female (n=28)
Mean Complement C3 (mg/dl)	135.648± 9.0637#	143.615± 8.5841#†	102.791± 7.7780	101.600± 5.7152
	139.95±9.59*		102.12±6.65	

Table 15: Complement C3 in cases and controls

*p < 0.001 C3 between cases and controls

#p<0.001 C3 between same gender in cases and controls

†p<0.001 C3 between female cases and male cases

The mean complement C3 level in cases was 139.95±9.59 and in controls was 102.12±6.65. A statistically significant difference of p < 0.001 was noted between the two groups.

On comparing male cases and male controls; statistically significant difference (p<0.001) was observed between the two groups. Similar result was found between female cases and female controls.

On comparing female cases with male cases, higher C3 was found in female cases and it was statistically significant. (p <0.001)

Subjects with metabolic syndrome had mild correlation between C3 and TC, TGL, LDL and inverse correlation with HDL.

Highly significant correlation was found between C3 and BMI, Waist circumference and Total count and inverse correlation with height in subjects with metabolic syndrome. C3 correlated significantly with total count in control subjects as well.

Number of components of metabolic syndrome	Number of patients (n=50)	Complement C3 (Mean±SD)
2 components	9	133.256±5.7907
3 components	19	139.353±9.0536
4 components	22	143.205±10.0586
Inference	Mean Complement C3 is increasing significantly with increasing number of components of metabolic syndrome with P=0.02 (ANOVA)	

As the number of MetS components increased, Complement C3 levels also increased in a significant manner ($p<0.05$)

As the number of MetS components increased, serum triglycerides and VLDL increased in a significant manner ($p<0.05$)

DISCUSSION

Indians have higher incidence of atherogenic dyslipidemia characterised by high triglycerides, low HDL, with normal or low total cholesterol and LDL. The same can be noted from our study. Compared to South Asian subjects studied in Ajjan et al, the subjects in our study have higher triglyceride levels in both groups.

Compared to study by Oostrom et al, which mainly included Caucasians; our subjects have lower total cholesterol and LDL levels[137].

The mean complement C3 level in subjects with MetS was 139.95±9.59 mg/dl and in those without MetS was 102.12±6.65 mg/dl, and this difference was statistically significant ($p < 0.001$). Thus it indicates that Complement C3 is elevated in subjects with metabolic syndrome.

Increased plasma concentrations of complement C3 have been associated with insulin resistance. Elevated C3 concentration levels have been reported in individuals with obesity, type 2 diabetes, hypertension, dyslipidemia, and coronary artery disease, stroke, all of which are known to be associated with insulin resistance. An elevated C3 concentration has also been found to predict myocardial infarction. C3 has been hypothesised to be an immune mediator in the development of atherosclerosis. Clustering of the traditional risk factors like diabetes, hypertension, dyslipidemia constitutes metabolic syndrome. Studies indicate that C3 levels increase as the number of MetS components increase. Thus, it is postulated that C3 can be used as a marker to assess the cardiovascular risk and those at risk of metabolic syndrome.

This study aims to assess the levels of C3 in subjects with metabolic syndrome and its association with individual components of metabolic syndrome.

In our study, subjects were of age between 35 years to 70 years. Mean age of subjects with metabolic syndrome was 50.76 ± 10.36 years. Mean age in the control group was 50.78 ± 10.58. There was no significant difference between the two groups ($p=0.998$) and hence they were matched with respect to age. It was noted that as the age increases, C3 increases in both the groups; thus increasing age is an important cardiovascular risk factor.

The metabolic syndrome group consisted of 23 males and 27 female subjects whereas the control group and 22 males and 28 females subjects. The difference was not significant.

In a study by Ajjan R et al, where they studied complement C3 levels in South Asian subjects with presence/absence of metabolic syndrome. There were 95 subjects in the study groups; with 150 subjects in the control group. The mean age was 41 years[13].

Oostrom et al, studied complement C3 in 40 subjects with metabolic syndrome and 70 subjects without metabolic syndrome. There were 25 male and 15 female subjects with metabolic syndrome; 55 male and 15 female subjects without metabolic syndrome[137].

Mean BMI in the subjects with metabolic syndrome was 29.78±4.06 kg/m^2. In the control group the mean BMI and in control group was 20.78±2.07

kg/m^2.

In the study by Oostrom et al, mean BMI in MetS group was 26.5±2.0 and the mean BMI in the control group was 24.5±2.5[137]

On measuring waist circumference we found that the MetS group had mean WC of 101.56 cm; the control group had 80.18 cm.

In South Asians with metabolic syndrome mean WC was 95 cm whereas in subjects without metabolic syndrome mean WC was 86 cm[135].

In the Utrecht study by Oostrom et al, mean WC in the presence/absence of MetS was 97 cm and 90 cm respectively[137].

In our study 66% (33) of patients had blood pressure recording of more than 135/85mmhg. Thirty five patients (70%) patients were known hypertensive's on treatment. None had hypertension in the control group. Mean SBP was 135.16 mmHg and mean DBP was 85.24 mmHg. C3 levels were higher in the subjects with hypertension compared to those without hypertension

In the Ajjan R et al study, in South Asian subjects with MetS the mean SBP was 135 mmHg, and the mean DBP was 88 mmHg[135].

The mean SBP was found to be 136 mmHg and the mean DBP to be 88 mmHg in the Oostrom et al study[137].have history of diabetes. 2 patients with metabolic syndrome who did not have diabetes had impaired fasting glucose (101-125). There were no diabetics in the control group. Mean FBS was 166.22 mg/dl in the MetS group whereas it was 85.22 mg/dl in the control group. However the significance in the C3 levels between the diabetics and non diabetics could not be ascertained as the two groups were statistically not comparable.

In the Ajjan R et al study, mean glucose was 100.90 mg/dl in the MetS group whereas it was mg/dl in the control group. 12 subjects had impaired fasting glucose[135].

In the Oostrom et al study, mean glucose in the MetS group was 99.10 mg/dl and in the control group was 91.89 mg/dl[137].

Majority of patients with metabolic syndrome had hypertension or diabetes for duration of 1- 5 years (42.85% and 50% respectively). 30 subjects had both hypertension and diabetes, 14 had only hypertension and 5 had only diabetes. There was a linear increase in the C3 levels on comparison of subjects with hypertension, diabetes and subjects with diabetes and hypertension; however the increase was not statistically significant.

Muscari et al, found that C3 levels were significantly higher if the subjects had either diabetes or hypertension[15].

In subjects with metabolic syndrome, 25(50%) patients had abnormal total cholesterol (>200mg/dl), 41(82%) patients had abnormal triglycerides (>150mg/dl), 37(74%) patients had abnormal HDL (<40mg/dl in males, <50 in females). It was found that there was significant difference in the lipid profile parameters between the case and control group (p<0.001).

We compared the lipid profile parameters in the both groups in various studies.

Study	Complement C3 levels (mg/dl)	
	Cases (MetS+)	Controls (MetS -)
Ajjan et al[135]	131	117
Oostrom et al[137]	121	91
Van Greevenbroek et al[132]	194	163
Our study	139.95	102.12

Our results are comparable with Ajjan et al study in subjects with MetS, both address the same ethnic group.

Significant difference in the complement C3 was noted among male and female groups as well. (Male cases 135.648±9.06, male controls 102.791±7.77; p <0.001. female cases 143.615±8.58, female controls 101.600±5.71; p

< 0.001)

It was of interest to note the gender difference in our study; that the female subjects with Mets had significantly higher C3 levels compared to male subjects with Mets. (p < 0.001)

On assessment of Complement C3 levels in non diabetic Pima Indians by Weyer et al, female subjects had a higher C3 levels compared to male subjects (180:165, p not significant)[141].

In the Ajjan et al study, female subjects with MetS had higher C3 of 136 mg/dl compared to male subjects with 125 mg/dl (p value not mentioned)[135].

It is postulated that enhanced systemic low-grade inflammation may lead, especially in women, to the dysfunction of the anti-inflammatory and atheroprotective properties of apo A- I and HDL particles, which increases the risk of diabetes and CVD even more. In a meta-analysis the stated cardiovascular risk in subjects with MetS was higher for women compared with men (RR = 2.63 vs 1.98;p = 0.09)[142].

On studying the correlation between individual components and C3 levels; significant correlation was found between C3 and Waist circumference, BMI, and Total count. (p<0.001)

We compared the correlations between C3 and various parameters in other studies.

The study population was categorized into those having 2, 3 and 4 components of metabolic syndrome. It was found that C3 levels increased significantly as the subjects had more components of metabolic syndrome. (p<0.05)

levels with increasing number of MetS components[137].

In the study by Herna´ndez-Mijares et al, on comparing the levels of C3 depending on the number of associated disorders (hypertension, type 2 diabetes and dyslipidemia), significant differences were found between patients with only obesity, and those who had the obesity associated with one, or several of these disorders[125].

Ohsawa et al, in a study in Japan with subjects with Mets observed that as the number of Mets diagnostic criteria increases, serum concentration of C3 increased[136].

It was of interest to note that, there was a significant relationship between Triglycerides, VLDL and the number of components of MetS (p<0.001) with higher Triglyceride and VLDL level with increasing components of MetS.

CONCLUSION

The present study concluded that

1. Complement C3 levels are significantly elevated in subjects with metabolic syndrome.
2. Female subjects with metabolic syndrome have higher C3 levels compared to male counterparts.
3. Significant correlation exists between C3 levels and waist circumference, BMI and total count.
4. C3 levels are elevated in subjects with both diabetes and hypertension compared to subjects with either risk factor.
5. C3 levels increase as the number of components of metabolic syndrome increases.
6. With the increase in number of metabolic syndrome components, serum triglyceride and VLDL increase significantly.

Thus, Complement C3 levels can be used as a biomarker to assess the risk and severity of metabolic syndrome and hence help in assessment of the cardiovascular risk in these subjects

SUMMARY

Global incidence of metabolic syndrome is at rise. This is associated with increasing incidence of hypertension, diabetes, dyslipidemia and cardiovascular disease.

There is increasing evidence that elevated complement C3 is a marker of chronic low grade inflammation and correlates with traditional risk factors i.e. diabetes, hypertension and dyslipidemia as well as increase in incidence of cardiovascular disease.

Thus it was of interest to find if levels of complement C3 was associated with subjects of metabolic syndrome and to determine the relation between complement C3 levels and individual components of metabolic syndrome.

The study was conducted in Victoria hospital and Bowring and Lady Curzon hospital attached to Bangalore medical college and Research Institute. The study included 50 subjects with metabolic syndrome and 50 age and sex matched controls.

Subjects with metabolic syndrome had significantly higher C3 levels compared to their counterparts. C3 levels were more in the female subjects compared to male subjects ($p<0.001$)

C3 had significant positive correlation with waist circumference, BMI, total count. ($p<0.001$)

C3 levels were higher in subjects with both hypertension and diabetes compared to subjects who had either diabetes or hypertension. However the difference was not statistically significant.

As the number of components of metabolic syndrome increased, C3 levels significantly increased in these subjects. Also serum triglyceride and VLDL showed similar increase.

Longitudinal follow up studies with a larger sample population will need to be undertaken in order to evaluate the role of serum complement component 3 as a marker of clinical severity in metabolic syndrome

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