To Compare the Level of Cystatin C in Type 2 Diabetes Mellitus with Obesity

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Abstract

Background: Cystatin C is a non-glycosylated basic protein that is produced and secreted at a constant rate by all nucleated cells. Cystatin C is a more reliable marker than the serum creatinine because it is less affected by external factors such as gender, race and muscle mass. However, the comparison of serum cystatin C level in type 2 diabetes mellitus is not well known in people with obesity. **Objectives:** To estimate the level of cystatin C in type 2 diabetes and that can be explained by the change in obesity. Thus, this current study aimed to determine and compare the level of cystatin C in type 2 DM with obesity and also correlate the cystatin C level with the quality of life in type 2 DM and obesity. **Material and Methods:** We have taken three groups: Group A containing type 2 DM, Group B containing obesity, and Group C containing type 2 DM with obesity. In all, 25 patients in each group were selected and analyzed for cystatin C. **Results:** Cystatin C level was very high in type 2 DM with obesity group. The *P* value was 0.008 in type 2 DM with the obesity group and it has shown a highly significant correlation with BMI. In our study, we have also seen the positive correlation of cystatin C with BMI in Group B plain obese and Group C diabetes obese than Group A diabetes non-obese. We have seen in our study and found a poor correlation between HbA1c, RBS and cystatin C. **Conclusion:** The level of cystatin C is much higher in type 2 DM with obese patient as compared with type 2 DM and obese patients.

Keywords: Body mass index, cystatin C, HbA1c, obesity, type 2 diabetes mellitus

INTRODUCTION

Obesity is a major factor for metabolic disorders and premature deaths in developing countries.[1] The risk of type 2 diabetes increases many folds in association with obesity. Obesity is the fifth leading cause of global deaths. Approximately, 90% of people suffering from type 2 diabetes mellitus (DM) are overweight or have obesity.[2] Cystatin C is a nonglycosylated basic protein that is produced and secreted at a constant rate by all nucleated cells.[3] Cystatin C is more reliable marker than the serum creatinine because it is less affected by external factors such as gender, race and muscle mass.[4] It is the early marker for nephropathy and it can also predict peripheral neuropathy, retinopathy and arterial sclerosis.[5] Apart from renal function, cystatin C is also associated with cardiovascular events and limb ischemia.[6,7] Associated complications deteriorate the quality of life of patients and increase the morbidity rate.[8] Cystatin C is involved in arterial wall remodeling, blood vessel integrity, neovascularization, inflammation and neuronal degenerative pathology.[9] Moreover, serum cystatin C level is the superior marker of GFR than serum creatinine or serum

creatinine-based estimates of GFR, and a better predictor of cerebrovascular disease deaths, coronary heart disease, lower extremity arterial disease.[10] Cystatin C, though counted a predictor of renal disease, is primarily associated with obesity irrespective of gender.[11] Cystatin C level is widely studied in European and non-European populations. Indian currently has the largest number of people with diabetes in the world. Diabetes in Asian Indians is characterized by younger age at onset, increased insulin resistance. Previous studies have shown the association of cystatin c with increased Body Mass Index (BMI). However, the comparison of serum cystatin C level in type 2 DM is not well known in people with obesity. So, we aim to estimate the level of cystatin C in type 2 diabetes

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and that can be explained by the change in obesity. Thus, in this current study, we aimed to determine and compare the level of cystatin C in type 2 DM with obesity and also correlate the cystatin C level with the quality of life in type 2 DM and obesity.

MATERIALS AND METHODS

This was a cross-sectional and observational study designed to compare the level of cystatin C in obesity and type 2 DM. The study population consisted of 50 newly diagnosed patients with type 2 DM and 25 patients with obesity compared with age and sex matches. All patients were screened according to our inclusion and exclusion criteria of the study. The diagnosis of type 2 DM was done according to American Diabetes Association[12] and obesity (BMI >30) according to World Health Organization.[13] The study was conducted in the outpatient department of the medicine ward of Maharishi Markandeshwar Institute of Medical Science and Research (MMIMSR), Mullana, Ambala, Haryana, India. The study protocol was approved by Institutional Ethics Committee (IEC no. 1325) and written informed consent was taken from all the participants. The patients were informed and clarified about the purpose of this study in the language understood by the patient, prior to enrolment. The study was conducted under the Declaration of Helsinki and the code of Good Clinical Practice.

All the screened patients meeting the inclusion criteria of this study were divided into three groups: Group A (type 2 diabetes non-obese patients), Group B (non-diabetic obese patients) and Group C (type 2 diabetic with obesity). Each patient was assessed for cystatin C level and WHOQOL-BREF[14] patients >18 and <70 years and of either gender, or newly diagnosed patients with type 2 diabetes were included in the study. Patients receiving any drug therapy other than oral hypoglycemic agents were rejected. Patients with type 1 DM, acute complications of diabetes including diabetic ketoacidosis and acute foot ulcers, patients with pre-existing renal failure (GFR <60 ml/min), liver failure (alcohol abuse and liver cirrhosis), pregnant and lactating mothers, patients with the history of peptic ulcers, pulmonary tuberculosis, thyroid disorders, uremia, paraneoplastic neuropathy and patients on steroid therapy, HIV-infected patients, acute hepatitis A, B or C infection.

Obesity (BMI >30) has been considered according to World Health Organization guidelines,[13] glycated hemoglobin (HbA1c), complete blood count (CBC), liver and renal function tests and serum lipids were also assessed.

Biochemical estimation of serum Cystatin C

Serum cystatin C estimation was done by enzyme-linked immunosorbent assay (ELISA). Pipet 100 µl of diluted Standards, Quality Controls, Dilution Buffer (Blank) and samples, preferably in duplicates, into the appropriate wells. Incubate the plate at room temperature (ca. 25°C) for 30 minutes, shaking at 300 rpm on an orbital microplate shaker. Wash the wells three times with Wash Solution (0.35 ml per well).

After the final wash, invert and tap the plate strongly against paper towel. Add 100 µl of Conjugate Solution into each well. Incubate the plate at room temperature (ca. 25°C) for 30 minutes, shaking at 300 rpm on an orbital microplate shaker. Wash the wells three times with Wash Solution (0.35 ml per well). After the final wash, invert and tap the plate strongly against paper towel. Add 100 µl of Substrate Solution into each well. Avoid exposing the microtiter plate to direct sunlight. Covering the plate with, such as aluminum foil is recommended. Incubate the plate for 10 minutes at room temperature. The incubation time may be extended (up to 20 minutes) if the reaction temperature is below than 20°C. Do not shake with the plate during the incubation. Stop color development by adding 100 µl of Stop Solution. Determine the absorbance of each well using a microplate reader set to 450 nm, preferably with the reference wavelength set to 630 nm (acceptable range: 550-650 nm). Subtract readings at 630 nm (550-650 nm) from the readings at 450 nm. The absorbance should be read within 5 minutes.[15]

Assessment of health-related quality of life (HRQOL) questionnaire

World Health Organization Quality of Life Questionnaire - short version[13] was used to assess the quality of life. The four domains of the WHOQOL-BREF are physical health, psychological social relationships and environment. Subjects rated all questions on a 5-point Likert-type scale. Questions were asked in vernacular language of patients. Four domains were assessed which contains physical health, psychological, social relationships, and environmental factors.

Other diagnostics tests like HbA1c, uric acid, total cholesterol, low-density lipoprotein, high-density lipoprotein, serum creatinine, etc. were also assessed according to manufacturer's kit instructions.

Statistical analysis

The data were compiled and proposed as the mean ± standard error of mean (SEM). The difference between Group A, Group B and Group C were analysed using One-way analysis of variance (ANOVA). Correlation analysis was used to find the correlation between the various parameters. Statistical analyses were performed using the Graph Pad Prism Version 5.1 software program (Graph PadPrism, San Diego, CA, USA).

RESULTS

A total of 152 patients were screened for eligibility amongst 75 patients were recruited based on the inclusion and exclusion criteria, to participate in the present study. Twenty five patients with mean age 52 ± 1.6 (9 males and 14 females) in Group A (Diabetes non-obese patients), 25 patients in Group B (Plain obese patients) with the mean age 58 ± 1.7 (12 males and 13 females) and Group C (Diabetes obese patients) with the mean age of 53 ± 2.1 (12 males and 13 females) completed the study [Table 1]. As is evident from the data presented, All study subjects were not significantly differ regarding

Table 1: Demographic and clinical characteristics of diabetes, obese and diabetic obese patients

Variables	Mean±SEM		
	Group A (Type 2 DM)	Group B (Obesity)	Group C (Type 2 DM with obesity)
Age (years)	52±1.6	58±1.7	53±2.1
Gender (M/F)	12/13	12/13	12/13
BMI (kg/m²)	22±0.38	35±0.25[a]	33±0.39[a]
SBP (mm/Hg)	130±7.6	127±2.3	135±4.2
DBP (mm/Hg)	79±3.7	75±1.7	82±1.8
S. Cr. (mg/dl)	0.98±0.02	1.0±0.01	1.5±0.06[a,b]
S. uric acid (mg/dl)	6.1±0.82	4.9±0.23	6.7±0.81
GFR (ml/min)	90±2.4	104±0.60[a]	83±5.3
SGOT (IU/L)	22±0.75	21±1.0	21±1.0
SGPT (IU/L)	25±0.53	25±0.40	25±0.53
HbA1c (%)	8.5±0.15	6.1±0.05[a]	12±0.59[a,b]
TC (mg/dl)	150±5.0	239±1.2[a]	255±2.4[a,b]
HDL (mg/dl)	41±0.59	37±0.86[a]	39±0.74[a]
LDL (mg/dl)	135±9.6	147±1.6[a]	156±4.5[a,b]
VLDL (mg/dl)	33±0.75	53±1.2[a]	64±3.8[a,b]
Cystatin C (ng/ml)	752±9.9	942±12[a]	1112±24[a,b]

Values presented as mean±SEM, except gender which is expressed as the number M: Male, F: Female, BMI: Body mass index, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, SGOT: Serum glutamate oxaloacetic transaminase, SGPT: Serum glutamate pyruvate transaminase, RBS; Random blood glucose, S. cr: Serum creatinine, TC: Total cholesterol, LDL: Low density lipoproteins, VLDL: Very low density lipoproteins, HDL: High density lipoproteins, RBS: Random blood sugar, HbA1c: Glycosylated hemoglobin, GFR: Glomerular filtration rate. [a]$P<0.05$ Obesity and type 2 DM with obesity compared with type 2 DM with normal weight. [b]$P<0.05$ Obesity compared with type 2 DM with obesity

the basic demographic characteristics (age and sex). The demographic, clinical and laboratory characteristics of diabetes non-obese (Group A), plain obese (Group B) and diabetic obese patients (Group C) are summarized in Table 1 and 2.

Assessment of body mass index (BMI)
In the present study, BMI was assessed and there was a statistically significant difference between Group B (Plain obese), $P < 0.001$ and Group C (Diabetes obese) $P < 0.001$ compared with Group A (Diabetes non-obese) [Figure 1].

Assessment of serum cystatin C
We have measured the level of cystatin C in all the three groups and it was found that there was a statistically significant difference. The serum cystatin C values were significantly higher in Group B plain obese (942 ±12), $P < 0.001$ and Group C obese patients with diabetes (1112 ± 24), $P < 0.001$ compared with Group A diabetes non-obese (752 ± 9.9). There was also a significant difference in serum cystatin C values between Group B and Group C ($P < 0.001$) [Figure 2].

Pearson's correlation analysis between cystatin C and different demographic and clinical characteristics of Group A, Group B and Group C patients
Pearson's correlation was assessed between Group A diabetes non-obese, Group B plain obese, Group C diabetes obese patients [Tables 3 and 4]. The correlation was assessed with the level of cystatin C to the clinical characteristics. The positive correlation was found in Group C in age ($r = 0.44$, $P = 0.02$), BMI ($r = 0.51$, $P = 0.008$), HbA1c ($r = 0.65$, $P < 0.001$), LDL ($r = 0.63$, $P = 0.006$), VLDL ($r = 0.05$, $P = 0.60$). The
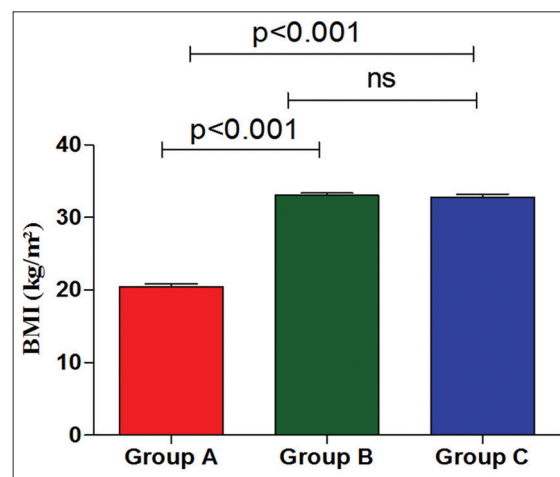

Figure 1: Body mass index (BMI) was a statistically higher in Group B (Obese only and Group C (Diabetes with obesity) compared with Group A (Type-2 DM)

negative correlation was found in Group A in Sr. Cr ($r = -0.09$, $P = 0.66$), Sr. Uric acid ($r = -0.31$, $P = 0.12$), GFR ($r = -0.01$, $P = 0.93$). The values were summarized in Table 3 and 4.

Pearson's correlation analysis of cystatin C with Age, BMI, physical health
In our study, cystatin C is postively associated with BMI in all three groups: group A (P value = 0.07, r value = 0.36), group B (P value = 0.01, r value = 0.41) and group C (P value = 0.008, r value = 0.51), age in group A (P value = <0.001, r value = 0.75), group B (P value = 0.04, r value = 0.39) and group C (P value = 0.02, r value = 0.44), while physical

Table 2: The status of health-related quality of life in diabetes, obese and diabetic obese patients

Variables	Group A (DM non-obese)	Group B (Plain obese)	Group C (DM obese)
Physical Health (Domain 1)	21±0.51[b]	19±0.53[a]	18±0.63[a]
Psychological Health (Domain 2)	16±2.0	17±1.5	18±1.8
Social Relationship (Domain 3)	9.2±0.21	9.0±0.28	8.4±0.28
Environment Health (Domain 4)	22±0.49	22±0.49	21±0.56

[a]$P<0.05$ Obesity and type 2 DM with obesity compared with type 2 DM with normal weight. [b]$P<0.05$ Obesity compared with type 2 DM with obesity

Table 3: Pearson's correlation analysis of different clinical characteristics

Variables	Group A Diabetes non-obese		Group B Plain obese		Group C Diabetes obese	
	r	*P*	*r*	*P*	*r*	*P*
Age (years)	0.75	<0.001	0.39	0.04	0.44	0.02
Gender (M/F)	0.31	0.121	-0.12	0.54	-0.27	0.19
BMI (kg/m²)	0.36	0.07	0.41	0.01	0.51	0.008
SBP (mm/Hg)	0.29	0.14	-0.30	0.13	0.08	0.69
DBP (mm/Hg)	0.20	0.32	0.02	0.90	0.06	0.74
Sr.cr (mg/dl)	-0.09	0.66	0.14	0.47	0.05	0.79
Sr. uric acid (mg/dl)	-0.31	0.12	-0.04	0.84	0.24	0.24
GFR (ml/min)	-0.01	0.93	-0.30	0.14	-0.44	0.02
SGOT (IU/L)	-0.16	0.43	-0.27	0.17	0.26	0.19
SGPT (IU/L)	0.19	0.33	0.13	0.52	-0.22	0.28
HbA1c (%)	0.75	0.53	0.01	0.94	0.65	0.04
TC (mg/dl)	0.07	0.73	0.73	0.01	0.14	0.48
HDL (mg/dl)	0.25	0.21	0.10	0.62	-0.12	0.53
LDL (mg/dl)	0.21	0.30	0.39	0.04	0.63	0.06
VLDL (mg/dl)	0.33	0.09	0.53	0.05	0.05	0.80
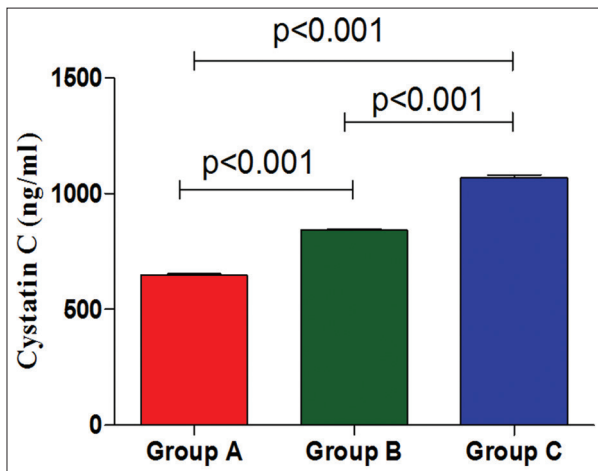
Figure 2: Level of Cystatin-C in group A, Group B and Group C

Figure 3: Level of Cystatin C was not positively correlated with BMI ($r = 0.36$, $P = 0.07$) in Group A (Type-2 DM patients)

health in group B (P value = 0.04, r value = −0.40) group C (P value = 0.03, r value = −0.03) is negatively correlated with cystatin C [Figures 3-5].

DISCUSSION

In the present study, there was no statistically significant difference in age gender, blood pressure, serum uric acid and liver function test in all three groups (non-obese type 2 diabetic, plain obese and type 2 diabetes with obesity). In this study, BMI
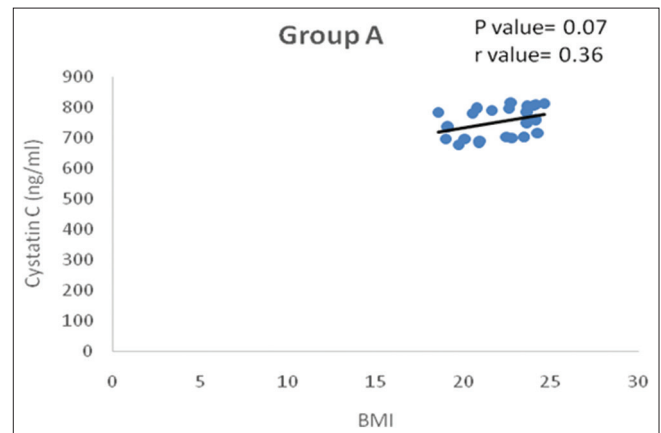
is higher in Group B (plain obese) and Group C (type 2 diabetes with obesity) as compared with Group A (diabetes patients). It is because of groups B and C contain obese patient instead of Group A contains only non-obese patients with type 2 diabetes. The reported findings in patients were independent of known cardiovascular risk factors (hypertension, hyperlipidemia, diabetes and smokers).

The incidence of type 2 diabetes is higher in the increased body weight or person with belly shape.[16] In many instances, obesity is the proximal trigger that culminates ultimately in

diabetes and cardiovascular disease.[17] Elevated cystatin C level indicates a useful biomarker in identifying an increased cardiovascular risk and increased morbidity and mortality in diabetic and obese patients whose serum creatinine levels and glomerular filtration rate indicate a low cardiovascular risk.[18,19] To the best of our knowledge, this is the first study to compare the cystatin C level between the non-obese type 2 diabetic, plain obese and type 2 diabetes with obesity.

Cystatin C is a small molecular protein that is produced by all nucleated cells at a constant rate and a protease inhibitor that is freely filtered by the renal glomeruli, metabolized by the proximal tubule and identified as a promising marker of renal failure. It is stored in the adipose tissues (mainly belly fat) after being metabolized by the proximal tubules.[1] It is a well-known established marker for renal disease, nephropathy, neuropathy and retinopathy.[20] Obesity is the main cause for many metabolic syndromes. It increases the risk of diabetes mellitus, hypertension and cardiovascular diseases to up to three- to four-fold. Mahajan *et al.* and Al wakeel *et al.* have demonstrated and reported the significant correlation between serum cystatin C level and increase in BMI or obesity. Cystatin C concentration is less affected by gender, muscle mass and diet than serum creatinine.[21] It is also reported to have association with cardiovascular disease and peripheral vascular disease. It is also found to be the early predictor of cardiovascular disease with diabetes mellitus irrespective of renal function.[22] Previous studies have shown that increased level of cystatin C cause insulin resistance, as Insulin resistance also have a strong association in developing cardiovascular diseases, so

this finding clears the relationship between cystatin C and cardiovascular diseases.[23] Obesity is also a major factor that causes insulin resistance. These both commonly play a major role in the occurrence of diabetes and the increase in the level of HbA1c in the population.[24] Our objective was to clarify the reason for the increase level of cystatin C in the population having diabetes and obesity.

In the current study, we have found a positive correlation in cystatin C with BMI in all three groups. As previous studies have observed that with the increase in adipose mass there is a significant increase in the cystatin C level in both genders. In our study, we have also seen the positive correlation of cystatin C with BMI in Group B plain obese and Group C diabetes obese than Group A diabetes non-obese. These reported studies suggest the role of adipose tissues in increasing the level of cystatin C.[25] A recent study has shown that cystatin C is significantly associated with the incidence of diabetes in patients having high fat mass.[1]

We have seen in our study and found a poor correlation between HbA1c, RBS and cystatin C. We have found that cystatin C is not much significant with HbA1c of diabetes non-obese and diabetes obese and in plain obese patients. Previous studies have shown that an increase level of cystatin C cause insulin resistance although the mechanism is unclear. Insulin resistance also has a strong association in developing cardiovascular diseases, so this finding clears the relationship between cystatin C and cardiovascular diseases.[23] Recent studies have shown that cystatin C is associated with the incidence of diabetes only in those patients who are obese or have insulin resistance.[26]
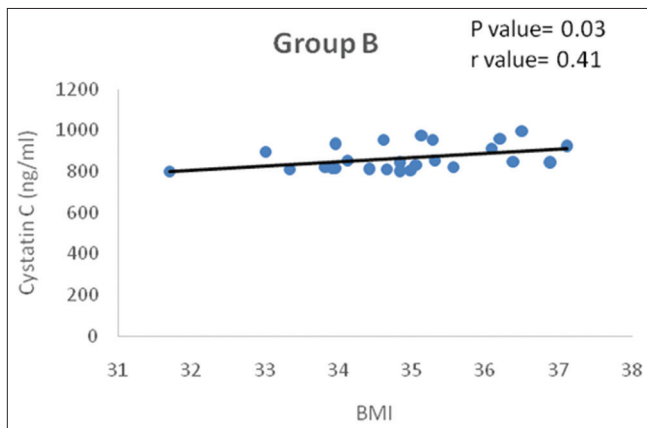

Figure 4: Cystatin C level in plain obese patients showed positive correlation with BMI ($r = 0.41$, $P = 0.03$)
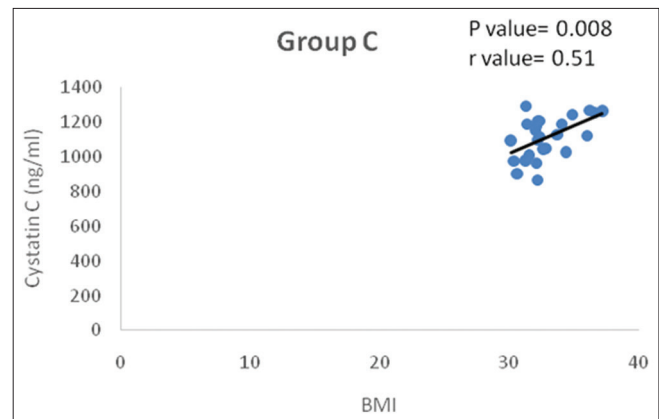

Figure 5: Cystatin C level in Type-2 DM with obesity showed a high positive correlation with BMI ($r = 0.51$, $P = 0.008$)

Table 4: Pearson's correlation analysis of health-related quality of life in diabetes, obese and diabetic obese patients						
Variables	Group A Diabetes non-obese		Group B Plain obese		Group C Diabetes obese	
	r	*P*	*r*	*P*	*r*	*P*
Physical health (Domain 1)	-0.41	0.60	-0.40	0.04	-0.43	0.03
Psychological health (Domain 2)	-0.20	0.33	0.06	0.76	0.19	0.35
Social relationship (Domain 3)	-0.05	0.79	0.10	0.62	0.37	0.06
Environment health (Domain 4)	-0.34	0.08	-0.12	0.55	0.19	0.35

Obesity is also a major factor in causing insulin resistance. These both play a major role in the pathophysiology of diabetes and the increasing level of HbA1c.[24] In our study, the HbA1c level was high because we took the rural population in our study and they have poor eating habits and they are not even aware of their disease and the reason for poor correlation was that we have taken the naïve patients.

The increased level of cystatin C also affects the quality of life measured by using WHOQOL-BREF. The physical activity of the patients having diabetes and obesity deteriorates with the increase in age.[27] In our study, we have also found the significant and the inverse correlation between cystatin C and the physical health of the patient in Group A and Group C. We have also found a significant correlation between the cystatin C and GFR, total cholesterol and age. It was observed that the patient having diabetes and are obese with the add on the factor of increased cystatin C level are more prone to have cardiovascular risk.[28]

Literature has reported that the patients who are obese or have abnormal metabolic function have an increase in the level of LDL, total cholesterol and VLDL and a significant low level of HDL.[29] There is a significant increase in the level of total cholesterol and LDL in diabetic obese patients than plain obese patients and there is a decrease in the formation of HDL in diabetic obese patients, this will lead to a substantial decrease in the level of HDL in type 2 diabetes obese patients.[30] In the present study, we have found a significant positive correlation of cystatin C with LDL and total cholesterol in Group C than Group B plain obese and there was no significant correlation with Group A diabetes non-obese. These raised parameters are associated with diabetes and obesity which can further lead to an increase in cardiovascular risk.

This study has several limitations. As it is a cross-sectional study the observation of the impact of cystatin C levels on the actual cardiovascular diseases could not be possible.

Conclusion

To the best of our knowledge, this is the first study to compare the cystatin C level between the newly diagnosed type 2 DM and type 2 DM with obese patients and plain obese patients. Cystatin C, though considered a renal marker, is associated with obesity and also causes insulin resistance in diabetes. The level of cystatin C is much higher in type 2 DM with obese patients as compared with type 2 DM and obese patients. Therefore, cystatin C adds to the list of adipose-secreted factors with the potential to affect adipose tissue biology and obesity-linked complications and could be a predictor of cardiovascular disease and metabolic syndrome. Furthermore, when the quality of life was assessed, it also shows that with the increase in the level of cystatin C that is found much more in type 2 DM obese patients there was a decline in the quality of life. Our study shows that with the increase in cystatin C level quality of life of person deteriorates. Obesity causes insulin resistance and cystatin C itself is a cause of insulin resistance

so if the person having obesity and high level of cystatin C is at increased risk of metabolic syndrome and cardiovascular diseases. Therefore, it can be counted as the early predictor of cardiovascular risk.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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