Study of Cyclophilin-A, High Sensitivity C-reactive Protein, and Malondialdehyde in Obese and Nonobese Type 2 Diabetes Mellitus Patients

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ABSTRACT

Aim and objectives: To study cyclophilin-A, high sensitivity C-reactive protein (hsCRP) and malondialdehyde in obese and nonobese type 2 diabetes mellitus (DM) patients.

Material and methods: A total of 120 subjects aged 35–65 years were enrolled and grouped as:
- Obese subjects with diabetes,
- Nonobese subjects with diabetes,
- Obese subjects without diabetes,
- Nonobese subjects without diabetes.

Their serum was tested for estimation of 4 biochemical parameters, namely cyclophilin-A, hsCRP, malondialdehyde, and lipid profile.

Observation and results: The mean cholesterol, triglycerides (TG), VLDL, LDL levels were significantly higher in obese diabetics as compared to obese nondiabetics, normal body mass index (BMI) diabetics, and control group. The mean HDL was higher in nondiabetic obese as compared to obese diabetic patients, normal BMI diabetics and control group and this difference was statistically insignificant. Cyclophilin-A and hsCRP were significantly higher in obese diabetics as compared to non-diabetic obese patients, Normal BMI diabetics and control group. The mean lipid peroxide (malondialdehyde) was significantly higher in nondiabetic obese patients, as compared to obese diabetic patients, normal BMI diabetics, and control group.

Conclusion: Cyclophilin-A (CypA) and hsCRP were significantly elevated in obese type 2 diabetics while malondialdehyde was significantly higher in nondiabetic Obese patients. Lipid profile parameters were also significantly elevated in obese type 2 diabetics. These inflammatory cardiometabolic risk biomarkers can be used for diagnostic, therapeutic and prognostic decision-making, especially in the context of inadequate quantitative risk assessments available to clinicians.

Keywords: Cyclophilin-A, High sensitivity C-reactive protein, Malondialdehyde, Obese, Type-2 diabetes mellitus patients.

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INTRODUCTION

According to the latest 2016 World Health Organization (WHO) data, an estimated 422 million adults are living with diabetes mellitus globally.1 Diabetes is growing alarmingly in India. India had 69.2 million people living with diabetes (8.7%) as per the 2015 data. Calling India, the diabetes capital of the world, the International Journal of Diabetes in Developing Countries say that there is an alarming rise in incidence in India.2 WHO project that diabetes will be the 7th leading cause of death in 2030.3 Obesity is a major independent and modifiable risk factor for type 2 diabetes mellitus (T2DM) and many epidemiological studies have suggested a progressive increase in the prevalence of T2DM with obesity.4,5

The etiology of obesity-related type 2 diabetes is multifactorial. Factors, such as insulin resistance, β-cell dysfunction, physical inactivity and body fat distribution are all inter-related and play a causal role in its development. Chronic hyperglycemia in diabetes is linked to long-term damage, dysfunction and failure of different organs, especially kidneys, nerves, eyes, heart and blood vessels.6

Type 2 diabetes mellitus (T2DM) is associated with the development of premature atherosclerosis, adding further to cardiovascular morbidity and mortality. The most common lipid abnormality noted in diabetics is Hypertriglyceridermia. In obese diabetics, the hepatic clearance of lipids and insulin are decreased as evident by studies showing increased levels of portal free fatty acids (FFA).

Cyclophilins are proteins belonging to the superfamily of immunophilins. The CypA is normally an intracellular protein. In diabetes, high glucose levels and reactive oxygen species activate monocytes to secrete CypA via vesicles. Secreted CypA acts as a proinflammatory cytokine that activates endothelial cells and leukocytes.

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increasing inflammation in vessels and promoting atherogenesis. Diabetes and atherosclerosis can affect one another, with CypA being one of the factors connecting diabetes and atherosclerosis. Therefore, we conducted a present study to evaluate CypA, hs-CRP, malondialdehyde and lipid profile in diagnosed cases of Obesity and T2DM and compare with healthy control.

**AIMS AND OBJECTIVES**

- To estimate levels of cyclophilin-A, high sensitivity C-reactive protein, malondialdehyde and lipid profile in diabetes and obese patients.
- To compare and correlate CypA, high sensitivity C-reactive protein (CRP), malondialdehyde and Lipid profile in study groups.

**MATERIALS AND METHODS**

A cross-sectional study was conducted in the Department of Biochemistry and the Department of General Medicine at MGM Hospital, Kamothe, Navi Mumbai from February 2017 to March 2018. The study was approved by the Institutional Ethics Review Committee. Patients from groups 1, 2 and 3 were enrolled from medicine ward. Group 4 was from the general population as well as the medicine ward. Total of 120 subjects was enrolled and grouped as mentioned below aged 35–65 years.

- **Group 1**: Obese subjects with diabetes
- **Group 2**: Nonobese subjects with diabetes
- **Group 3**: Obese subjects without diabetes
- **Group 4**: Nonobese subjects without diabetes

**Inclusion Criteria**

- **Group 1 (Obese subjects with diabetes)**: Proven diabetics as per WHO criteria, BMI >25 kg/m².
- **Group 2 (nonobese subjects with diabetes)**: Proven Diabetics as per WHO criteria and BMI <25 kg/m².
- **Group 3 (obese subjects without diabetes)**: BMI >25 kg/m².
- **Group 4 (nonobese subjects without diabetes)**: Patients without any cardiovascular event and BMI <25 kg/m².

**Exclusion Criteria**

Age less than 35 years and more than 65 years, with chronic liver disease, chronic renal disease, HIV patient, tuberculosis, asthma, malignancy, pPreanant women, any chronic inflammatory disease.

**Sample Collection**

Venous blood was collected under strict aseptic conditions in the plain bulb for serum for estimation of biochemical parameters—cyclophilin-A, CRP, malondialdehyde, lipid profile. All estimations were analyzed in the central clinical laboratory of MGM Hospital, Navi Mumbai. Plasma glucose, serum cholesterol, serum triglyceride, and serum HDL cholesterol were analyzed on AU480 autoanalyzer. Serum CypA was analyzed by Allianz-bio human CypA ELISA kit. Serum hsCRP was analyzed by turbidimetric immunoassay.

**Statistical Analysis**

All the collected data were entered in the Microsoft Excel sheet and then transferred to Statistical Package for the Social Sciences (SPSS) software ver. 17 for analysis. Qualitative data were presented as frequency and percentages and analyzed using the Chi-square test. Quantitative data were presented as mean and standard deviation (SD) and compared by analysis of variance ANOVA test. A p value <0.05 was taken as a level of significance.

**OBSERVATION AND RESULTS**

In our study, there were no significant differences in the mean age and sex distribution among the groups.

**ANOVA Test**

As seen in the Table 1, the mean cholesterol, TG, VLDL, LDL was significantly higher in obese diabetics as compared to obese patients, normal BMI diabetics and control group. The mean HDL was higher in obese as compared to obese diabetics patients, normal BMI diabetics and control group and this difference was statistically insignificant, depicted in Graph 1.

As seen in Table 2, the mean hsCRP and cyclophilin-A were significantly higher in obese diabetics as compared to obese patients, normal BMI diabetics and control group. The mean lipid peroxide (malondialdehyde) was significantly higher in obese as compared to obese diabetics patients (2.86 ± 0.65), normal BMI diabetics and control group, depicted in Graph 2.

**DISCUSSION**

Obesity is associated with a chronic low-grade inflammation, as evidenced by an increase in circulating inflammatory markers, such as CRP. The presence of systemic inflammation in visceral obesity has been linked to an increased risk of developing CVD and type 2 diabetes. Obesity results when there is an imbalance between energy ingested and energy expended. A relative excess of energy (either genetic or diet-induced) results in two major cellular features; adipocyte expansion and infiltration of inflammatory cells into adipose tissue in both mice and humans.
Adipocytes and macrophages both generate inflammatory molecules, which lead to insulin resistance and systemic inflammation.

In this study, there were no significant differences in the mean age and sex distribution among the groups. These findings are in agreement with the study conducted by Lamiaa et al.20

In the present study, lipid profile parameters like cholesterol, TG, VLDL, LDL was significantly higher in obese diabetics as compared to obese patients, normal BMI diabetics and control group. While the mean HDL was higher in obese as compared to obese diabetics patients, normal BMI diabetics and control group and this difference was statistically insignificant (p < 0.001). This finding is consistent with the Lamiaa et al. study, which showed a significant increase in total cholesterol, LDL-C, non-HDL-C and triglycerides in obese subjects with and without diabetes, while HDL-C was significantly reduced.20

The mean cholesterol, TG and LDL was higher in obese diabetics as compared to obese patients and control group. The significant differences in lipid profile markers between healthy and obese non-diabetic groups were consistent with the study of Khan and Khaleel et al., whereas the study of Songa et al. did not show significant differences in lipid profile markers between healthy and obese nondiabetic groups.21,22 This did not match our results. Both studies and many other similar studies, Yadav et al however, agrees with our findings regarding the significant difference in the lipid profile of diabetic patients and controls.23 Lipid profile parameters differ significantly in obese diabetic and nondiabetic subjects, due to the fact that the insensitivity of adipose cells and other target tissues to insulin (insulin resistance), clearly seen in obesity and T2DM, leads to dysregulation of enzymes such as lipoprotein lipase, leading to increased and extended lipemia and failure to clear plasma triglycerides rapidly.24

Dyslipidemia in the two diabetic groups is supported by the following facts and theories. Insulin is known to have major regulatory influence on lipid metabolism, and

| Table 1: Lipid profile parameters amongst different study population |
|-----------------------|--------|--------|--------|--------|------------|
|                       | Healthy control | Obese patients | Normal BMI diabetics | Obese diabetics | p value   |
| Cholesterol (mg/dL)   | 147.16 ± 31.65 | 195.13 ± 21.27 | 167.86 ± 39.10 | 216.16 ± 27.44 | 0.0001    |
| TG (mg/dL)            | 101.53 ± 21.47 | 151.23 ± 59.04 | 118.36 ± 52.88 | 175.2 ± 92.16  | 0.0001    |
| HDL (mg/dL)           | 4.16 ± 11.41  | 5.76 ± 63.52   | 5.46 ± 16.93   | 52.1 ± 63.52   | 0.633     |
| VLDL (mg/dL)          | 20.16 ± 4.29  | 32.33 ± 17.18  | 23.76 ± 10.53  | 48.16 ± 41.43  | 0.0001    |
| LDL (mg/dL)           | 92.76 ± 9.96  | 122.03 ± 33.85 | 106.96 ± 89.22 | 122.36 ± 42.23 | 0.0001    |

| Table 2: Mean hsCRP, cyclophilin-A, and malondialdehyde amongst different study population |
|-----------------------------------------------|-----------------|-----------------|-----------------|-----------------|-----------|
|                                               | Healthy Control | Obese patients | Normal BMI diabetics | Obese diabetics | p value |
| hsCRP (mg/L)                                 | 1.06 ± 0.52     | 7.8 ± 1.91      | 2.73 ± 0.73      | 8.5 ± 2.11      | 0.0001   |
| Cyclophilin-A (ng/mL)                        | 3.33 ± 2.13     | 17.9 ± 9.42     | 12.44 ± 3.96     | 17.13 ± 5.07    | 0.0001   |
| Malondialdehyde (μmol/L)                     | 1.39 ± 0.16     | 3.20 ± 0.35     | 2.38 ± 0.24      | 2.86 ± 0.65     | 0.0001   |
the effects of primary abnormalities in lipid metabolism on insulin resistance contribute to pathogenesis of diabetes. Dyslipidemia in type 2 diabetes is characterized by an increased level of triglyceride and decreased high-density lipoprotein cholesterol (HDL-C), which is known to be present for many years before diabetic hyperglycemia begins. The mechanism by which hypertriglyceridemia occurs in diabetes is fairly well understood. Levels of non-esterified fatty acids (NEFAs) are increased because, in many patients, the adipose tissue mass from which these are released, particularly the more metabolically active and centrally distributed adipose, is increased. Hormone-sensitive lipase, the intracellular lipase present in adipose tissue is activated by the insulin deficiency or resistance present in diabetes. This increases the release of NEFAs from triglyceride stored in adipose tissue. High circulating levels of NEFAs increase hepatic triglyceride production. In the present study, biomarkers like hsCRP and CypA was significantly raised in obese diabetics as compared to obese patients, normal BMI diabetics and control group while the mean malondialdehyde was significantly higher in obese as compared to obese diabetics patients, normal BMI diabetics and control group. These findings are consistent with the study conducted by Lamiaa et al., in which the inflammatory markers (hsCRP) in the obese group increased compared to the controls. Similarly, Ramachandran et al. in their report showed that CypA which is secreted by monocytes and plays vital role in proinflammatory stimulus for vascular inflammation and diabetes. Satoh et al., also reported higher level of CypA in diabetic patients with coronary artery disease as compared to healthy volunteers, signifying its role in increasing vascular disease in type 2 diabetes. The hsCRP appears strongly associated with diabetes mellitus and resistance to insulin. These findings are consistent with the study conducted by Safiullah Amanullah et al., the hsCRP levels in diabetic subjects were increased in comparison with non-diabetic subjects. Several studies have shown that hsCRP predicts diabetes in western populations earlier. Goodarzi et al. support the correlation between the degree of hyperglycemia and oxidative stress. Gillery et al., explains that hyperglycemia generates an increase of the intensity of the reactions of nonenzymatic glycation proteins that are associated with oxidative stress, well described in patients with diabetes. Abdul-Ghani et al., in their study reported that type 2 diabetic participants had an increased rate of mitochondrial ROS production compared with age and BMI-matched non-diabetic participants.

CONCLUSION
C-reactive protein (CRP), CypA were significantly elevated in obese type-2 diabetics while malondialdehyde was significantly higher in obese patients. Lipid profile parameters were also significantly elevated in obese type-2 diabetics. These cardiometabolic risk inflammatory biomarkers play a critical role in diagnostic, therapeutic and prognostic decision making, especially in the context of insufficient quantitative risk assessments available to clinicians. In order to reduce modifiable risk factors, this could significantly reduce the burden of complications on patients and the healthcare system.

REFERENCES