Section: Biochemistry

Evaluation of Inflammatory Marker hsCRP, MDA, HbA1c and Lipid Profile in Type 2 Diabetic Subjects: A Comparative Study.

Uday Kumar Gupta[1], Tapan Kumar Mohapatra[2], Pratishtha Potdar[3]

[1]PG Student, Department of Biochemistry, Santosh Medical College & Hospital, Ghaziabad, U. P.
[2]Professor and Head, Department of Biochemistry, Santosh Medical College & Hospital, Ghaziabad, U. P.
[3]Associate Professor, Department of Anatomy, Santosh Medical College & Hospital, Ghaziabad, U. P.

ABSTRACT

Background: Diabetes mellitus is considered to be a state of persistent low grade inflammation which contributes to the pathogenesis of disease. Inflammation is a state of local protective response to tissue injury. In addition to local response, systemic response called as acute-phase response is depicted by the changes in levels of acute phase reactants like C-Reactive Protein(CRP), complement system proteins, serum amyloid A, haptoglobin and fibrinogen. **Methods:** 50 Type 2 Diabetic patients and 50 non diabetic subjects between the age group of 30-65 years who were all attending in OPD at Santosh Medical college and Hospital Ghaziabad, were selected for the study. **Results:** Both the study and control groups were comparable with respect to age (p 0.012). The study group had higher body mass index(p 0.0003), higher fasting blood glucose (p<0.0001), higher 2 –hour postprandial blood glucose (p<0.0001), higher HbA1c levels (p<0.0001), higher serum total cholesterol (p<0.0001), higher triglyceride levels(p<0.0001), higher LDL cholesterol(p<0.0001), and lower HDL cholesterol levels(p<0.0001), higher hsCRP level (p<0.0001), higher MDA level (p<0.0001) and Lower Thiols level (p<0.0001) in type 2 DM subjects. **Conclusion:** In conclusion, the mean concentrations of fasting & postprandial serum glucose, lipids, high-sensitivity C-reactive protein, MDA, & glycated hemoglobin were significantly increased in type 2 diabetic subjects when compared to controls. There was no significant increase in plasma protein Thiols levels found in type 2 diabetic subjects when compared to controls.

Keywords: Type 2 Diabetic, hs-CRP, MDA.

INTRODUCTION

Diabetes mellitus is considered to be a state of persistent low grade inflammation which contributes to the pathogenesis of disease.[1] Inflammation is a state of local protective response to tissue injury.[2] In addition to local response, systemic response called as acute-phase response is depicted by the changes in levels of acute phase reactants like C-Reactive Protein(CRP), complement system proteins, serum amyloid A, haptoglobin and fibrinogen.[3] Patients with diabetes mellitus aggravate other co-morbidities like hypertension, obesity and dyslipidemia which in turn increase the risk for Cardio Vascular Disease (CVD).[4] C reactive protein, measured as high sensitivity C reactive protein (hs-CRP), an acute phase protein is produced by the liver and their levels increase whenever there is instances of inflammation in the body.[5] CRP may also rise in acute Coronary Syndrome, arthritis, autoimmune disease, inflammatory bowel disease, pancreatitis, colitis and carcinoma. CRP testing cannot be used to diagnose specific diseases but serves more as a general indicator of inflammation or infection.[6] Numerous epidemiologic studies done in United States and Europe have concluded high-sensitivity C-reactive protein (hs-CRP) to be a predictor of future coronary events among apparently healthy individuals.[7]

In type-2 DM, insulin resistance is the primary event, followed by increasing degree of β-cell dysfunction.[8] Chronic, systemic subclinical inflammation has also been identified as a driving force for insulin resistance, metabolic syndrome, and type 2 DM.[9] The process of inflammation induces hepatic synthesis of various acute phase proteins such as serum ferritin and high sensitivity C-reactive protein (hs-CRP), which is believed to play a role in insulin resistance as well as atherosclerosis.[10] Serum levels of hs-CRP have been found to be a strong predictor for increased cardiovascular disease risk associated with type 2 DM. Higher incidence of

Name & Address of Corresponding Author
Dr Uday Kumar Gupta
PG Student, Department of Biochemistry,
Santosh Medical College & Hospital,
Ghaziabad-201009,
U. P.

type 2 DM has been observed with high levels of hs-CRP.[9,11]

These high-sensitivity assays help quantify low grades of systemic inflammation, in the absence of overt systemic inflammatory or immunologic disorders.[12] The hs-CRP provide a sensitive marker of increased inflammatory activity in the arterial wall.[13,14] The hs-CRP is the measurement of CRP level with greater accuracy. On the basis of data obtained from population based studies, the American Heart Association/Centres for Disease Control working group on markers of inflammation in CVD has classified serum hs-CRP levels <1, 1–3 and >3 mg/L as low-, intermediate-, and high-risk groups for global CVD, respectively.[15] There is no enough data of serum hs-CRP level in normal non-diabetic people, but in some studies have shown that Asian people have comparatively higher (17%) level of CRP.[16-18] Elevated levels of hs-CRP have been observed in Indians.[19-21] Aim of this present was to estimate the levels of Inflammatory Marker hsCRP, MDA, HbA1c and Lipid Profile in Type 2 Diabetic Subjects and controls.

MATERIALS AND METHODS

This present study was conducted in the Department of Biochemistry, Santosh Medical College and Hospital, Ghaziabad. 50 Type 2 Diabetic patients and 50 non diabetic subjects between the age group of 30-65 years who were all attending in OPD at Santosh Medical College and Hospital Ghaziabad, were selected for the study. All the patients were included as cases evaluated and diagnosed as Type 2 Diabetes mellitus on the basis of history and Biochemical investigations during the period July-2017 to August-2018 according to inclusion and exclusion criteria. The study was approved by Santosh Medical College and Hospital, Ghaziabad. Informed consent was obtained from all the participants.

Biochemical Investigation:

5ml of fasting venous blood samples were collected in clot activator coated polypropylene tubes by venupuncture under strict aseptic precaution as soon as the subjects got admitted as per the inclusion criteria. Similar way 2 hours post prandial also collected. Blood samples were centrifuged at 3000 rpm used for 10 minutes and serum was separated. 8-12 hours fasting samples, 2 hours post prandial samples were collected from all subjects during their hospital visit and analysis of below said parameters were done. (Fasting and Post prandial blood glucose, Glycated Hemoglobin (HbA1c), Fasting Serum Lipid parameters which include (Total cholesterol, Triglycerides, LDL-cholesterol, HDL- cholesterol), Serum high sensitivity C-reactive protein (hs-CRP), Serum Malondialdehyde (MDA) and Plasma protein

Thiols.

Statistical Analysis

All the data was initially entered to Microsoft Excel 2010 and later these spreadsheets were used for analysis. Statistical analysis was done by using SPSS version 20.0. We used student t-test and pearson's correlation coefficient to find the statistical significance. A P-value <0.05 was to be considered statistically significant.

RESULTS & DISCUSSION

This present study consists of 50 type 2 diabetic patients which included 27 males and 23 females. Similarly, the control group also had 50 (29 males and 21 females) Non diabetic subjects. [Figure 1]
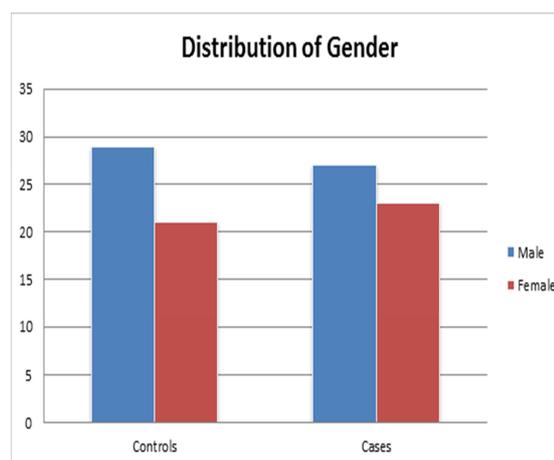

**Figure 1: Shows the distribution of gender in the study group and control group.**

Table 1: Distribution of cases and controls by age group

Age group in years	Control group		Study group	
	Number (n=50)	Percentage (%)	Number (n=50)	Percentage (%)
30-35	08	16.0	05	10.0
35-40	14	28.0	10	20.0
40-45	14	28.0	12	24.0
45-50	09	18.0	10	20.0
50-55	03	6.0	04	8.0
55-60	02	4.0	09	18.0
60-65	0	00	0	00
Total	50	100	50	100

[Table 1 & Figure 2] In the study group, age of the patients ranged from 30 to 59 years, with a mean and standard deviation of 44.36 and 7.57. The majority of the patients belonged to the age group 35-45 years (56%). Among the controls, age ranged from 30 to 59 years, the mean and standard deviation were 41.0 and 6.2 respectively. Majority were in the age group 40-45 years (24%).

Table 2: Distribution of BMI across the groups (n=100)

BMI (kg/m2)	Control group		Study group	
	Number (n=50)	Percentage (%)	Number (n=50)	Percentage (%)
<18.5	0	00	0	00
18.5 – 24.9	38	76.0	23	46.0
25 – 29.9	12	24.0	25	50.0
30 -34.9	0	00	2	4.0
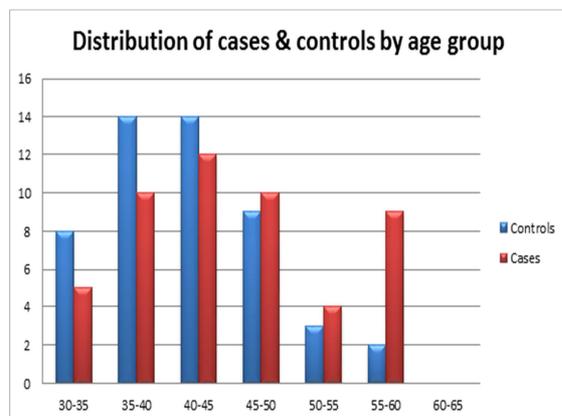
Figure 2: Shows the distribution of cases and controls by age group.
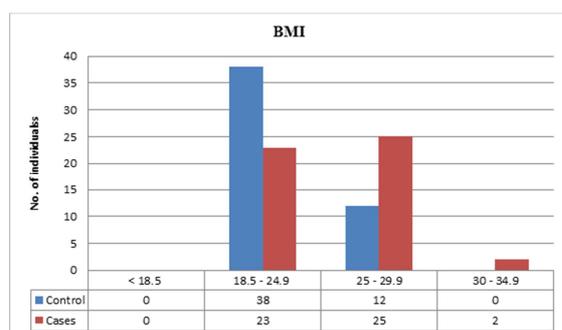
Figure 3: Distribution of cases and controls according to Body Mass Index.

[Table 2 & Figure 3] Shows the BMI of the study group ranged from 19.8 to 32.9 kg/m2. The mean and standard deviation were 23.93 and 1.26 respectively. Among the controls, BMI varied from 21.5 to 26.2 kg/m2 with a mean of 25.54 and a standard deviation of 2.59.

Blood Glucose
The study group had fasting blood glucose of 138.3 ± 17.05 mg/dL and 2-hour postprandial blood glucose of 221.2 ± 33.27 mg/dL. In the control group, the values were 83.16 ± 7.65 mg/dL and 115.9 ± 8.32 mg/dL respectively [Table 3].

Glycemic Control
Glycemic control was measured by using the level of HbA1c (glycosylated haemoglobin) in blood. In the study group, HbA1c varied from 6.6 to 9.2 % with a mean and standard deviation of 7.95 and 0.84 % respectively.
In the control group, the value ranged from 4.1 to 5.4 % with a mean of 4.91 and a standard deviation

of 0.33 %. Patients in the study group were divided into two groups based on the level of HbA1c. 28 patients had HbA1c ≥8 %, implying poor glycemic control.

Lipid profile
Serum total cholesterol (TC)
In the study group, the value of serum total cholesterol ranged between 156 to 281 mg/dL with a mean value of 211.76 mg/dL and a standard deviation of 33.81 mg/dL. In the control group, the value ranged from 152 to 212 mg/dL with a mean value of 177.0 mg/dL and a standard deviation of 13.33 mg/dL.

Table 3: Comparison of study variables in test and control groups

Variables	Control group(n=50) [Mean ± S.D.]	Study group(n=50) [Mean ± S.D.]	P value
Age in years	41.0± 6.2	44.36 ± 7.57	0.012
Height in cm	161.36 ± 5.95	159.52 ± 8.83	0.25
Weight in Kg	62.4 ± 5.47	64.54 ± 5.62	0.06
BMI	23.93 ± 1.27	25.54 ± 2.60	0.0003
FBS mg/dl	83.16 ± 7.65	138.3 ± 17.05	0.0001
PPBS mg/dl	115.9 ± 8.32	221.2 ± 33.27	0.0001
HbA1c %	4.91 ± 0.33	7.95 ± 0.84	0.0001
T. cholesterol mg/dl	177.0 ± 13.33	211.76 ± 33.81	0.0001
Triglycerides mg/dl	138.06 ± 18.29	184.48 ± 31.81	0.001
HDL mg/dl	51.48 ± 5.26	38.54 ± 9.01	0.0001
LDL mg/dl	96.48 ± 10.89	138.32 ± 34.82	0.0001
hs-CRP mg/L	2.32 ± 0.63	3.40 ± 1.14	0.0001
MDA nmol/ml	1.66 ± 0.35	2.93 ± 0.56	0.0001
Thiols µmol/L	705.32 ± 159.50	542.78 ± 155.09	0.0001

Staitistically significant P <0.05

Triglycerides (TG)
In the study group, the value of triglycerides ranged from 131 to 243 mg/dL with a mean value of 184.48 mg/dL and a standard deviation of 31.81 mg/dL.
In the control group, the value ranged from 84 to 188 mg/dL with a mean value of 138.06 mg/dL and a standard deviation of 18.29 mg/dL.

High Density Lipoprotein (HDL)
In the study group, HDL value ranged from 30 to 56 mg/dL with a mean value of 38.54 mg/dL and a standard deviation of 9.01 mg/dL. In the control group, HDL value ranged from 40 to 58 mg/dL with a mean value of 51.48 mg/dL and a standard deviation of 5.26 mg/dL.

Low Density Lipoprotein (LDL)

In the study group, LDL values ranged from 93 to 202 mg/dL with a mean value of 138.32 mg/dL and a standard deviation of 34.82 mg/dL. In the control group, LDL values ranged from 75 to 131 mg/dL with a mean value of 96.48 mg/dL and a standard deviation of 10.89 mg/dL.

Hs- CRP:

The mean hsCRP level in type 2 DM subjects was 3.43 mg/L and standard deviation is 1.14 mg/L. In the control group, the value was 2.32 mg/L and standard deviation is 0.63 mg/L. For 42 diabetic patients, hsCRP was > 3.0 mg/dL (high risk).

MDA:

The mean MDA level in type 2 DM subjects was 2.93 nmol/ml and standard deviation is 0.56 nmol/ml. In the control group, the value was 1.66 nmol/ml and standard deviation is 0.35 nmol/ml.

Thiols:

The mean Thiols level in type 2 DM subjects was 542.78 µmol/L and standard deviation is 155.09 µmol/L. In the control group, the value was 705.32 µmol/L and standard deviation is 159.50 µmol/L.

Both the study and control groups were comparable with respect to age (p 0.012). The study group had higher body mass index(p 0.0003), higher fasting blood glucose (p<0.0001), higher 2 –hour postprandial blood glucose (p<0.0001), higher HbA1C levels (p<0.0001), higher serum total cholesterol (p<0.0001), higher triglyceride levels(p<0.0001), higher LDL cholesterol(p<0.0001), and lower HDL cholesterol levels(p<0.0001), higher hsCRP level (p<0.0001), higher MDA level (p<0.0001) and Lower Thiols level (p<0.0001) in type 2 DM subjects.

Nowadays, diabetes mellitus is one of the most important causes of death all over the world because of its adverse effects on cardiovascular system and is one of the important factors that may influence metabolic status. Type 2 diabetes mellitus is a chronic metabolic disease and is associated with numerous micro-vascular and macro-vascular complications. This reduces the life expectancy of the patients by ten years. Inflammatory biomarkers may be of valuable tool for risk evaluation. Among them best evidence to date supports the use of high-sensitivity C-reactive protein to monitor cardiovascular risk in diabetic and non-diabetic individuals. Various studies have shown the relationship between oxidative stress, inflammation and the pathogenesis of type 2 diabetes mellitus, implicating their role in the occurrence of complications.[22,23] Cardiovascular complications have a major role in increasing the morbidity and mortality in type 2 diabetes mellitus patients.[24] Persistent hyperglycemic state is known to induce Oxidative stress which in turn has been shown to cause endothelial damage. This initiates the events Leading to the pathogenesis of micro and macro-vascular diseases. Oxidative stress is known to increase the expression of pro-inflammatory markers and pro-coagulant factors. It also induces apoptosis and impairs the release of nitric oxide.[25] This study was done to compare the oxidant and inflammatory status in type 2 diabetes mellitus patients, by measuring their fasting blood sugar, postprandial blood sugar, HbA1c, lipids, MDA, plasma protein thiols and hs-CRP levels.

CONCLUSION

In conclusion, the mean concentrations of fasting & postprandial serum glucose, lipids, high-sensitivity C-reactive protein, MDA, & glycated hemoglobin were significantly increased in type 2 diabetic subjects when compared to controls. There was no significant increase in plasma protein Thiols levels found in type 2 diabetic subjects when compared to controls. Our study shows that significant increase in LDL Cholesterol and high sensitive C-reactive protein may lead to cause risk of Cardio Vascular Disease and atherosclerosis etc., Elevated hsCRP levels in addition to lipid profile screening may be a valuable tool to predict future CVD risk.

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