



## Original Research Article

## Study of hs-CRP and insulin resistance in type 2 diabetic subjects

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## ABSTRACT

**Introduction:** A main cause of morbidity and mortality in Worldwide is Diabetes mellitus. Acute phase reactant like hs-CRP acts as a non-specific marker of inflammation. Action of insulin becomes resistant on peripheral tissues of human body in Insulin resistance (IR). Pathogenesis of type - 2 diabetes mellitus is strongly linked with Insulin resistance.

**Aim:** The aims of this study is to assess between type-2 diabetic subjects and non-diabetic healthy subjects about the status of pro- inflammatory markers as hs-CRP and IR.

**Material and Methods:** This study was conducted in Govt. Medical College, Shivpuri (MP). A total of 200 subjects were included in this study. Out of these, 100 were type-2 diabetes mellitus cases and 100 were non diabetic healthy subjects. They were evaluated by measurement of several physiological and biochemical parameters such as Blood Pressure, Body Mass Index, blood parameters as –Fasting Blood Sugar, Post Prandial, HbA1c, Triglyceride, Total Cholesterol, Low Density Lipoprotein, Very Low Density Lipoprotein, High Density Lipoprotein, high sensitive-C Reactive Protein, Fasting Insulin level and Homeostasis Model Assessment of Insulin Resistance.

**Result:** hs-CRP is usually extensively increased in diabetic cases. The raised level of hs-CRP is noteworthy in diabetes mellitus cases as compared to healthy subjects. Serum Total Cholesterol, Triglyceride, VLDL and LDL levels were highly increased, whereas HDL level were significantly lower in patients of diabetes when compared to controls. hs-CRP had considerable positive correlation with insulin resistance markers and whereas negative associations with insulin sensitivity markers.

**Conclusion:** hs-CRP and IR were strongly related with the metabolic variables and markers of Type- 2 diabetes mellitus subjects with and without complication. Results of this study show that hs-CRP can play a major role in the early detection of IR in diabetes mellitus cases.

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## 1. Introduction

Diabetes mellitus occurs due to insufficient or inefficient insulin leads to hyperglycaemia. The prevalence of type- 2 diabetes mellitus has been increasing day by day worldwide at this time.<sup>1</sup> Macro and micro vascular complications in patients of type - 2 diabetes mellitus shows that it leads to heart disease, retinopathy, neuropathy and nephropathy etc.<sup>2</sup>

Insulin resistance is a condition in which the peripheral tissues of the human body shows resistance to the action

of insulin, it is aggravated factors like type - 2 diabetes mellitus, obesity, hypertension etc. Insulin resistance is strongly related with the development of type- 2 diabetes. Therefore, it is necessary to identify the various risk factors for developing insulin resistance, so that preventive procedures can be developed.

Obesity also acts as a risk factor for various chronic diseases including type-2 diabetes and insulin resistance.<sup>3,4</sup> An increase in level of serum high-sensitivity C-reactive protein (hs-CRP) is broadly accepted as a significant risk factor for the development of cardiovascular disease (CVD) also.<sup>5-7</sup>

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High sensitive C-reactive protein (an acute-phase reactant protein produced by liver) is an extreme sensitive marker for inflammation. It is found that chronic inflammation of low grade as revealed by increased level of hs-CRP might potentially be a cause underlying the etiology and manifestation of type- 2 diabetes.<sup>8,9</sup>

hs-CRP, an acute phase protein produced primarily by liver cells under the influence of cytokines such as Tumor Necrosis Factor  $-\alpha$  and Interleukin-6.<sup>10</sup> Major features of T-2DM include insulin resistance, glucose intolerance, hyperinsulinemia, hyperglycemia, and dyslipidemia.<sup>11</sup>

The aim was to assess whether high sensitive-C Reactive Protein can be used as a biomarker to detect insulin resistance in type - 2 diabetes mellitus and healthy adults in Greater Gwalior region in India.

## 2. Materials and Methods

The study was carried out in Government Medical College, Shivpuri (MP). Subjects were selected from Department of Medicine of Medical College and those attending the medical outpatient camps at Shivpuri as well as Gwalior region of MP. The study included a total number of 200 subjects, diagnosis being based on duration of type-2diabetes mellitus. The patients were divided into two groups-

Group I: 100 Healthy individuals free from any diabetic complications and diabetes

Group II: 100 type- 2 Diabetic patients with complications (60men and 40 women).

The inclusion criteria for the selection of diabetic subjects was high blood sugar value – fasting  $> 126\text{mg/dl}$  and Post Prandial  $>200\text{ mg/dl}$ , duration of diabetes mellitus. The exclusion criteria- diabetic subjects having other complications, smokers, more alcohol intake etc.

A written consent was obtained from the patients. A questionnaire regarding the demographic data like age, sex, height (cm), body weight (kg) and duration of diabetes mellitus were measured while using light weight clothing but not shoes. Smoking habit, vegetarian and non-vegetarian, family history of diabetes mellitus, Blood pressure, hypertension, obesity, MI and renal disease were also noted for each patient. Approval for conducting this study was granted from the Institutional Ethical Committee of Government Medical College, Shivpuri (MP).

To evaluate the association between T-2DM and hs-CRP levels were used by Multivariate logistic regression models, both as continuous and discrete traits, with age, sex, height (cm), body weight in kg and BMI as covariates. hs-CRP levels were classified into three groups, low risk ( $<1\text{ mg/liter}$ ), intermediate risk ( $1-3\text{ mg/liter}$ ), and high risk ( $>3\text{mg/liter}$ ), according to the AHA and CDC recommendations. All statistical values were analysed by using SPSS version 22.0. The P- values  $<0.001$  were noted as the level of significance.

Fasting blood samples (venous) were collected from both study groups, the samples were centrifuged, serum was separated and kept at  $4^{\circ}\text{C}$ .

hs-CRP (by using a latex-immunoturbidimetric high sensitivity method based on the principle of agglutination reaction) were measured in the laboratory according to manufacturer's protocol(POINTE SCIENTIFIC, INC.5449 RESEARCH DRIVE, CANTON, MI 48188). Plasma levels of fasting insulin were measured by using a sandwich ELISA kit method (LDN LABOR DIAGNOSTIKA NORD, ELISA Kit, GmbH & Co. KG) based on the sandwich principle). Estimation of serum glucose was done by Glucose Oxidase- Peroxidase (GOD -POD) method.

Lipid profile were determined according to manufacturer's protocol such as Serum triglycerides by Tinder's GPO-POD enzymatic method, Serum total cholesterol by Cholesterol Oxidase –Peroxidase enzymatic method and serum HDL by Phosphotungstic acid method. Serum LDL and VLDL values were calculated by Friedewald's formula.

## 3. Result

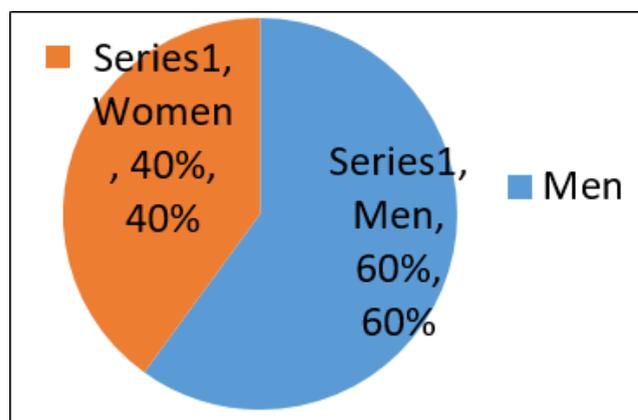
The study was done on 100 patients of type-2 diabetes cases and 100 controls. The mean age of the patients was  $52\pm 8.24$  years (Range- 35 to 75). Comparison of means of serum biochemical markers between type-2 diabetic group and healthy groups is presented in Table 1. The values of all these biochemical study parameters such as FBS, Glycosylated hemoglobin (HbA1c), TC, TG, VLDL, LDL, hs-CRP, Fasting plasma Insulin level and HOMA-IR except HDL were increased in diabetic cases as compared to healthy subjects and the observations were found to be statistically significant (P value  $<0.01$  and  $<0.001$ ).

The P value of 0.01 and 0.001 was found statistically significant. Simple (Pearson Correlation) correlation coefficients between hs-CRP and various parameters were calculated. We observed positively correlated with fasting glucose (0.684), HbA1c (0.612), total cholesterol (0.696), triglycerides (0.658), LDL (0.524), fasting insulin (0.386) and homeostasis model assessment of insulin resistance (HOMA-IR) (0.422) and negatively with HDL (-0.432) among groups.

Insulin resistance was calculated by using HOMA-IR calculator.

$$\text{HOMA-IR} = \text{Fasting plasma insulin } (\mu\text{U/L}) \times \text{fasting glucose (mg/dl)} / 405$$

The diabetic group had significantly higher BMI, FBS, fasting insulin ( $17.32\pm 2.10\mu\text{U/ml}$  vs  $6.13\pm 1.26\mu\text{U/ml}$ ),HOMA-IR and hs-CRP ( $3.64 \pm 1.38\text{ mg/l}$  vs  $1.28 \pm 0.68\text{mg/l}$ ) than the healthy control group.



**Fig. 1:** Hundred diagnosed type-2 diabetes mellitus patients age between 35-75 years were selected for this cross sectional study. Out of which 60 (60%) were males and 40 (40%) females

**Table 1:** Comparison of physiological and biochemical parameters between study groups

Study Variables	Healthy Controls=100	Type 2 Diabetic Subjects=100
Age (years)	48±8.80	52±8.24
Duration of diabetes (years)	-	12.5±4.32
BMI (kg/m <sup>2</sup> )	21.42± 1.56	27.62± 3.68*
BP Systolic (mm/Hg)	106.6±4.18	166.85±3.89
BP Diastolic (mm/Hg)	78.6±1.28	110.85±4.85
FBS(mg/dl)	86.22±12.26	188.24±37.7**
HbA1c(%)	4.6±2.26	10.2±2.26**
Total Cholesterol (mg/dl)	158.3±24.09	252.4±41.9**
TG (mg/dl)	108.04±19.49	212.7±45.05*
HDL (mg/dl)	46.45±6.14	28.27±7.02
LDL (mg/dl)	110.1±16.20	158.7±39.3**
VLDL (mg/dl)	29.38±4.09	45.89±11.3
Fasting plasma Insulin(μU/ml)	6.14±1.24	16.42±2.12**
HOMA-IR	1.58 ± 0.88	6.50 ± 1.64
hs-CRP (mg/l)	1.28 ± 0.68	3.64 ± 1.38*

\* Significant at P<0.01, \*\* significant at P<0.001

**Table 2:** Pearson correlation studied of hs-CRP with the other biochemical parameters in Type-2 Diabetic groups

Biochemical Parameters	r
FBS	0.684*
HbA1c	0.612*
Total Cholesterol	0.696*
Triglyceride	0.658*
LDL	0.524*
HDL	-0.432*
Fasting plasma Insulin	0.386*
HOMA-IR	0.422*

\* Significant at P<0.001

#### 4. Discussion

Our study showed Fasting Blood Glucose levels in type 2 diabetic group (mean 188.24±37.7) were higher than control group (mean 86.22±12.26). A significant difference was observed (p<0.001) in mean FBG level in Type-2 diabetic cases when compared with controls.

Glycosylated haemoglobin (HbA1c) was also elevated in diabetic groups.<sup>12-14</sup> The increased level of HbA1c is due to post-translational, spontaneous and nonenzymatic (Glycation) addition of glucose present in both outside and inside of the cells favouring attachment of glucose with each β-chain of HbA at N-terminal valine, resulting in progressive glycation end products (HbA1c).<sup>15</sup> We also found that the average values of Total Cholesterol, Triglycerides, Low Density Lipoprotein and Very Low Density Lipoprotein were significantly increased whereas High Density Lipoprotein was also found decreased in type- 2 diabetic subjects as compared to healthy and the results were statistically significant. The Positive correlation coefficient of Total Cholesterol, Triglycerides, Low Density Lipoprotein and Very Low Density Lipoprotein were observed with hs-CRP in type-2 diabetic patients.

Insulin resistance and Inflammation are commonly found to be associated with various chronic diseases as type-2 diabetes.<sup>16-18</sup> Measurement of inflammatory markers might be helpful for the prediction of the vascular risk in Type-2 diabetes mellitus cases. Inflammation is related with pathogenesis of Type-2 diabetes mellitus. IR and hyperglycemia are also promoting inflammation by increased oxidative stress and that may link diabetes mellitus to the atherosclerosis development.<sup>19</sup> Increased levels of hs-CRP commonly well-established risk factors of T2DM as well as IR, obesity and hypertension.<sup>20</sup> Obesity and IR have been well known risk factors for the major health issues such as type -2 diabetes, dyslipidemia, hypertension and cardiovascular diseases etc. Central obesity is linked and played a main role in the development of IR and Type-2 diabetes mellitus.<sup>21</sup> Increased levels of hs-CRP is responsible for the development of systemic inflammation as it is a well known risk factor for the development of Type-2 diabetes.<sup>9,22,23</sup>

#### 5. Conclusion

The results showed that hs-CRP strongly related with type-2 diabetes patients and the metabolic variables of Type- 2 diabetes mellitus patients with and without complications in subjects. The hs-CRP may be considered as a predictor for the development of cardiovascular diseases, nephropathy, retinopathy and neuropathy in Type - 2 diabetic populations. Clinical study observations indicate the benefit of early insulin resistance treatment to oppose Type- 2 Diabetes in patients with raised level of hs-CRP.

## 6. Source of Funding

None.

## 7. Conflict of Interest

None.

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