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"TO STUDY THE EFFECT OF INSULIN RESISTANCE AND HS-CRP IN TYPE 2 DIABETES MELLITUS AND PRE-DIABETES INDIVIDUALS."

Poorvi Gupta* (PhD Scholar) Department Of Biochemistry, People's College of Medical Sciences & Research Center, Bhopal(MP). *Corresponding Author

Dr. P.J.Hisalkar (HOD &Professor), Department of Biochemistry,Government Medical College & Associated Group of Hospitals Dungarpur, (Rajasthan).

Dr. Neerja Mallick Professor & Registrar, People's University, Bhopal(MP).

ABSTRACT **Background/Aim**- CRP is considered as a prime inflammatory marker for T2DM. The objective of this study was to identify the association of hs-CRP with insulin resistance in subjects with pre-diabetes and compare to it with the levels in newly diagnosed type 2 diabetes and healthy subjects.

Material & Methods: In this study total 900 subjects were distributed into three groups as per ADA criteria. FPG, 2-hr glucose (after 75 gm oral glucose intake), HbA1c and fasting insulin were analyzed and HOMA-IR was used to calculate insulin resistance mathematically. Anthropometric measurements were done. The Immunoturbidimetric assay method was used to analyze serum hs-CRP by c311 fully automated analyzer (Roche diagnostics).

Results: Hs-CRP concentration was significantly increased in patients with type 2 diabetes mellitus and pre-diabetes in comparison to the control group, p value < 0.001. And hs-CRP showed a positive correlation with HOMA -IR.

Conclusion: The findings of this study illustrates the significance impact of the Hs-CRP in the development of insulin resistance (HOMA-IR) in pre-diabetic patient and strongly correlate with Type 2 diabetes mellitus at p < 0.001.

KEYWORDS : pre-diabetes, Inflammation, hs-CRP, IR, HbA1c

INTRODUCTION

Pre-diabetes is the progeny of diabetes. It is also termed as Impaired Glucose Regulation (IGR). It is a reversible condition that increases the risk for diabetes which is associated with insulin resistance or decline in insulin sensitivity (1,2). An important reason why CRP, measured by highly sensitive assays, emerged as a biomarker in clinical practice was that CRP has a relatively long half-life without diurnal variation, and remains stable over long periods of time in people without acute infections or inflammatory diseases (3). Several studies have confirmed that T2D is an inflammatory condition. DSouza *et al.,* (4) have proposed that variation in the concentration of inflammatory markers, c-peptide and fatty acid binding protein are participated factor in pathogenesis of IR in T2DM (4).

Many previous researches have mentioned that the progression and severity of metabolic disorder are related with increased level of inflammatory parameters (5,6). Elevated levels of inflammatory proteins are found in chronic low-grade inflammation and presence of these proteins has been found in the development of T2DM (7). But little information is available on the relationships between low-grade inflammation and pre-diabetes patients. Therefore, the present study was undertaken to evaluate Serum hs-CRP levels in pre-diabetes patients.

In this study we have highlighted the role of CRP in progression of type 2 diabetes in subjects of Bhopal region. We also studied associations of CRP level with insulin resistance in all study subjects divided in three groups.

MATERIAL & METHODS:

This cross sectional study was carried out in the Department of Biochemistry, People's College of Medical Sciences & Research Centre and Centre for Scientific Research & Development (CSRD), People's University, Bhopal during July 2017 to July 2019. The blood sample was collected from the outpatient department (OPD) and inpatient department (IPD) of People's Hospital. The study was designed taking 900 human subjects, in which, 300 ages matched healthy subjects were considered as control group, 300 as pre-diabetic subjects and 300 as type 2 diabetic subjects according to ADA criteria (8). Ethical principles such as respect for the persons, beneficence and justice were adhered. Ethical clearance was obtained from the research committee and the Institutional Review Board of People's University. Written informed consent was taken from all the subjects. The evaluation involved a full medical history and anthropometric measurements and arterial blood pressure.

INCLUSION CRITERIA:

- Patients diagnosed with pre-diabetes according to the ADA (American Diabetes of Association) values of FPG(100-125mg/dl), 2 hr glucose(140-199mg/dl) and HbA1c (5.7-6.4%) are taken into consideration for selection of patient.
- Patients newly diagnosed with type 2 diabetes mellitus as per ADA criteria(FBG ≥126 mg/dl , 2-hr glucose ≥ 200 mg/dl, HbA1c ≥ 6.5%) and
- Patients aged between 30-60 years are taken up into the study

EXCLUSION CRITERIA:

- Patients with diagnosis of any other disease other than pre-diabetes & type 2 diabetes mellitus (based on their medical history and physical examination) are excluded.
- Patients on antidiabetic drugs, insulin and corticosteroids are excluded from the study.
- Patients below 30 years and above 60 years are excluded from the study.

All the biochemical parameters as FBG, 2-hr Glucose (9), HbA1c and Hs-CRP were estimated by Standard Kit method by using Cobas c311 fully automated analyzer (Roche diagnostics). Serum Insulin was assayed on Cobas c411 fully automated immunoassay analyzer (Roche diagnostics) by using cobas kits. Insulin resistance was estimated by the **Homeostasis model assessment (HOMA-IR)** and calculated as Fasting Insulin (microU/L) x Fasting glucose (mg/dl)/405.

Table no.1: Biochemical Parameters and Methods:

Sr No.	Biochemical Parameters	Methodology
1.	Glucose	Enzymatic Method (Hexokinase)(10)
2.	HbA1c	Turbidimetric inhibition immunoassay(11)
3.	Insulin	Electrochemiluminescence Method (ECL)(12)
4.	Hs-CRP	Immunoturbidimetric assay method (13)

Calculation and Statistical analysis:

The data was entered into Microsoft Excel software package. The entered data were transferred to SPSS 24.0 software (SPSS Inc., Chicago, Illinois, USA) package for analysis ANOVA test was applied to proportions to test the level of significance. Pearson's correlation was used to study the strength of association. The level of significance was fixed at 0.05. Confidence interval (CI) was set at 95%.

RESULTS:

In present study a total of 900 subjects participated, and out of these 300 were type II diabetes patients, 300 were pre-diabetes and 300 were controls.

Table 2: Comparison between the three groups selected for the study

Parameters	Healthy controls	Pre-diabetes	Diabetes	ANOVA
WC (cm)	74.87 ± 7.4	79.95 ± 5.7	84.2 ± 5.4	0.001*
WHR	0.82 ± 0.09	0.87 ± 0.06	0.98 ± 0.23	0.001*
BMI (kg/m2)	22.22 ± 2.79	24.89 ± 2.4	29.25 ± 3.06	0.001*
FBG (mg/dl)	83.62 ± 7.7	114.58 ± 7.3	149.78±30.27	0.001*
2-hr Glucose (mg/dl)	120.72 ± 10.05	163.2 ± 14.77	255.58±40.07	0.001*
HbA1c (%)	4.5 ± 0.63	6.10 ± 0.25	8.85 ± 1.39	0.001*
Fasting insulin	6.09 ± 2.13	7.19 ± 3.63	29.006 ± 5.06	0.001*
HOMA-IR	1.48 ± 0.80	2.04 ± 0.98	10.67 ± 2.7	0.001*
Hs-CRP (mg/L)	0.53±0.17	0.37±0.05	0.29±0.11	0.001*

*** p value significant < 0.001**

- In our study have compared anthropometric and biochemical parameters in IGT (Impaired Glucose Tolerance), type 2 DM and healthy control groups. As shown in Table 2, anthropometric parameters (BMI, waist circumference and waist to hip ratio) are statistically significantly differed in pre-diabetes and DM groups.
- There is a significant increase ($p<0.001$) in Hs-CRP and IR in diabetic group as compare to pre-diabetic and healthy control.

DISCUSSION

In our study we compared serum hs-CRP levels in diabetic, pre diabetic and in healthy controls. Mean values of hs-CRP showed a gradual significant increase among diabetic, pre-diabetic patients when compared with normal controls (table-2).

Several possible mechanisms are found by which we can mention chronic low-degree inflammation might be induced diabetes and its complications. In a hyperglycaemic state, the concentration of advanced glycation end products raises. These end products have been shown for activation of macrophages, raise oxidative stress, and up regulate the synthesis of interleukin-1, interleukin-6, and tumour necrosis factor and after this, production of CRP is occur (14).Another possible mechanism is that increases in CRP concentrations are related to cytokines which are derived from adipose tissue (15). CRP, tumour necrosis factor-alpha, and IL-6 get triggered by the excessive adipose tissue to activate insulin signaling pathways. This result in resistance to insulin that eventually progresses into a hyperglycemic state called as T2D. Many recently published articles have demonstrated a relationship between elevated CRP levels and increased diabetes risk. For chronic inflammation an etiologic role in the development of IR has been hypothesized [16, 17] our results also support this hypothesis. It has been accepted that rising of CRP concentrations is an independent predictive parameter of T2DM, which is also related with different components of the metabolic syndrome ie obesity, IR, and dyslipidemia [18, 19, 20, 21, 22]. However, the role of adipose tissue as a possible cause of the chronic inflammatory condition in patients with pre diabetic with and without early diabetic changes requires further investigation.

CONCLUSION

In our study we conclude that there is a significant change in both hs-CRP and IR levels in healthy, pre-diabetic, and diabetic population of Bhopal region. There is a significant but gradual change during the progression of healthy towards diabetic population via pre-diabetic condition. Thus systemic inflammation has an important role in the pathogenesis of pre diabetic. There is a progressive raises value of hs-CRP in pre diabetic and diabetic patients. Therefore, inflammation reflects the severity of the disease and signifies the presence of ongoing disease process. Screening of these biomarkers at an early stage might prove fruitful in the early detection of the development of insulin resistance and type 2 diabetes mellitus and positively delay the onset of this non communicable disease. Further, this will provide an opportunity for the research and invention of drugs to block this inflammatory pathway and in turn the development of type 2 diabetes.

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