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# ROLE OF INFLAMMATORY CYTOKINES IN PRE-DIABETES AND TYPE 2 DIABETES

**Poorvi Gupta** Research Scholar,Dept of Biochemistry, PCMS & RC, Bhopal.

**Dr Prashant Hisalkar*** Professor & Head, Dept Biochemistry, GMCH Dungarpur. *Corresponding Author

**Dr Neerja Mallick** Professor & Registrar, People's University Bhopal.

**ABSTRACT**

**Background**- Inflammation and type 2 diabetes mellitus (T2DM) has been extensively investigated for over a decade. However, the relationship between inflammatory biomarkers, including C-reactive protein (CRP) and tumor necrosis factor alpha (TNF-α), pre-diabetes and T2DM is still inconsistent and limited. Thus, this study is aimed to find out the role of pro- inflammatory cytokines ie.Hs-CRP and TNF- in pre-diabetes and type 2 diabetes subjects.

**Material & Methods:** The hospital based analytical cross-sectional study was conducted in the Department of Biochemistry, People's College of Medical Science and Research Center (PCMS and RC) and Centre for Scientific Research and Development (CSRD), People's University, Bhopal. Total 900 subjects were distributed into three groups (300 pre-diabetic subjects, 300 type 2 diabetic subjects and 300 healthy subjects) as per ADA criteria. The biochemical parameters as FBG, 2-hr glucose (after 75 gm oral glucose intake), HbA1c and fasting insulin were analyzed. HOMA-IR was used to calculate insulin resistance mathematically. Anthropometric measurements were done. TNF- was done by ELISA method and Immunoturbidimetric assay method was used to analyze serum hs-CRP by c311 fully automated analyzer (Roche diagnostics).

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

In conclusion, besides consideration of CRP levels alone, our findings suggested that elevated TNF- and CRP levels could be a potential predictor of T2DM and at pre-diabetes stage we can reduce the risk of T2D.

## INTRODUCTION

The fasting blood glucose level between 100 and 125mg/dL indicates pre-diabetes. It is asymptomatic disease and there are around 80 million peoples with prediabetes in India. The Indian Diabetes Prevention Programme-1 (IDPP-1) has revealed a high number of PD patients (IGT) converting into diabetes patients (18% per year) [1-2]. The most distressing fact that the onset age for diabetes has shifted down to a younger age [3].

Pre-diabetes is a dysmetabolic state of glucose level between diabetes mellitus (DM) and normal glucose tolerance (NGT) which includes IFG and IGT. Those subjects with IGT have impaired late-phase insulin secretion and increased insulin resistance (IR) in skeletal muscle. On the contrary, subjects with IFG have impaired early-phase insulin secretion and increased IR in the liver. They confirm the role of cytokines in the causation of IR and thereby type 2 diabetes mellitus (T2DM).

T2DM is a complex disease in which both genetic and environmental factors interact in determining impaired - cell insulin secretion and peripheral insulin resistance [4-6]. It is also a metabolic disorder between pro- and anti-inflammatory characterized by chronic hyperglycemia and increased or decreased levels of circulating cytokines. The rise in the proinflammatory cytokines (e.g., IL-6, tumor necrosis factor- (TNF-) , C-reactive protein (CRP),etc) or the fall in anti-inflammatory cytokines is the essential step in glucotoxicity and lipotoxicity induced mitochondrial injury, oxidative stress, and beta cell apoptosis [5-7]. These pro- and anti-inflammatory cytokines can enhance insulin resistance directly in adipocytes, muscle, and hepatic cells, leading to systemic disruption of insulin sensitivity and impaired glucose homeostasis. It is reported that TNF- is a possible mediator of insulin resistance and diabetes since it inhibits insulin signaling and impairs its secretion [8].

Effects of the inflammatory markers such as C-reactive protein (CRP), tumor necrosis factor alpha (TNF-α) and interleukin 6 (IL6) that are triggered by excessive adipose tissue have been reported on insulin signaling pathways, resulting in insulin resistance and eventually progressing to type 2 diabetes mellitus (T2DM) [9-10].

Elevation of CRP was related to an increased risk of developing T2DM and was suggested as an independent risk determinant for newly diagnosed patients. However, these results are controversial. Some studies stated a link between CRP and increased risk of prolonged T2DM development [11-15].

TNF-α was believed to induce insulin resistance by inhibiting phosphorylation of IRS-1 and Akt substrate 160 on insulin signaling cascade [16-17]. A previous study also revealed the role of TNF-α in reducing insulin production from β-cell [18]. Therefore, this marker is suspected to be a possible mediator between insulin resistance and diabetes. Hence this study was conducted to find out the role of pro- inflammatory cytokines ie. Hs-CRP and TNF-α in pre-diabetes and type 2 diabetes subjects.

## MATERIAL AND METHODS

This study was done in the Department of Biochemistry, People's College of Medical Science and Research Center (PCMS and RC), Centre for Scientific Research and Development (CSRD), People's University, Bhopal. This is a cross-sectional hospital based descriptive study includes 300 type 2 diabetes patients, 300 pre-diabetes persons, and 300 controls during the period June 2017 to April 2019. Written informed consent was taken from all participants after applying inclusion and exclusion criteria. Socio demographic data were collected by a self-designed questionnaire.

### Inclusion Criteria for Prediabetes According to American Diabetes Association

Age: between 30 years and 60 years

Fasting blood sugar level: 100-125 mg %

HbA1c: 5.7% to 6.4%
Postprandial blood sugar level (after 2 hours of 75 g oral glucose): 140 to 199 mg%

**Exclusion Criteria for Prediabetes**
Age more than 60 years and age less than 18
Diagnosed diabetic patients
Pregnant women
HIV-positive patients

**Inclusion Criteria for Type II Diabetes According to American Diabetes Association**
Age: between 30 years and 60 years
Newly diagnosed cases of type 2 diabetes and HbA1c 6.5%

**Exclusion Criteria for Type II Diabetes**
Age not more than 60 years and age less than 30
Pregnant women, HIV-positive patients
Prolonged diabetes
Patients on statin therapy

The study protocol was approved by Institutional Ethics Committee. All the participants were screened for age, gender, fasting blood glucose level, 2hr-glucose level, HbA1c, family history, and any medication history. Pre-diabetic and T2D cases were included and excluded with the help of physician, Department of Medicine, PCMS and RC. Biochemical parameters investigations are as follows :

| Sr No. | Biochemical Parameters | Methodology |
|---|---|---|
| 1. | Glucose | Enzymatic Method (Hexokinase)(19) |
| 2. | HbA1c | Turbidimetric inhibition immunoassay(20) |
| 3. | Insulin | Electrochemiluminescence Method (ECL)(21) |
| 4. | Hs-CRP | Immunoturbidimetric assay method (22) |
| 5. | TNF-α | ELISA(23) |

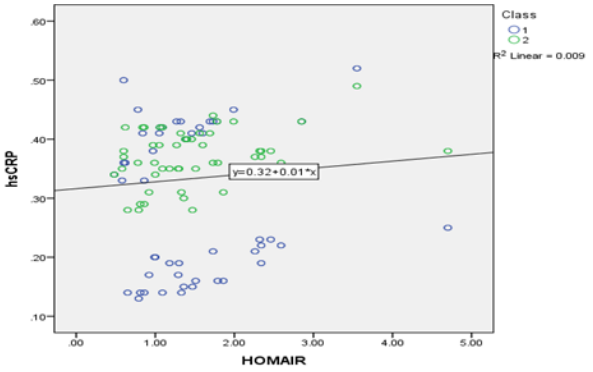
**Statistical Analysis** was done using statistical package SPSS 24.0 and Microsoft Excel 2010. Independent *t* test was used for comparison. "*p*" value <0.05 was used for level of significance.

**Observations**
A total of 900 subjects enrolled during the period July- 2017 to July -2019. The ratio of pre-diabetes: diabetes: control is 1:1:1.

**Table 2: Distribution of Demographic characteristics and Biochemical parameters in Controls, Prediabetes and T2D Patients**

| Parameters | Healthy controls | Pre-diabetes | Type 2 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) | 0.29±0.11 | 0.37±0.05 | 0.53±0.17 | 0.001* |
| TNF-α (pg/ml) | 255.74± 29.07 | 265.21± 34.20 | 296.74 ± 33.33 | 0.001* |

This table shows distribution of the biochemical parameters and anthropometric parameters (BMI, waist circumference and waist to hip ratio) are statistically significantly higher in to pre-diabetic and T2D patients as compared healthy control. The mean values of Hs-CRP, TNF-α and IR were significantly higher in diabetic group as compare to pre-diabetic and healthy control.[Fig.1 & 2]




- **Fig1- correlation between hs-CRP and HOMA-IR in pre-diabetes**

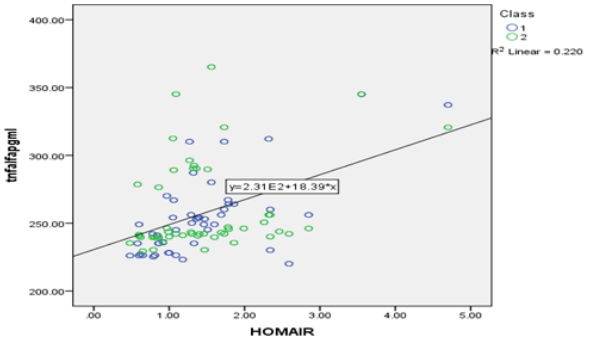



**Fig-2 correlation between TNF-alpha and HOMA-IR in pre-diabetes**

**DISCUSSION**
It has been recommended that T2DM is responsible for an continuing cytokine mediated low-grade chronic inflammation, Epidemiological facts suggests that inflammatory markers such as TNF-a and hs-CRP lead to the development of diabetes and glucose disorders . same found in present study (table-2) .

And after seeing the correlation in PD a positive correlation of IR with TNF-alpha (fig-2) and weak with hs-CRP (fig-1) found which is a significant, these result shows that at pre-diabetes stage we can stop to precede diabetes. Mostly found T2DM patients are obese with high BMI and high IR levels. Promotion of thrombogenic agent production, enhancement of endothelial adhesion molecules expression, activation of the complement cascade, and reduction of endothelial nitric oxide synthase (eNOS) (24) these are the possible mechanisms through which we can understand that increased CRP level induce IR.

For T2DM, CRP is considered as a prime inflammatory marker, and production is done by the liver cell. And regulation of this protein's expression is done by interleukin-6 (IL-6) and tumour necrosis factor (TNF-α) and their production is done by adipocytes (25). An increased level of C-reactive protein (CRP) and TNF-α has been connected with obesity, hypertension, heavy drinking, smoking and less physical activity. A most accepted mechanism is that increase level of CRP concentrations is related to cytokines which are derived from adipose tissue (19). CRP and TNF-α gets triggered by the excessive adipose tissue to activate insulin signaling pathways. These results 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 (26) our results also support this hypothesis.

It has been accepted that rising of CRP and TNF-

concentrations is an independent predictive parameter of T2D. However, the role of adipose tissue is a possible cause of the chronic inflammatory condition in patients with pre diabetic with and without early diabetic changes requires further investigation. (27, 28, 29, 30, 31).

Inflammation and their cytokines can be one of the reasons for leading to insulin resistance and T2D and above mentioned results support this hypothesis because with cytokines insulin resistance is also increasing in pre-diabetes and diabetes mellitus subjects when compare with healthy subjects (32).

## CONCLUSION

Results showed a strong relationship between these pro-inflammatory cytokines and T2D and support this hypothesis that these inflammatory markers may initiate T2D. Thus these cytokines may be a good marker for T2D and can secure at pre-diabetic stage.

## REFERENCES


1. Unwin N, Whiting D, Guariguata L, Ghyoot G, Gan D (Eds). The IDF Diabetes Atlas, 5th edition. Brussels, Belgium: International Diabetes Federation; 2011.pp.7-12.
2. Ramachandran A, Snehalatha C, Mary S, et al. The Indian Diabetes Prevention Programme shows that lifestyle modification and metformin prevent type 2 diabetes in Asian Indian subjects with impaired glucose tolerance (IDPP-1). Diabetologia. 2006;49(2):289-97. Epub 2006.
3. Diabetes, India Country Profile [Internet]. World Health Organization. 2016 [cited 2019 Nov 6]. p. 81–90. Available from: https://www.who.int/ diabetes/ country-profiles/ind_en.pdf.
4. A. Nadeem, S. Mumtaz, A. K. Naveed et al., "Gene-gene, geneenv ironment,gene-nutrient interactions and single nucleotide polymorphisms of inflammatory cytokines," *World Journal of Diabetes*, vol. 6, no. 4, pp. 642–647, 2015.
5. M. Banerjee and M. Saxena, "Genetic polymorphisms of cytokine genes in type 2 diabetes mellitus," *World Journal of Diabetes*, vol. 5, no. 4, pp. 493–504, 2014.
6. A. Brunetti, E. Chiefari, and D. Foti, "Recent advances in the molecular genetics of type 2 diabetes mellitus," *World Journal of Diabetes*, vol. 5, no. 2, pp. 128–140, 2014.
7. M. Saxena, N. Srivastava, and M. Banerjee, "Association of IL-6, TNF- and IL-10 gene polymorphisms with type 2 diabetes mellitus," *Molecular Biology Reports*, vol. 40, no. 11, pp. 6271–6279, 2013.
8. P. Arora, B. Garcia-Bailo, Z. Dastani et al., "Genetic polymorphisms of innate immunity-related inflammatory pathways and their association with factors related to type 2 diabetes," *BMC Medical Genetics*, vol. 12, article 95, 2011.
9. Esser N, Legrand-Poels S, Piette J, Scheen AJ, Paquot N. Inflammation as a link between obesity, metabolic syndrome and type 2 diabetes. Diabetes Res Clin Pract. 2014;105:141–50.
10. Arora P, Garcia-Bailo B, Dastani Z, Brenner D, Villegas A, Malik S, et al. Genetic polymorphisms of innate immunity-related inflammatory pathways and their association with factors related to type 2 diabetes. BMC Med Genet. 2011;12:95.
11. Belfki H, Ben Ali S, Bougatef S, Ben Ahmed D, Haddad N, Jmal A, et al. Association between C-reactive protein and type 2 diabetes in a Tunisian population. Inflammation. 2012;35:684–9.
12. Dongway AC, Faggad AS, Zaki HY, Abdalla BE. C-reactive protein is associated with low-density lipoprotein cholesterol and obesity in type 2 diabetic Sudanese. Diabetes Metab Syndr Obes. 2015;8:427–35.
13. Hu FB, Meigs JB, Li TY, Rifai N, Manson JE. Inflammatory markers and risk of developing type 2 diabetes in women. Diabetes. 2004;53(3):693–700.
14. Doi Y, Kiyohara Y, Kubo M, Ninomiya T, Wakugawa Y, Yonemoto K, et al. Elevated C-reactive protein is a predictor of the development of diabetes in a general Japanese population: The Hisayama Study. Diabetes Care. 2005;28(10): 2497–500.
15. Aruna DP, JoAnn EM, Nader R, Julie EB, Paul MR. C-Reactive Protein, Interleukin 6, and Risk of Developing Type 2 Diabetes Mellitus. J Am Med Assoc. 2001;286(3):327–34.
16. Smitka K, Maresova D. Adipose Tissue as an Endocrine Organ: An Update on Pro-inflammatory and Anti inflammatory Microenvironment. Prague Med Rep. 2015;116(2):87–111.
17. Ibfelt T, Fischer CP, Plomgaard P, van Hall G, Pedersen BK. The acute effects of low-dose TNF-alpha on glucose metabolism and beta-cell function in humans. Mediat Inflamm. 2014;2014:295478.
18. Hajer GR, van Haeften TW, Visseren FL. Adipose tissue dysfunction in obesity, diabetes, and vascular diseases. Eur Heart J. 2008;29:2959–71.
19. Standard operating protocol for the quantitative determination of glucose in human serum, plasma, urine and CSF on Roche/Hitachi cobas c systems. 2009;1–4.
20. Standard Operating Procedure for the quantitative determination of % hemoglobin A1C (DCCT/NGSP) in whole blood on Roche/Hitachi cobas c systems. Gunderson Heal Syst. 2017;2:1–4.
21. Elecsys Insulin - Immunoassay for the in vitro quantitative determination of human insulin in serum and plasma. Cobas. 2017;1–5.
22. Immunoturbidimetric assay for the in vitro quantitative determination of CRP in human serum and plasma on Roche/Hitachi cobas c systems. 2017;4–7.
23. Human TNF- (Tumor Necrosis Factor Alpha) ELISA Kit. Elabscience [Internet]. 2018;(7th edition). Available from: www.elabscience.com.
24. Fukuchi, Y. *et al*. Immuno histochemical detection of oxidative stress biomarkers, Dityrosine &N(epsilon)-(hexanoyl)lysine & CRP in rabbit atherosclerotic lesions. *J.Atheroscl.* 15, 185–192 (2008).
25. Freeman DJ, Norrie J, Caslake MJ, Gaw A, Ford I, Lowe GDO, et al. C-Reactive Protein Is an Independent Predictor of Risk for the Development of Diabetes in the West of Scotland Coronary Prevention Study. Diabetes [Internet]. 2002 May 1 [cited 2019 Apr 29];51(5):1596–600. Available from: http:// diabetes. diabetesjournals.org/cgi/doi/10.2337/diabetes.51.5.1596.
26. Phosat, C., Panprathip, P., Chumpathat, N. *et al.* Elevated C-reactive protein, interleukin 6, tumor necrosis factor alpha and glycemic load associated with type 2 diabetes mellitus in rural Thais: a cross-sectional study. *BMC Endocr Disord* **17,** 44 (2017). https://doi.org/10.1186/s12902-017-0189-z
27. Yu,Z. *et al*. Associations of physical activity with inflammatory factors, adipocytokines, and metabolic syndrome in middle-aged and older chinese people. *Circulation.* 119, 2969–2977 (2009).
28. Nakanishi, S., Okubo, M., Yamane, K., Kohno, M. & Kamei, N. Elevated C-reactive protein is a risk factor for the development of type 2 diabetes in Japanese Americans. *Diab Care.* 26, 2754–2757 (2003)
29. Thorand, B. *et al*. Sex differences in the prediction of type 2 diabetes by inflammatory markers: results from the MONICA/KORA Augsburg case-cohort study, 1984–2002. *Diab Care.* **30**, 854–860 (2007).
30. Doi, Y. *et al*. Elevated C-reactive protein is a predictor of the development of diabetes in a general Japanese population. *Diab Care.* 28, 2497–2500 (2005).
31. Duncan, B. B. *et al*. Low-grade systemic inflammation and the development of type 2 diabetes. *Diabetes.* 52, 1799–1805 (2003).
32. Al-Ozairi E, Middelbeek R, & Horton E (2006). Cardiovascular risk assessment in type 2 diabetes mellitus. *Current Diabetes Reports* **6**, 333-336.