Original Research Article

Evaluation of inflammatory mediators in newly diagnosed type II diabetic patients without hypertension

Amit Varshney[1*], Ramakant Rawat[2]

[1]Associate Professor, Department of General Medicine,United Institute of Medical Sciences and Hospital, Prayagraj, Uttar Pradesh, India

[2]Associate Professor, Medicine, U P University of Medical Sciences, Saifai Etawah, Uttar Pradesh, India

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Abstract

Background: The present study was conducted to assess the role of inflammation in T2DM by measuring the levels of cytokines, including IL-6 (pg/ml),hs-CRP(mg/L), TNF- alpha (pg/ml) in T2DM. **Materials & Methods:**34 type II diabetes mellitus patients and equal numbers of normotensive non diabetic subjects were recruited. Glycated hemoglobin (HbA1c) levels, IL-6 (pg/ml),hs-CRP(mg/L), TNF- alpha (pg/ml) were evaluated. **Results:** IL-6 level of 16.2±4.2 in Group I and 3.8±1.4 in control patients. In diabetic patients HS CRP levels were observed to be 1.5±0.24 (mg/L) and 0.5±0.04 (mg/L) in control subjects. TNF- alpha levels were observed to be 34.6 in diabetic patients and 14.2 in control subjects. All inflammatory markers studied in our study showed statistically significant difference.**Conclusion:**Patients with established T2DM, had different cytokine profile than healthy controls. Further large prospective studies are required to evaluate a temporal relation between baseline levels of inflammatory markers and incidence of T2DM.

Key words: Cytokine,diabetic, inflammatory markers, interleukins, normotensive, TNF- alpha.

Introduction

Type 2 diabetes mellitus (T2DM) is a complex disease in which both genetic and environmental factors interact in determining impaired β-cell insulin secretion and peripheral insulin resistance. It is a leading cause of premature deaths worldwide, and its exceptional upsurge poses a severe threat and imposes a huge economic burden worldwide (825 billion dollars per year)[1].According to a recent estimation of the World Health Organization (WHO), 422 million people globally are affected from diabetes with a prevalence rate of 8.5% and 46.3% still remains undiagnosed and the number is projected to rise to 552 million in 2030[2]. Inflammation is considered to be a key regulator of the pathogenesis of T2DM, but what triggers this inflammation still unknown. However, it may be related to obesity. Obesity is associated with enlargement of adipose tissue and consequently increases the number of adipose tissue macrophages. These macrophages are responsible for almost all adipose tissue tumor necrosis factor- (TNF-) expression, significant amounts of interleukin-6 (IL-6), and other acute-phase response markers and mediators of inflammation[3]. Many proinflammatory cytokines play a central role in inflammatory reaction and were shown to increase the risk of T2DM. These pro-Inflammatory cytokines can enhance insulin resistance directly in adipocytes, muscle and hepatic cells, leading to systemic disruption of insulin sensitivity and impaired glucose homeostasis. Increased levels of these pro-inflammatory cytokines lead to hepatic production and secretion of acute-phase proteins such as C-reactive protein (CRP), plasminogen activator inhibitor-1 (PAI-1), amyloid-A, 1-acid glycoprotein, and haptoglobin. These proteins appear in the early stages of T2DM, and their circulating concentrations increase as the disease progresses[3,4].Few studies reported that it has an association with the pathogenesis of T2DM. On the other hand, many studies suggest that

**Correspondence*

Dr. Amit Varshney

Associate Professor, Department of General Medicine,United Institute of Medical Sciences and Hospital,Prayagraj, Uttar Pradesh, India.

E-mail: firstamit21@yahoo.co.in

IL-6 has a dual role of anti-inflammatory and improves glucose metabolism in T2DM patients[4]. IL-6 has a great impact on glucose homeostasis and metabolism by acting indirectly on the β-cells of the pancreas and on adipocytes[5]. However, its role as an early biomarker for T2DM is still doubted and more such studies are needed in different populations[6]. The present study was conducted to assess the role of inflammation in T2DM by measuring the levels of cytokines, including IL-6 (pg/ml),hs-CRP(mg/L), TNF-alpha (pg/ml) in T2DM

Materials & Methods

The present study was conducted in the Department of General Medicine of United Institute of Medical Sciences and Hospital, Prayagraj, Uttar Pradesh, Indiaamong 34Type II diabetes mellitus patients of both genders. Equal numbers of normotensive non diabetic subjects were also recruited. All involved patients were informed regarding the study and their consent was obtained. Data such as name, age, gender etc. was recorded. 5 ml of venous blood was collected from all enrolled subjects. Glycatedhemoglobin (HbA1c) levels, IL-6 (pg/ml),hs-CRP(mg/L), TNF-alpha (pg/ml)were determined. Results were statistically analyzed for correct inference. P value less than 0.05 was considered significant.

Results

Table 1:Distribution of patients

Groups	Group I (Diabetics)	Group II (Control)
M:F	20:14	19:15

Table 1 shows that there were 20 males and 14 females in group I and 19 males and 15 females in group II.

Table 2:Assessment of parameters

Parameters	Group I (Diabetics)	Group II (Control)	P value
IL-6 (pg/ml)	16.2±4.2	3.8±1.4	0.001*
HS CRP (mg/L)	1.5±0.24	0.5±0.04	0.015*
TNF- alpha (pg/ml)	34.6±6.8	14.2±4.2	0.003*

Table 2 showed IL-6 level of 16.2±4.2 in Group I and 3.8±1.4 in control patients. In diabetic patients HS CRPlevels were observed to be 1.5±0.24 (mg/L) and 0.5±0.04 (mg/L) in control subjects. TNF- alpha levels were observed to be 34.6 in diabetic patients and 14.2 in control subjects. All inflammatory markers studied in our study showed statistically significant difference.

Table 3: Karl Pearson correlation between inflammatory markers and HbA1c levels

Parameters	r value
IL-6 (pg/ml)	+0.8
HS CRP (mg/L)	-
TNF- alpha (pg/ml)	-

HbA1c was positively correlated with only IL-6 with a significant -value of 0.005.

Discussion

With the associations between inflammatory cytokines (e.g., IL-6, TNF-alpha, and hs-CRP) and insulin resistance, prediabetes or T2DM had been widely researched[5,6]. Most studies found that subjects with insulin resistance, prediabetes, or T2DM had increased levels of IL-6, TNF-α, and hs-CRP. A study reported that high levels of inflammatory cytokines appeared in early stage of T2DM and were capable of predicting the development of type 2 diabetes through diminishing insulin sensitivity[7-10]. Trend of T2DM is changed from metabolic disorder to inflammation as effects of the pro and anti-inflammatory cytokines like tumor necrosis factor alpha (TNF-α), interleukin-6 (IL-6) and C-reactive protein (CRP) has been reported in insulin signaling pathways, cross-linking and ultimately developing insulin resistance in β-cells of pancreas which further risks to T2DM.[9]The present study was conducted to assess the role of inflammation in T2DM by measuring the levels of IL-6 (pg/ml), hs-CRP (mg/L), TNF- alpha (pg/ml) in T2DM.In present study, there were 20 males and 14 females in group I and 19 males and 15 females in group II.Darko et al[10] examined the associations of IL-6 and TNF-

alpha with anthropometric measurement and the effect of co-morbidity with hypertension using rural and urban dwellers in participants aged 25–70 years consisting of 77 T2DM ± hypertension patients and 112 controls were selected from a larger study on Research on Obesity and Diabetes among African Migrants (RODAM). Anthropometric measurements, blood pressure and body fat percentage were measured. Fasting blood samples were analyzed for glucose, IL-6 and TNF-alpha levels. The median level of IL-6 was significantly higher ($p < 0.0001$) among rural dwellers compared to urban dwellers. Inversely, urban dwellers had significantly higher ($p = 0.0424$) median level of TNF-α compared to rural cases. No significant differences were observed in IL-6 ($p = 0.3571$) and TNF-α ($p = 0.2581$) among T2DM patients compared with T2DM ± hypertension patients. A weak negative correlation was found between IL-6 and BMI in urban T2DM. The average level of IL-6 was higher in rural T2DM participants compared with those in urban setting. However, higher levels of TNF-alpha was observed among the study participants with T2DM in urban settings compared to those of rural. In this study, we observed that co-morbidity of hypertension had no significant effect on the levels of IL-6 and TNF-alpha. We found that meanIL-6 level of 16.2±4.2 in Group I and 3.8±1.4 in control patients. In diabetic patients HS CRP levels were observed to be 1.5±0.24 (mg/L) and 0.5±0.04 (mg/L) in control subjects. TNF- alpha levels were observed to be 34.6 in diabetic patients and 14.2 in control subjects. All inflammatory markers studied in our study showed statistically significant difference.

Lainampetch et al[11] evaluated the association of baseline inflammatory marker levels and variation with incidence of T2DM. After the 2-year follow-up, 18.6% of total subjects had developed T2DM. The risk of developing T2DM was significantly increased in subjects with a high level of baseline CRP and a stronger impact was found with the combination of high CRP and IL-6 levels. One-year inflammatory marker variation analysis also revealed the significant association of elevated TNF-alpha and risk of developing T2DM. Findings suggested that IL-6 outstandingly plays a contributing role in T2DM progression and elevated TNF-alpha levels over time could be a potential predictor of T2DM.Similar to our study Mavridis et al. investigated inflammatory cytokines in insulin-treated T2DM patients and showed increased levels of IL-6, TNF-alpha in insulin-treated T2DM patients compared to sulfonylurea-treated patients[12].Bashir et al[13] a total of 340 subjects were selected in this study among them 160 were T2DM cases and 180 were healthy controls. Serum expression of inflammatory mediators (TNF-alphaand IL-6) were quantified by ELISA technique. The expressions of candidate cytokines (TNF-α, IL-6, CRP, and WBC) were highly significant ($p<0.001$) in T2DM. Among inflammatory mediators, TNF-α shows a positive correlation ($p<0.001$) with glycemic profile and insulin sensitivity in T2DM cases in comparison with healthy normal. Biochemical (fasting sugar, HbA1c, insulin resistance, lipid profile) and anthropometric (BMI) parameters were highly significant ($p<0.001$) in T2DM cases as compared to non-diabetic normal.Al-Shukaili et al assessed the inflammatory markers in type 2 diabetes mellitus (T2DM) by measuring some cytokines concentrations and lymphocytes subset and correlate them with other laboratory investigations. Fifty-seven patients with type-2 diabetes and 30 healthy volunteers were enrolled in this study. Data for the C-reactive protein (CRP), haemoglobin, HbA1c, and autoantibody levels were obtained from the patients files. The levels of IL-1, IL-6, IL-15, and TNF- alphawere found to be decreased in T2DM patients, whereas the levels of IL-10, IFN-alpha, and caspase-1 were increased, compared to normal controls. No significant differences in lymphocytes subset between cases and normal control were observed. Significant correlations were found between HbA1c and IL-6; body mass index (BMI) was significantly correlated with CRP, TNF-alpha, and phosphate; the weight (Wt) was associated with CRP and IFN-alpha. They concluded that, an alteration in the function of the immune system was observed in T2DM patient[8].The shortcoming of the study was small sample size.

Conclusion

In conclusion, we found that, patients with established T2DM, had different cytokine profile than healthy controls. Further large prospective studies are required to evaluate a temporal relation between baseline levels of inflammatory markers and incidence of T2DM.

References

1. Al-Shookri, G. L. Khor, Y. M. Chan, S. C. Loke, and M. Al-Maskari, “Type 2 diabetes in the Sultanate of Oman,” Malaysian Journal of Nutrition, 2011;17(1): 129–141.
2. J. A. Al-Lawati, M. N. Barakat, M. Al-Maskari, M. K. Elsayed, A. M. Al-Lawati, and A. J. Mohammed, “HbA1c levels among primary healthcare patients with type 2 diabetes mellitus in

Oman," Oman Medical Journal, 2012;27(6):465–470.
3. Pradhan AD, Manson JE, Rifai N, Buring JE, Ridker PM. C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. JAMA. 2001;286:327–34.
4. Salomaa V, Havulinna A, Saarela O, Zeller T, Jousilahti P, Jula A, et al. Thirty-one novel biomarkers as predictors for clinically incident diabetes. PLoS One. 2010;5:e10100.
5. Nadeem A, Naveed AK, Hussain MM, Raza SI. Correlation of inflammatory markers with type 2 Diabetes Mellitus in Pakistani patients.Journal of Postgraduate Medical Institute (Peshawar-Pakistan). 2013;27(03):267–273.
6. Bertoni AG, Burke GL, Owusu JA, Carnethon MR, Vaidya D, Barr RG, et al. Inflammation and the incidence of type 2 diabetes: the Multi-Ethnic Study of Atherosclerosis (MESA). Diabetes Care. 2010;33:804–10.
7. M. Mishra, H. Kumar, S. Bajpai, R. K. Singh, and K. Tripathi, "Level of serum IL-12 and its correlation with endothelial dysfunction, insulin resistance, proinflammatory cytokines and lipid profile in newly diagnosed type 2 diabetes," Diabetes Research and Clinical Practice, 2011;94(2):255–261.
8. Ahmed Al-Shukaili, Saif AL-Ghafri, Safia Al-Marhoobi, Said Al-Abri, Jawad Al-Lawati, Masoud Al-Maskari, "Analysis of Inflammatory Mediators in Type 2 Diabetes Patients", International Journal of Endocrinology, 2013:1-
9. Agyemang C, Beune E, Meeks K, Owusu-Dabo E, Agyei-Baffour P, Aikins A, et al. Rationale and cross-sectional study design of the Research on Obesity and type 2 Diabetes among African Migrants: the RODAM study. BMJ open. 2014;4(3):e004877.
10. Darko SN, Yar DD, Owusu-Dabo E, Awuah AA, Dapaah W, Addofoh N, Salifu SP, Awua-Boateng NY, Adomako-Boateng F. Variations in levels of IL-6 and TNF-α in type 2 diabetes mellitus between rural and urban Ashanti Region of Ghana. BMC endocrine disorders. 2015 ;15(1):50.
11. Lainampetch J, Panprathip P, Phosat C, Chumpathat N, Prangthip P, Soonthornworasiri N, Puduang S, Wechjakwen N, Kwanbunjan K. Association of Tumor Necrosis Factor Alpha, Interleukin 6, and C-Reactive Protein with the Risk of Developing Type 2 Diabetes: A Retrospective Cohort Study of Rural Thais. Journal of Diabetes Research. 2019 ;8(2):9.
12. G. Mavridis, E. Souliou, E. Diza et al., "Inflammatory cytokines in insulin-treated patients with type 2 diabetes," Nutrition, Metabolism and Cardiovascular Diseases, 2008;18(7):471–476.
13. Bashir H, Bhat SA, Majid S, Hamid R, Koul RK, Rehman MU, Din I, Bhat JA, Qadir J, Masood A. Role of inflammatory mediators (TNF-α, IL-6, CRP), biochemical and hematological parameters in type 2 diabetes mellitus patients of Kashmir, India. Medical Journal of the Islamic Republic of Iran. 2020;34:5.

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