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CORRELATIONS BETWEEN PRO-INFLAMMATORY MARKERS AND GLYCATED HEMOGLOBIN IN TYPE 2 DIABETES PATIENTS

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Abstract

Background and Aim. *Markers of inflammation are predictive of diabetic macrovascular complications. In type 2 diabetes, inflammation might be responsible for enhancing insulin resistance and may contribute to the reduction of islet cells secretory function. In the present study, we investigated the correlation between the pro-inflammatory markers and glycated hemoglobin in patients with type 2 diabetes mellitus.* ***Materials and Methods.*** *66 patients (32 women and 34 men) with type 2 diabetes were recruited for this study. On fasting plasma samples C-reactive protein, interleukin-6, tumor necrosis factor-α (TNF-α) and glycated hemoglobin (HbA1c) were assayed. C-reactive protein, interleukin-6 and TNF-α were measured using enzyme-linked immunosorbent assays. The HbA1c were measured using a high-performance liquid chromatography method. Results were compared to the corresponding parameters obtained for 38 subjects (18 women and 20 men) without type 2 diabetes (control group).* ***Results.*** *The groups were similar in terms of age and sex but there were statistically significant differences in the recorded parameters in diabetic patients compared to the control group. Circulating levels of C-reactive protein, interleukin-6, TNF-α and HbA1c were higher ($p<0.05$) in patients with type 2 diabetes mellitus compared to normally metabolic patients. In the patients with type 2 diabetes mellitus HbA1c were significantly correlated with circulating C-reactive protein ($r=0.909$, $p<0.001$) and interleukin-6 ($r=0.883$, $p<0.001$) levels.* ***Conclusion.*** *The markers of inflammation are significantly higher in patients with type 2 diabetes compared to subjects without diabetes. The elevated markers of inflammation (C-reactive protein and interleukin-6) were significantly correlated with glycemic control (HbA1c).*

Key words*: markers of inflammation, glycated hemoglobin, type 2 diabetes mellitus.*

Introduction

Type 2 diabetes mellitus is associated with an increased risk of atherosclerotic cardiovascular disease. Several epidemiological studies have documented the association between inflammatory markers and atherosclerotic cardiovascular disease

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[1,2,3]. Early endothelial dysfunction and inflammation secondary of the risk factors such hyperglycemia, atherogenic lipoproteins, hypertension, cigarette smoking result in the recruitment of leucocytes and expression of adhesion molecules which facilitate the adhesion to the endothelium and their migration into the intima. The secretion of inflammatory cytokines promotes the proliferation of smooth muscle cells and the formation of the atheromatous plaque. In the end stage of the pathogenesis of atherosclerosis, inflammatory mediators weaken the extracellular matrix of the atheroma, causing its eventual rupture and formation of a thrombus [4].

The key mediator of inflammation at the cellular level is the transcription nuclear factor kB which activates the transcription of genes involved in the expression of pro-inflammatory markers [4]. Numerous biomarkers of subclinical inflammation have been associated with an increased risk of atherosclerotic cardiovascular disease in patients with type 2 diabetes such oxidized low-density lipoproteins, pro-inflammatory cytokines (interleukin-1, tumor necrosis factor-α), adhesion molecules (intercellular adhesion molecule-1, vascular cell adhesion protein 1, selectins: E-selectin, P-selectin), inflammatory stimuli with hepatic effects (interleukin-6) or the products of the hepatic stimulation, such as high-sensitivity C-reactive protein, serum amyloid A and a host of other acute-phase reactants [5,6].

In 1998, a hypothesis was proposed suggesting that chronic inflammation is associated with the development of type 2 diabetes [7]. The mechanisms by which chronic inflammation can generate type 2 diabetes are not clear, but a number of experimental and clinical studies have demonstrated that to pro-inflammatory cytokines are involved in multiple metabolic pathways relevant to insulin resistance, including insulin regulation, reactive oxygen species, lipoprotein lipase action adipocyte function and may contribute to the reduction of islet cells secretory function [8,9,10].

Recent studies have shown that pro-inflammatory cytokines play a determinant role in the development of microvascular diabetic complications. Regarding diabetic neuropathy experimental investigations have shown that pro-inflammatory cytokines are produced locally by resident and infiltrating cells and exhibit pleiotropic effects on homeostasis of glia and neurons in the central, peripheral and autonomic nervous systems. *Satoh Jet* al. [11] demonstrated *the role of tumor necrosis factor-alpha (*TNF-α) *in* the development of *diabetic polyneuropathy. Clinical and experimental studies* have shown that pro-inflammatory cytokines could participate in the development of diabetic retinopathy. The levels of pro-inflammatory cytokines are elevated in the vitreous fluid of patients with proliferative diabetic retinopathy and messenger ribonucleic acid (mRNA) expression for TNF-α and interleukin-1 is increased in the retina early in the course of diabetes [12,13]. In the last decade most of the attention has been focused on the implications of pro-inflammatory cytokines in the setting of diabetic nephropathy. *Royall JA* et al. [14] demonstrated that "interleukin-1 increases vascular endothelial permeability and has been involved in the proliferation of mesangial cells and matrix synthesis, as well as in the development of intraglomerular microcirculatory abnormalities" and *Dipetrillo K* [15], *Kalantarinia K* [16] and our group have found

that TNF-α play a determinant role to sodium retention and renal hypertrophy, important renal alterations that occur during the initial stage of diabetic nephropathy and that increased urinary as well as renal interstitial concentrations of TNF-α precede the rise in albuminuria.

Materials and Methods

66 patients (32 women and 34 men) with type 2 diabetes were recruited for this study. On fasting plasma samples C-reactive protein, interleukin-6, tumor necrosis factor-*α (*TNF-α) and glycated hemoglobin (HbA1c) were assayed. C-reactive protein, interleukin-6 and TNF-α were measured using enzyme-linked immunosorbent assays. The HbA1c were measured using a high-performance liquid chromatography method. Results were compared to the corresponding parameters obtained for 38 subjects without type 2 diabetes (control group).

Statistical analyses

Data are presented as mean ± SD. Clinical characteristics were compared using the t Student Test. Pearson's moment-product correlation coefficients were calculated to evaluate relationships between variables. Significance was defined at the 0.05 level of confidence. All calculations were performed using the Statistical Package for Social Sciences Software (SPSS) version 15.

Results

The average age of the participants was 54.48 ± 3.68 years for the patients with type 2 diabetes and 53.76 ±4.01 years in population without type 2 diabetes The groups were similar in terms of age and sex but there were statistically significant differences in the recorded parameters in diabetic patients compared to the control group. Circulating levels of C-reactive protein (7.90 ± 2.89 mg/L vs, 6.37 ± 1.77 mg/L), interleukin-6, (5.88 ± 2.11 pg/ml vs. 4.89 ± 1.17 pg/ml), TNF-α (9.10 ± 4.67 pg/ml vs 7.11 ± 1.27 pg/ml) and HbA1c (7.83 ± 1.63 % vs. 5.37 ± 0.32) were significantly higher ($p<0.05$) in patients with type 2 diabetes mellitus compared to normally metabolic patients. In the patients with type 2 diabetes mellitus, HbA1c were significantly correlated with circulating C-reactive protein ($r=0.909$, $p<0.001$) and interleukin-6 ($r=0.883$, $p<0.001$) levels.

The characteristics of the patients with type 2 diabetes and healthy controls are shown in Table 1.

Discussion

Several data support the role for inflammation in diabetogenesis. Our results are consistent with several, large, prospective studies that have reported that circulating levels of C-reactive protein, interleukin-6 and TNF-α were higher in patients with type 2 diabetes mellitus compared to normally metabolic patients.

The prototypic marker of inflammation is C-reactive protein. Several studies have shown that C-reactive protein levels are increased in population with diabetes or with impaired fasting glucose compared with those with a normal fasting glucose level diabetes. [17,18,19,20]. Elevated C-reactive protein levels have not only been reported in diabetes, but also appear to predict type 2 diabetes [21,22]. A significant positive correlation was found between C-reactive protein level and HbA1c level ($r=0.909$, $p<0.001$). Our results are consistent with several, large, prospective studies that have reported the association

between C-reactive protein levels and glycated hemoglobin (HbA1c). Data collected from the Third National Health and Nutrition Examination Survey have shown that elevated C-reactive protein levels was associated with higher HbA1c [23].

Table 1. The characteristics of the patients with type 2 diabetes and healthy controls

	Characteristics of diabetic patients	Characteristics of control group
Mean age (years)	54.48 ± 3 .68	53.76 ± 4.01
C-reactive protein (mg/L)	7.90 ± 2.89	6.37 ± 1.77
Interleukin-6 ((pg/ml)	5.88 ± 2.11	4.89 ± 1.17
Tumor necrosis factor-*α (pg/ml)*	9.10 ± 4.67	7.11 ± 1.27
Glycated hemoglobin (%)	7.83 ± 1.63	5.37 ± 0.32

The prototypic marker of inflammation is C-reactive protein. Several studies, have shown that C-reactive protein levels are increased in population with diabetes or with impaired fasting glucose compared with those with a normal fasting glucose level diabetes. [17,18,19,20]. Elevated C-reactive protein levels have not only been reported in diabetes, but also appear to predict type 2 diabetes [21,22]. A significant positive correlation was found between C-reactive protein level and HbA1c level ($r=0.909$, $p<0.001$). Our results are consistent with several, large, prospective studies that have reported the association between C-reactive protein levels and glycated hemoglobin (HbA1c). Data collected from the Third National Health and Nutrition Examination Survey have shown that elevated C-reactive protein levels was associated with higher HbA1c [23].

Circulating interleukin-6 levels have been reported to be elevated in subjects with type 2 diabetes and correlate with insulin resistance [24,25] Joseph J. Senn *et al* [26] reported that interleukin-6 inhibit signal transduction and insulin action in experimental models (primary mouse hepatocytes and the human hepatocarcinoma cell line), The interleukin-6 effect is characterized by a decreased tyrosine phosphorylation of insulin receptor substrate-1 and decreased association of the p85 subunit of phosphatidylinositol 3-kinase with insulin receptor substrate-1 in response to physiologic insulin levels.

Results show that there is a significant positive correlation between interleukin-6 level and HbA1c level ($r=0.883$, $p<0.001$).

TNF-α is the prototypical member of a family of cytokines and induce apoptosis, differentiation, cell activation, and inflammation [27]. TNF-α is found in the extra cellular matrix, endothelium, and vessel walls of fibro vascular tissue of proliferative diabetic retinopathy [28]. Several data support the role of TNF-α in the insulin resistance and in the pathogenesis of micro vascular and macro vascular complications of type 2 diabetes [5,6,15,16,29].

No correlation was found between the HbA1c level and serum TNF- α levels.

Conclusion

The markers of inflammation are significantly higher in patients with type 2

diabetes compared to subjects without diabetes. The results indicate that hyperglycemia significantly causes increase in inflammatory markers like C-reactive protein and interleukin-6, which further contribute to pathogenesis of microvascular and macrovascular complications.

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