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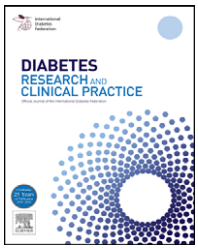

NF-κB binding activity and pro-inflammatory cytokines expression correlate with body mass index but not glycosylated hemoglobin in Chinese population

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ABSTRACT

Aims: Chronic inflammation is linked to type 2 diabetes (T2DM), so we investigated correlations between obesity, blood glucose levels, and inflammation in T2DM patients.
Methods: Peripheral blood mononuclear cells (PBMCs) were collected from 40 T2DM patients (27 men, 13 women; mean age 49.63 years), and 10 non-diabetic controls (all men; mean age 38.60 years). Inflammation was measured as DNA-binding activity of nuclear factor κB (NF-κB), a key transcription factor in inflammation. Protein levels of NF-κB subunit p65, and NF-κB inhibitor IκBα were assessed by Western blot. Transcript levels for p65, IκBα, and the NF-κB target genes TNF-α, MMP-9, IL-6, IL-8, and IL-18 were measured by real-time PCR. Body mass index (BMI) and glycohemoglobin were measured for all the subjects.
Results: NF-κB DNA-binding activity, p65 and IκBα protein levels, and expression of IL-6, TNFα and MMP-9 were significantly higher in PBMCs from T2DM patients, than from non-diabetic controls. NF-κB binding was significantly positively associated with both BMI and homeostasis model assessment of insulin resistance (HOMA-IR).
Conclusions: Inflammation was observed in PBMCs in T2DM patients in a Chinese population, and correlated independently with obesity and blood glucose levels. Lack of correlation with glycohemoglobin suggested that moderate-term blood glucose control did not mitigate inflammation.

1. Introduction

Insulin resistance (IR) [1] and insulin deficiency [2] are the two major pathophysiological causes of type 2 diabetes (T2DM) [3,4]. However, the relationship between these factors and a host of clinical and biochemical characteristics such as central obesity, hypertension, accelerated atherosclerosis, and lipid metabolism disorders, and the mechanism by which they cause T2DM remains unclear [5–9]. The contribution of inflammation has been the focus of recent research [10–12]. T2DM is a low-grade chronic inflammatory disease activated by the immune system [13]. In individuals susceptible to T2DM, the innate immune system is activated by environmental factors such as aging and overnutrition. Tumor necrosis factor-α, (TNF-α), interleukin-6 (IL-6) and other inflammatory cytokines are secreted by sentinel cells such as macrophages, T lymphocyte and adipocytes, initiating IR, insulin secretion deficiency, T2DM and metabolic syndrome.

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Consistent with this model, several clinical epidemiological studies have confirmed that IL-6 and C-reactive protein (CRP) are strong predictors of T2DM [14,15]. Elevated serum levels of TNF-α and IL-6 are also associated with IR and T2DM.

TNF-α and IL-6 [16] are target genes of the regulatory nuclear factor κB (NF-κB), which is a critical transcription factor in inflammation and the immune response [17,18]. The activation of NF-κB upregulates gene expression of a variety of pro-inflammatory cytokines. NF-κB is significantly activated in the peripheral blood mononuclear cells (PBMC) of obese people, leading to an increase in the mRNA and protein levels of TNF-α, IL-6 and CRP [19]. However, no studies have reported the activation state of NF-κB in primary and uncomplicated cases of T2DM.

Hyperglycemia is a common feature of diabetes mellitus (DM), and a main pathogenic factor of diabetes and its complications. For cells in a long-term high glucose environment, changes to the cell structure and function, and the specific pathogenic responses of a variety of cytokines and vasoactive factors are not yet known [20]. Acute hyperglycemia can raise TNF-α and IL-6 levels in the blood of healthy adults and adults with impaired glucose tolerance. It can enhance NF-κB binding activity in the PBMC of healthy subjects, and increase the production of reactive oxygen species in PBMC [21]. Reduction of postprandial hyperglycemia in patients with type 2 diabetes can reduce NF-κB activation in PBMCs [22]. Some research found that in type 1 diabetes patients, NF-κB binding activity in PBMCs was higher in patients with a glycosylated hemoglobin (HbA1c) level greater than 10%, than in patients with a HbA1c of 6–8% [23]. However, the effect of controlling blood glucose in T2DM patients on the level of inflammatory factors regulated by NF-κB is still controversial and no information has been reported for Chinese populations.

This study observed PBMC NF-κB DNA-binding activity, protein levels of the NF-κB subunit p65 and inhibitor of NF-κB (IκBα), and mRNA levels of downstream inflammatory factors such as TNF-α and IL-6, in 40 T2DM patients and 10 controls Subjects had a range of body mass indexes (BMIs) and blood glucose control levels. The aim of the study was to investigate the impact of obesity, blood glucose control and T2DM on patient inflammatory status, in order to investigate possible means of preventing T2DM and macro-vascular complications.

2. Subjects, materials and methods

2.1. Patients and groups

Subjects were 40 patients with incipient T2DM (27 men and 13 women) aged 26–72 years (mean 49.63 years), recruited from the First Affiliated Hospital of the Medical College of Xi'an Jiaotong University (Shaanxi Province, China). T2DM was diagnosed by the American Diabetes Association (ADA), 2007 criteria [24]. The disease course of all the recruited patients was less than 5 years. Subjects with heart, brain, kidney or other major organ disease, or ketoacidosis, vascular complications or neuropathy such as diabetic nephropathy and diabetic ophthalmopathy, were excluded. Patients were equally divided by body mass index (BMI), with 20 subjects classified as obese (OB, BMI $\geq$ 25 kg/m^2), and 20 as normal (NOR, BMI $<$ 25 kg/m^2) [25]. The OB and NOR groups each had 10 patients with high glycohemoglobin levels (HbA1c $>$ 7%). For controls, 10 healthy male volunteers aged 31–47 years (mean 38.60 years) were recruited. Based on BMI as described above, five were OB, and five were NOR. The 10 healthy controls met the following criteria: fasting plasma glucose $<$ 5.6 mmol/l and 2-h plasma glucose $<$ 7.8 mmol/l in a 75 g, oral glucose-tolerance test; no obvious infection or definite potential infection; no other acute or chronic inflammatory diseases; and no arteriosclerosis and other diabetic risk diseases. Informed consent was obtained from all the subjects, and the study protocol approved by the Xi'an Jiaotong University Ethics Committee.

2.2. Blood samples and insulin resistance index calculation

Insulin was measured from fasting plasma samples with the use of an Radio immuno assay kit (Union Medical & Pharmaceutical Tech Ltd.; Tianjin, China). Glucose was measured in Peripheral blood by ACCU-CHEK® Advantage Glucometer (Roche Diagnostics Ins). Venous blood samples were obtained for measurement of total cholesterol and triglyceride use Automatic biochemical analyzer (HITACHI 7600; Tokyo, Japan) while HbA1c was measured by high-performance liquid chromatography (Adams A1c HA-8160; Arkray, Kyoto, Japan). Homeostasis model assessment of insulin resistance (HOMA-IR), an index of insulin resistance, was calculated according to Matthews et al. [26] with the use of the following:

$$\text{HOMA} - \text{IR} = \frac{\text{fasting insulin}(\mu\text{U/ml}) \times \text{fasting glucose}(\text{mmol/l})}{22.5}$$

2.3. Protein and RNA isolation

Peripheral blood was collected from patients and control volunteers. PBMC were separated by density gradient centrifugation with Lymphocyte isolation solution (Shanghai Huajing Biotech Co; Shanghai, China) and stored at −80 °C. Nuclear and cytoplasmic proteins were isolated using NE-PER Nuclear and Cytoplasmic Extraction Reagents (Pierce, Rockford, IL) according to the manufacturer's instructions, added protease inhibitor cocktail (Roche Molecular Biochemicals; Mannheim, Germany) to all the samples, and used the BCA protein assay reagent kit (Pierce; Rockford, IL) to quantified total proteins. Total RNA was isolated by the RNA Fast 200 total RNA isolation kit (Fastgen Shanghai, China) following the kit instructions. All the samples were stored at −80 °C.

2.4. Electrophoretic mobility shift assay (EMSA)

The EMSA method was used to characterize NF-κB transcription factor binding activity in nuclear extracts using the LightShift chemiluminescent EMSA Kit (Pierce, Rockford, IL) as described by the manufacturer. Briefly, biotin-labeled probes (5′-AGT TGA GGG GAC TTT CCC AGG C-3′; 3′-TCA ACT CCC CTG