

Short Communication

No Association between Oxidative Stress and IL-6 in NIDDM Patients with Nephropathy

(IL-6 / oxidative stress / diabetic nephropathy)

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Abstract. Oxidative stress is hypothesized to play a role in the development of diabetes with and without nephropathy. In addition, it has been suggested that some metabolic abnormalities associated with diabetes may be due to cytokine overproduction. In the light of this knowledge, we aimed to measure MDA levels as a marker of oxidative stress and the IL-6 level in diabetes with and without different stages of nephropathy. Plasma MDA levels in the group of NIDDM patients with advanced nephropathy were significantly higher than in the group of NIDDM patients without nephropathy, which had significantly higher levels compared with the control group. Although IL-6 levels were elevated in diabetic groups with and without nephropathy in comparison with the control, no significant difference was found between patient groups. As a conclusion, oxidative stress may play an important role in diabetes with and without nephropathy, but the IL-6 level may not be useful in the evaluation of diabetic nephropathy.

Diabetic nephropathy is an important complication of both types of diabetes (Krolewski, 1999). Several hypotheses concerning the development of diabetic nephropathy have been proposed. However, the pathogenesis of diabetic nephropathy is not known clearly.

Oxidative stress has been considered to be a pathogenic factor of diabetic complications including nephropathy (Cooper, 2001). Increased oxidative stress has also been shown to induce cytokine expression in diabetes (Lehmann and Schleicher, 2000). Cytokines

are known to play an important role in the regulation of the immune and inflammatory response and may be involved in the pathogenesis of diabetes (Sekizuka et al., 1994). Interleukin 6 (IL-6) is a multifunctional cytokine (Hirano et al., 1990). In the kidney, IL-6 is synthesized by mesangial cells and acts as an autocrine growth factor (Fukatsu et al., 1991). Although there has been little work on the relationship between IL-6 and diabetic nephropathy, many studies have already shown that it is involved in the proliferation of mesangial cells (Shikano et al., 2000). In addition, recent studies suggest that IL-6 may be an early indicator for diabetic nephropathy (Sekizuka et al., 1994).

Taking together these observations, we aimed to investigate the relationship between oxidative stress and the IL-6 level in diabetes with and without nephropathy and to determine whether IL-6 may be useful for monitoring the progression of this disease.

Material and Methods

The patients were recruited from the Nephrology Department of the Istanbul Faculty of Medicine and Experimental Medical Research Institute. All individuals gave informed consent to the present study.

They were classified into four groups. The first group consisted of healthy subjects (N = 38).

The second group included patients with non-insulin-dependent diabetes mellitus (NIDDM) of at least 5 years duration with normoalbuminuria (< 30 mg/day) (N = 38). The third group (incipient diabetic nephropathy) included NIDDM patients with microalbuminuria (30–300 mg/day) and glomerular filtration rate (GFR) greater than 60 ml/min (N = 36). The fourth group (advanced diabetic nephropathy) included NIDDM patients with persistent macroalbuminuria (> 300 mg/day) and GFR lower than 60 ml/min (N = 39).

Data on the blood pressure and body mass index (BMI, kg/m²) were recorded for each patient. Various clinical parameters including glucose, creatinine, blood urea nitrogen (BUN) were measured using an autoanalyzer

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Abbreviations: BMI – body mass index, BUN – blood urea nitrogen, DN – diabetic nephropathy, EDTA – ethylene diamine tetraacetic acid, GFR – glomerular filtration rate, IL-6 – interleukin-6, MDA – malondialdehyde, NIDDM – non-insulin-dependent diabetes mellitus.

Table 1. Biochemical parameters of control and patient groups (means \pm SD)

	Control	NIDDM	Incipient DN	Advanced DN
GFR (ml/min)	121.76 \pm 38.44 ^a	101.96 \pm 35.05 ^a	98.51 \pm 29.96 ^a	35.19 \pm 11.76 ^b
Glucose (mg/dl)	85.94 \pm 7.26 ^a	154.78 \pm 61.57 ^b	184.4 \pm 86.89 ^b	171.88 \pm 79.87 ^b
BUN (mg/dl)	14.47 \pm 3.84 ^a	17.66 \pm 4.44 ^a	21.76 \pm 8.64 ^a	40.92 \pm 15.14 ^c
Creatinine (mg/dl)	0.72 \pm 0.16 ^a	0.94 \pm 0.28 ^a	1.06 \pm 0.35 ^b	2.55 \pm 1.21 ^c
MDA (nmol/ml)	5.53 \pm 1.42 ^a	6.13 \pm 0.96 ^b	6.95 \pm 1.70 ^{bc}	6.97 \pm 1.44 ^c
IL-6 (ng/ml)	18.68 \pm 5.11 ^a	23.79 \pm 1.08 ^b	23.06 \pm 0.83 ^b	23.63 \pm 0.82 ^b

^{abc}Values not sharing a common superscript letter are significantly different by ANOVA (LSD) test. P < 0.05

(Hitachi 902, Mannheim, Germany). Plasma IL-6 levels were determined by the competitive enzyme immunoassay principle (ELISA) (Chemicon, Temecula, CA). Plasma levels of malondialdehyde (MDA) were measured according to the method described by Buege and Aust (1978) using a molar extinction coefficient of $1.56 \times 10^{-5} \text{ M}^{-1}\text{cm}^{-1}$.

Results and Discussion

The patient groups were well-matched for age and body mass index. Diabetic patients with advanced nephropathy had significantly higher blood pressure values as compared with diabetic patients with incipient nephropathy and without nephropathy (data not shown). Elevated serum creatinine levels were observed in patients with incipient and advanced diabetic nephropathy. As expected, GFR differed with nephropathy stage (Table 1).

The mechanisms involved in high glucose-induced oxidative stress are still unclear. Glomerular changes seen in long-term diabetes may be caused by increased non-enzymatic reaction of glucose and subsequent formation of advanced glycosylation end product (AGE) or increased metabolism of glucose leading to oxidative stress (Ha and Kim, 1999; Lehmann and Schleicher, 2000). Lipid peroxidation, one of the radical reactions *in vivo*, is an important index for oxidative stress (Baynes, 1991). However, data on lipid peroxidation in diabetic nephropathy is scant. Kedziora-Kornatowska et al. showed that lipid peroxide levels were significantly elevated in erythrocytes of NIDDM patients without nephropathy and higher in erythrocytes of NIDDM patients with nephropathy (Kedziora-Kornatowska et al., 1998). In our study, lipid peroxide levels rose significantly in diabetic patients. In addition, a significant increase in lipid peroxide levels was found in the advanced nephropathy stage as compared with diabetes without nephropathy (Table 1). On the other hand, it has been demonstrated that increased oxidative stress may lead to increased cytokine production (Feingold and Grunfeld, 1992). In addition, several but not all studies reported that cytokine levels are increased in diabetic

patients (Dasgubta et al., 1991; Mooradian et al., 1991). In our study, serum IL-6 levels rose in diabetic patients with and without nephropathy as compared with control subjects. However, there was no significant change in different nephropathy stages when compared with the diabetic group (Table 1). Contrary to some other findings (Feingold and Grunfeld, 1992; Lehmann and Schleicher, 2000), increased oxidative stress did not influence this cytokine level in diabetic nephropathy. According to our results, diabetes may be associated with an enhanced IL-6 level, but may not be an indicator for nephropathy.

In conclusion, oxidative stress may be important in the pathogenesis of diabetes with and without nephropathy. However, the IL-6 level may not be useful in the evaluation of diabetic nephropathy, but more detailed study will be needed.

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