

Relationship Between Interleukin-8 and the Oxidant-Antioxidant System in End-Stage Renal Failure Patients

Makbule Aydin,¹ Elif Ozkok,¹ Oguz Ozturk,² Bedia Agachan,² Hulya Yilmaz,²
Ilhan Yaylim,² Seher Kebabcioglu,³ Turgay Ispir²

Objectives: We aimed to evaluate the relationship between interleukin-8 and the oxidant-antioxidant system in end-stage renal failure patients with and without diabetes mellitus undergoing regular hemodialysis treatment.

Materials and Methods: Plasma levels of malondialdehyde and whole blood reduced glutathione were measured as markers of the oxidant and antioxidant systems, respectively. Plasma interleukin-8 levels were measured by enzyme-linked immunosorbent assay.

Results: When compared with controls, plasma interleukin-8 levels were elevated in both diabetic and nondiabetic end-stage renal disease patients. Plasma malondialdehyde levels were statistically significantly higher in end-stage renal disease patients with and without diabetes mellitus than they were in controls; however, reduced glutathione levels were statistically significantly lower in diabetic and nondiabetic end-stage renal disease patients than they were controls.

Conclusions: In end-stage renal disease patients with and without diabetes mellitus, elevated interleukin-8 levels and decreased reduced glutathione levels may be attributed to increased oxidative stress due to inflammation. In other words, increased reactive oxygen species may induce interleukin-8 production and result in diminished reduced glutathione levels. Our data suggest a relationship between interleukin-8 and the oxidant-antioxidant system in end-stage renal failure patients.

Key words: Cytokine, Malondialdehyde, Glutathione

Since 1997, accumulating experimental evidence has shown that reactive oxygen species (ROS) play a key role in the pathophysiological pathways of a wide variety of clinical and experimental renal diseases [1-3]. These ROS, including the superoxide anion, the hydroxyl radical, hypochlorous acid, and peroxy-nitrite may be generated by activated neutrophils, monocytes, and mesangial cells during metabolic processes [4]. ROS have been shown to be primary mediators in glomerulonephritis, and they are factors in the regulation of glomerular permeability to proteins, development of morphologic lesions, and alteration of glomerular hemodynamics (ie, reductions of glomerular blood flow and glomerular filtration rate) [5]. ROS, generated either extracellularly or intracellularly through a ligand-receptor interaction, can function as signal transduction molecules to activate chemotactic cytokine interleukin-8 (IL-8) [6].

IL-8 is an important cytokine in the process of inflammation. It is produced by a wide variety of cell types including monocytes, fibroblasts, endothelial cells, keratinocytes, and Langerhans cells in response to inflammatory stimuli [7]. Endothelial-derived IL-8 secreted into the subendothelial matrix or bound to the surface of the endothelium promotes neutrophil adherence and migration [8, 9].

It has been suggested that antioxidants inhibit production of IL-8 [10]. Glutathione (GSH) is an important antioxidant that eliminates toxic peroxides and aldehydes from the cell and indirectly maintains vitamins C and E in their reduced and functional forms in tissue [11-13]. In renal diseases, there is profound imbalance between the oxidant and the antioxidant systems [14]. In addition, elevated oxidative stress induces the synthesis of chemokines [15].

In this study, we evaluated the relationship between IL-8 and the oxidant-antioxidant system in patients with end-stage renal disease (ESRD) with

Departments of ¹Neuroscience and ²Molecular Medicine, Istanbul University, Institute for Experimental Medicine; and ³Department of Nephrology, University of Marmara, Istanbul, Turkey

Address reprint requests to: Assoc Prof Makbule Aydin, Istanbul University, Institute for Experimental Medicine, Department of Neuroscience, Vakıf Gureba Cad. 34280, Caba, Istanbul, Turkey

Phone: 00 90 212 414 20 00 / 33356 Fax: 00 90 212 532 41 71 E-mail: aydinm@istanbul.edu.tr

and without diabetes mellitus (DM) undergoing regular hemodialysis treatment.

Materials and Methods

Study Protocol

The study population included 71 ESRD patients with DM (21 with type 1 DM and 42 with type 2 DM) and without DM (4 with chronic glomerulonephritis, 3 with chronic pyelonephritis, and 1 with Alport's syndrome) undergoing regular hemodialysis and 94 healthy subjects who served as controls. Prior to the study, the study protocol was approved by our local institutional ethics committee. The protocol conforms with the ethical guidelines of the 1975 Helsinki Declaration. Written informed consent was obtained from all of the subjects.

Diabetic patients were divided into type 1 and type 2 to observe whether diabetes type had any effect on the parameters we measured. ESRD patients were recruited from various hemodialysis centers. Whole blood with ethylenediaminetetraacetic acid (EDTA) was obtained from the Istanbul Medical Faculty, the Marmara University School of Medicine, Okmeydani State Hospital, and Baskent University in Istanbul, Turkey. Control subjects were selected among people without a history of renal diseases and without DM in their first-degree relatives. Patients with ESRD with creatinine clearances of less than 15 mL/min were included in the study.

Biochemical determinations

Blood samples (10 mL) were taken just before the dialysis procedure, and whole blood reduced GSH levels were studied on the same day. Antioxidant status was determined by measuring reduced GSH levels in whole blood according to the method of Beutler and associates [16]. After whole blood was hemolyzed with distilled water, reduced GSH levels were determined using a 5,5'-dithio-bis-(2-nitrobenzoic acid) reagent. The formed colored complex

was measured at 412 nm by a spectrophotometer. The remainder of the blood was used to obtain plasma to investigate IL-8 and malondialdehyde (MDA) levels. The extent of plasma lipid peroxidation was assessed by measuring MDA, the end product of lipid peroxidation, using a thiobarbituric acid assay according to the method of Yagi [17]. After a reaction of thiobarbituric acid with MDA, the reaction product was extracted in butanol, and its absorbance was determined spectrophotometrically at 535 nm. Plasma IL-8 levels were determined with an enzyme-linked immunosorbent assay kit (Pelikine Compact, CLB, Amsterdam, Netherlands) according to the manufacturer's instructions. The detection limit of the assay was 0.6 pg/mL.

Statistical analyses

Statistical analyses were performed using SPSS software (Statistical Package for the Social Sciences, version 10.0, SSPS Inc, Chicago, IL, USA). Clinical laboratory data are expressed as means $\pm$ SD. Mean values between patients and controls were compared using an unpaired *t* test. Values for *P* less than .05 were considered statistically significant.

Results

The demographic characteristics of the study groups are given in Table 1. There were no significant differences with regard to age among diabetic ESRD, nondiabetic ESRD patients, and controls. Table 2 shows the biochemical parameters of the patient and the control groups. As expected, fasting plasma glucose levels were considerably higher in both type 1 and type 2 diabetic ESRD patients than they were nondiabetic ESRD patients and healthy controls ($P < .01$). When patients in the type 1 and type 2 diabetic groups were compared, no significant differences with regard to MDA, GSH, or IL-8 levels were found. Compared with controls, plasma IL-8 levels were elevated in both diabetic ESRD ($P < .001$) and

Table 1. Demographic characteristics of the subjects

Subjects' clinical parameters	ESRD patients (with type 1 DM)	ESRD patients (with type 2 DM)	ESRD patients (Nondiabetic)	Controls
Female: male:ratio	6:15	13:29	3:5	38:56
Age (y)*	47.6 $\pm$ 2.8	48.6 $\pm$ 7.5	45.6 $\pm$ 8.4	46.2 $\pm$ 2.3
Duration of diabetes (y)*	19.6 $\pm$ 1.8	15 $\pm$ 1.20	-	-
Duration of dialysis (y)*	3.7 $\pm$ 1.6	2.4 $\pm$ 0.4	3.5 $\pm$ 1.4	-
Dialysis frequency per week	3	3	3	-
Dialysis type for	Acetate	Acetate	Acetate	-
Hemodialysis	HCO ₃	HCO ₃	HCO ₃	-

*Values are presented as means $\pm$ SD.

ESRD, end-stage renal disease; DM, diabetes mellitus

Table 2. Biochemical parameters of subjects

Biochemical parameters	ESRD patients (with type 1 DM)	ESRD patients (with type 2 DM)	ESRD patients (Nondiabetic)	Controls
Fasting glucose (mmol/L)	9.59 ± 4.7*, ‡	7.89 ± 2.6*, ‡	4.81 ± 0.8	4.25 ± 0.4
Plasma IL-8 (pg/mL)	14.9 ± 7.9†	13.4 ± 8.7†	9.2 ± 6.4*	4.2 ± 1.7
Plasma MDA levels (μmol /L)	5.4 ± 1.8*	4.7 ± 1.1	5.0 ± 1.5*	4.3 ± 1.7
Whole blood reduced GSH (mmol/L)	0.128 ± 0.039*	0.111 ± 0.024*	0.096 ± 0.023*	0.160 ± 0.032

Values are presented as means ± SD.

IL-8, Interleukin-8; MDA, malondialdehyde; GSH, glutathione; ESRD, end-stage renal disease; DM, diabetes mellitus

* $P < .01$, compared with controls

† $P < .001$, compared with controls

‡ $P < .01$, compared with nondiabetic ESRD patients

nondiabetic ESRD patients ($P < .01$). Plasma MDA levels were statistically significantly higher in ESRD patients with and without DM than they were in controls ($P < .01$), but whole blood reduced GSH levels were significantly lower in diabetic and nondiabetic ESRD patients compared with controls ($P < .01$) (Table 2).

Discussion

An expanding body of data now strongly suggests that chemokines contribute to inflammatory glomerular as well as tubulointerstitial diseases [18-20]. All types of renal cells can produce chemokines [21]. ROS can upregulate chemokine expression and may represent a common mechanism of injury-induced chemokine generation [22].

In the present study, plasma IL-8 and MDA concentrations were markedly elevated in diabetic ESRD and nondiabetic ESRD patients compared with healthy controls. Increased MDA concentrations may be due to the elevated oxidative stress during inflammation in ESRD patients. Mezzano and associates [23] found that TNF- α , IL-8 inflammatory cytokines, and thiobarbituric acid-reactive substances, a marker of lipid peroxidation, were increased in patients with chronic renal failure compared with healthy controls. Oberg and associates [24] showed increased oxidative stress and acute-phase inflammation in patients with stage-3, stage-4, and stage-5 chronic kidney disease compared with healthy subjects. In our study, oxidative stress also may have induced IL-8 production by activating nuclear transcription factors in ESRD patients. Oxidative stress has been reported to mediate IL-8 synthesis [6]. Recent studies have demonstrated that oxidative stress generated directly by exogenous H₂O₂ differentially induces IL-8 synthesis in epithelial and endothelial cells [25, 26]. IL-8 induction is associated with the activation of nuclear transcription factors,

such as nuclear factor kappa B (NF- κ B) and activator protein-1 in response to oxidative stress in various cell types [6, 27]. Since the IL-8 gene promoter region contains binding sites for activator protein-1 and NF- κ B, activation of NF- κ B leads to the production of mRNA species encoding IL-8.

GSH concentrations are closely correlated with the degree of renal failure. Our study showed that whole blood GSH levels were significantly lower in ESRD patients with and without DM compared with controls. It has been reported that increased ROS result in depletion of antioxidants [28]. GSH is present in high concentrations in most prokaryotic and eukaryotic cells. Body fluids such as bile, glomerular filtrate, blood plasma, and epithelial cell lining also contain GSH. GSH can react with ROS in several ways. First, it can react as a reductant, reducing species such as H₂O₂ directly to water with the formation of oxidized glutathione (GSSG). Second, it can react directly with ROS, yielding GSSG [29]. The GSH:GSSG ratio is therefore an indicator of oxidative stress. One limitation of our study is that we could not measure the concentration of GSSG. In our study, GSH depletion could have arisen from a decreased GSH:GSSG ratio. Oxidative stress might result in a decrease in this ratio because of elevated GSSG [28]. GSH depletion also could be attributed to decreased GSH synthesis and/or increased GSH degradation. Oxidative stress or depletion of GSH and subsequent increases in cytosolic GSSG in response to oxidative stress lead to the activation of NF- κ B [30]. Depletion in GSH by itself could contribute to the progression of uremia because it has been demonstrated that GSH depletion in rats leads to acute renal failure [31, 32]. Some authors have suggested that a naturally occurring thiol antioxidant compound such as ergothioneine could inhibit transcriptional activation of IL-8 [10]. Redox-sensitive transcription factors such as NF- κ B and activator protein-1 (their activation is affected by the redox GSH balance) have been shown

to be activated in epithelial and inflammatory cells during oxidative stress and inflammation, leading to the upregulation of several proinflammatory genes [15].

In conclusion, in ESRD patients with and without DM, elevated IL-8 and decreased GSH levels may be attributed to increased oxidative stress, which results from inflammation during ESRD. In other words, increased MDA may induce IL-8 production and also result in decreased GSH. Our data suggest a relationship between IL-8 and the oxidant-antioxidant status in ESRD patients.

References

- Baud L, Ardaillou R. Reactive oxygen species: production and role in the kidney. *Am J Physiol* 1986; 251(5 Pt 2): F765-F776
- Nath KA, Salahudeen AK. Induction of renal growth and injury in the intact rat kidney by dietary deficiency of antioxidants. *J Clin Invest* 1990; 86(4): 1179-1192
- Yoshioka T, Bills T, Moore-Jarrett T, Greene HL, Burr IM, Ichikawa I. Role of intrinsic antioxidant enzymes in renal oxidant injury. *Kidney Int* 1990; 38(2): 282-288
- Andreoli SP. Reactive oxygen molecules, oxidant injury and renal disease. *Pediatr Nephrol* 1991; 5(6): 733-742.
- Baud L, Ardaillou R. Involvement of reactive oxygen species in kidney damage. *Br Med Bull* 1993; 49(3): 621-629
- Roebuck KA. Oxidant stress regulation of IL-8 and ICAM-1 gene expression: differential activation and binding of the transcription factors AP-1 and NF-kappaB (Review). *Int J Mol Med* 1999; 4(3): 223-230
- Matsushima K, Oppenheim JJ. Interleukin 8 and MCAF: novel inflammatory cytokines inducible by IL 1 and TNF. *Cytokine* 1989; 1(1): 2-13
- Urakaze M, Temaru R, Satou A, Yamazaki K, Hamazaki T, Kobayashi M. The IL-8 production in endothelial cells is stimulated by high glucose. *Horm Metab Res* 1996; 28(8): 400-401
- Larsen CG, Anderson AO, Appella E, Oppenheim JJ, Matsushima K. The neutrophil-activating protein (NAP-1) is also chemotactic for T lymphocytes. *Science* 1989; 243(4897): 1464-1466
- Rahman I, Gilmour PS, Jimenez LA, Biswas SK, Antonicevic F, Aruoma OI. Ergothioneine inhibits oxidative stress- and TNF-alpha-induced NF-kappa B activation and interleukin-8 release in alveolar epithelial cells. *Biochem Biophys Res Commun* 2003; 302(4): 860-864
- Reed DJ. Glutathione: toxicological implications. *Annu Rev Pharmacol Toxicol* 1990; 30: 603-631
- Wefers H, Sies H. Antioxidant effects of ascorbate and glutathione in microsomal lipid peroxidation are dependent on vitamin E. In: Poli G, Cheeseman KH, Dianzani MU, Slater TF, eds. *Advances in biosciences: free radicals in the pathogenesis of liver injury*. New York, Pergamon Press; 1989, pp 309-316
- Winkler BS, Orselli SM, Rex TS. The redox couple between glutathione and ascorbic acid: a chemical and physiological perspective. *Free Radic Biol Med* 1994; 17(4): 333-349
- Huang HS, Ma MC, Chen J, Chen CF. Changes in the oxidant-antioxidant balance in the kidney of rats with nephrolithiasis induced by ethylene glycol. *J Urol* 2002; 167(6): 2584-2593
- Lakshminarayanan V, Drab-Weiss EA, Roebuck KA. H₂O₂ and tumor necrosis factor-alpha induce differential binding of the redox-responsive transcription factors AP-1 and NF-kappaB to the interleukin-8 promoter in endothelial and epithelial cells. *J Biol Chem* 1998; 273(49): 32670-32678
- Beutler E, Duron O, Kelly BM. Improved method for the determination of blood glutathione. *J Lab Clin Med* 1963; 61: 882-888
- Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem* 1979; 95(2): 351-358
- Rovin BH, Phan LT. Chemotactic factors and renal inflammation. *Am J Kidney Dis* 1998; 31(6): 1065-1084
- Prodjosudjadi W, Gerritsma JS, Klar-Mohamad N, Gerritsen AF, Bruijn JA, Daha MR, van Es LA. Production and cytokine-mediated regulation of monocyte chemoattractant protein-1 by human proximal tubular epithelial cells. *Kidney Int* 1995; 48(5): 1477-1486
- Schmouder RL, Strieter RM, Kunkel SL. Interferon-gamma regulation of human renal cortical epithelial cell-derived monocyte chemotactic peptide-1. *Kidney Int* 1993; 44(1): 43-49
- Schlondorff D, Nelson PJ, Luckow B, Banas B. Chemokines and renal disease. *Kidney Int* 1997; 51(3): 610-621
- Satriano JA, Shuldiner M, Hora K, Xing Y, Shan Z, Schlondorff D. Oxygen radicals as second messengers for expression of the monocyte chemoattractant protein, JE/MCP-1, and the monocyte colony-stimulating factor, CSF-1, in response to tumor necrosis factor-alpha and immunoglobulin G. Evidence for involvement of reduced nicotinamide adenine dinucleotide phosphate (NADPH)-dependent oxidase. *J Clin Invest* 1993; 92(3): 1564-1571
- Mezzano D, Pais EO, Aranda E, Panes O, Downey P, Ortiz M, et al. Inflammation, not hyperhomocysteinemia, is related to oxidative stress and hemostatic and endothelial dysfunction in uremia. *Kidney Int* 2001; 60(5): 1844-1850
- Oberg BP, McMenamin E, Lucas FL, McMonagle E, Morrow J, Ikizler TA, Himmelfarb J. Increased prevalence of oxidant stress and inflammation in patients with moderate to severe chronic kidney disease. *Kidney Int* 2004; 65(3): 1009-1016
- Shimada T, Watanabe N, Hiraishi H, Terano A. Redox regulation of interleukin-8 expression in MKN28 cells. *Dig Dis Sci* 1999; 44(2): 266-273
- Lakshminarayanan V, Beno DW, Costa RH, Roebuck KA. Differential regulation of interleukin-8 and intercellular adhesion molecule-1 by H₂O₂ and tumor necrosis factor-alpha in endothelial and epithelial cells. *J Biol Chem* 1997; 272(52): 32910-32918
- Roebuck KA, Carpenter LR, Lakshminarayanan V, Page SM, Moy JN, Thomas LL. Stimulus-specific regulation of chemokine expression involves differential activation of the redox-responsive transcription factors AP-1 and NF-kappaB. *J Leukoc Biol* 1999; 65(3): 291-298
- Halliwell B. Free radicals, antioxidants, and human disease: curiosity, cause, or consequence? *Lancet* 1994; 344(8924): 721-724
- Cotgreave IA, Moldeus P, Orrenius S. Host biochemical defense mechanisms against prooxidants. *Annu Rev Pharmacol Toxicol* 1988; 28: 189-212
- Rahman I, MacNee W. Regulation of redox glutathione levels and gene transcription in lung inflammation: therapeutic approaches. *Free Radic Biol Med* 2000; 28(9): 1405-1420
- Abul-Ezz SR, Walker PD, Shah SV. Role of glutathione in an animal model of myoglobinuric acute renal failure. *Proc Natl Acad Sci U S A* 1991; 88(21): 9833-9837
- Yeung JH. Effects of glycerol-induced acute renal failure on tissue glutathione and glutathione-dependent enzymes in the rat. *Methods Find Exp Clin Pharmacol* 1991; 13(1):23-28