

# Leptin as an Inflammatory Marker in Dialysis Patients

## Diyaliz Hastalarında İnflamatuvar Belirteç Olarak Leptin

Gürsel YILDIZ, MD,<sup>a</sup>  
Abdülkerim YILMAZ, MD,<sup>b</sup>  
Naim NUR, MD,<sup>c</sup>  
Hüseyin AYDIN,<sup>d</sup>  
Mansur KAYATAŞ, MD<sup>a</sup>

Departments of  
<sup>a</sup>Nephrology,  
<sup>b</sup>Internal Medicine,  
<sup>c</sup>Public Health,  
<sup>d</sup>Biochemistry,  
Cumhuriyet University Faculty of Medicine,  
Sivas

Geliş Tarihi/Received: 16.06.2009  
Kabul Tarihi/Accepted: 19.10.2009

Yazışma Adresi/Correspondence:  
Gürsel YILDIZ, MD  
Cumhuriyet University Faculty of Medicine,  
Department of Nephrology, Sivas,  
TÜRKİYE/TURKEY  
drgursel@yahoo.com

**ABSTRACT Objective:** Markers of chronic inflammation, such as high-sensitive C-reactive protein (hs-CRP) or tumor necrosis factor-alpha (TNF- $\alpha$ ) and interleukin (IL)-6, are predictive for cardiovascular mortality and morbidity in patients with chronic renal failure. Leptin stimulates angiogenesis and accelerates vascular calcification and it plays an active role in the process of atherosclerotic disease. This study investigated the relationship between leptin and inflammatory markers (hs-CRP, TNF- $\alpha$  ve IL-6) and leptin as an inflammatory marker in dialysis patients. **Material and Methods:** We examined the relationship of serum leptin levels with inflammatory markers in dialyzed patients. Serum CRP or TNF- $\alpha$ , IL-6 and leptin levels were determined as well as other metabolic parameters. Correlation studies and multivariate regression analysis were performed among variables. **Results:** Our patients showed elevated serum leptin and inflammatory markers. Serum leptin levels were positively correlated with CRP, TNF- $\alpha$ , IL-6 and body mass index (BMI). Serum CRP levels were directly related to leptin levels. **Conclusion:** Both hemodialysis and continuous ambulatory peritoneal dialysis patients showed high levels of chronic inflammation markers. Serum leptin levels were elevated in dialysis patients and significantly correlated with inflammatory markers.

**Key Words:** Leptin; C-reactive protein; tumor necrosis factor alpha; IL-6 protein, human; dialysis; patients

**ÖZET Amaç:** Yüksek sensitif CRP (hs-CRP), tümör nekroz faktör-alfa (TNF- $\alpha$ ) ve interlökin (IL)-6 gibi kronik inflamasyon belirteçleri, son evre böbrek yetmezliği hastalarının kardiyovasküler mortalite ve morbiditelerini öngörmede etkindirler. Leptin anjiyogenezi uyarır, damarsal kalsifikasyonu hızlandırır ve aterosklerotik hastalık sürecinde aktif rol oynar. Bu çalışmada kardiyovasküler hastalık için çok yüksek risk taşıyan diyaliz hastalarında, serum leptin düzeyleri ile kardiyovasküler risk unsuru olan inflamatuvar belirteçler (hs-CRP, TNF- $\alpha$  ve IL-6) arasındaki ilişki ve diyaliz hastalarında inflamatuvar belirteç olarak leptin düzeyleri araştırıldı. **Gereç ve Yöntemler:** Serum CRP, TNF- $\alpha$ , IL-6 ve leptin değerleri, diğer metabolik parametrelerle birlikte ölçülüp değerlendirildi. İstatistiksel olarak, değişkenler arasında korelasyon analizi ve çok değişkenli varyans analizi uygulandı. **Bulgular:** Hastalarda serum leptin ve diğer inflamatuvar belirteçlerin seviyeleri yüksek bulundu. Serum leptin düzeyi ile serum CRP, TNF- $\alpha$ , IL-6 düzeyleri ve beden kitle indeksi arasında pozitif korelasyon saptandı. Serum CRP seviyesi ile serum leptin seviyesi arasında direkt bir ilişki belirlendi. **Sonuç:** Hemodiyaliz ve sürekli ayaktan periton diyaliz hastalarında kronik inflamatuvar belirteçler yüksek bulunmuştur. Serum leptin seviyeleri diyaliz hastalarında yüksek bulunmakla beraber inflamatuvar belirteçler ile de anlamlı olarak ilişkili bulunmuştur.

**Anahtar Kelimeler:** Leptin; C-reaktif protein; tümör nekroz faktörü alfa; IL-6 proteini, insan; diyaliz; hastalar

Chronic inflammation is increasingly rated as an important issue due to its role in various pathological states, such as cardiovascular disease, obesity, diabetes, cancer and malnutrition.<sup>1</sup> Cardiovascular disease is the major cause for mortality and morbidity in patients with end-stage renal failure (ESRF).<sup>2</sup> The causes of inflammation in dialysis patients are multiple and complex. The disease status of renal failure, the consequence of the treatment for renal failure such as hemodialysis, peritoneal dialysis or chronic infection may be involved in the pathogenesis of microinflammation in chronic dialysis patients.<sup>3</sup> Markers of an acute phase reaction, such as C-reactive protein (CRP) or tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and interleukin (IL)-6, are predictive for cardiovascular mortality and morbidity in chronic renal failure patients.<sup>4,5</sup> The inflammatory response is coordinated by cytokines, especially TNF- $\alpha$  and IL-6. CRP is synthesized by the liver and regulated by different cytokines, particularly interleukin (IL)-6.<sup>6</sup> Leptin, the adipocyte-derived protein product of the *ob* gene, is found to be associated with malnutrition in dialysis patients.<sup>7</sup> Recent data implicate the role of leptin as an independent risk factor for cardiovascular diseases.<sup>8</sup>

In the present study, we investigated the patterns of serum leptin, CRP and some cytokines such as TNF- $\alpha$  and IL-6 to assess chronic inflammation in both hemodialysis (HD) and continuous ambulatory peritoneal dialysis (CAPD) patients. Parameters such as albumin and hemoglobin levels and their relationship with inflammatory markers were determined.

## MATERIAL AND METHODS

### PATIENTS

All CAPD and HD patients were treated at the Hemodialysis Units of Cumhuriyet University, School of Medicine, Sivas. Dialysis Center, Sultan Izzettin Keykavus Hospital and the SSK Sivas Hospital were enrolled in this cross sectional study. A total of 178 patients (94 females, 84 males) who were diagnosed with end-stage renal failure were studied. Thirty patients (18 females, 12 males) aged 25 to 75 years (mean,  $43 \pm 4$  years) on maintenance

CAPD therapy for a mean of  $31.8 \pm 16.3$  months (range, 4 to 60 months), and 148 patients (76 females, 72 males) aged 18 to 82 years (mean,  $50 \pm 9$  years) on maintenance HD therapy for a mean of  $56.4 \pm 46.3$  months (range, 3 to 226 months) were included. HD patients in different HD units used the same dialysis membrane (hemaphen dialyzer membranes) and they were hemodialysed for 4-5 h a day, three times a week. CAPD patients were dialyzed using Fresenius ANDY Plus or Stay-Safe systems (Fresenius Medical Care GmbH, Bad Homburg, Germany). All patients were clinically stable during the period of at least two months before assessment. Patients were excluded if they had infectious or noninfectious inflammatory disease, including dialysis-related peritonitis, if they were treated with immunosuppressive drugs, if they were on dialysis with jugular, subclavian or long-term permanent port catheter. Written informed consents were obtained from all participants. The study was approved by Local Ethics Committee.

### ASSAYS

Weight and height were measured by standard technique in all subjects. Body mass index (BMI) was calculated by dividing the weight to square of height ( $\text{kg}/\text{m}^2$ ).

Blood samples were taken in the morning after 12 h of overnight fasting (non HD day) from all of the patients to establish levels of leptin, TNF- $\alpha$ , IL-6, hs-CRP and other parameters. Samples were stored at  $-20^\circ\text{C}$ . Serum levels of TNF- $\alpha$ , IL-6 and hs-CRP were determined by enzyme-linked immunosorbent assay. Primary and biotinylated antibodies against TNF- $\alpha$ , and IL-6 were purchased from R&D Systems Europe (Abingdon, UK). Primary and biotinylated antibodies against hs-CRP were purchased from Dako (Glostrup, Denmark). For TNF- $\alpha$ , and IL-6, the sensitivity ranged between 2 and 3 pg/ml. For hs-CRP, it was usually 1 ng/ml.

The level of serum leptin was analyzed by radioimmunoassay through employing a DPC Gambia-CR (Japan) gamma counter. The DSL-23100 RCA (Diagnostic systems Lab, USA) commercial kit was used for serum leptin levels. Albumin and he-

moglobin levels were assessed by means of standard laboratory methods.

## STATISTICAL ANALYSIS

The statistical analysis was performed with the SPSS 12.0 software. All data were presented as mean  $\pm$  standard deviation. Demographic and biochemical data between the groups were compared by Student's t-test. Relationship among the variables was analyzed by Pearson correlation analysis. Stepwise multiple regression analysis was performed for selected variables: hs-CRP, leptin, IL-6 and TNF- $\alpha$ . P values less than 0.05 were considered as statistically significant.

## RESULTS

Demographic and biochemical features of all patients, both CAPD and HD groups are summarized in Table 1. The dialysis duration was significantly longer in hemodialysis patients ( $56.4 \pm 46.3$  vs.  $31.8 \pm 16.3$  months) as well as the age ( $51 \pm 15$  vs.  $43 \pm 14$  years). Hemoglobin levels were significantly higher in CAPD patients than in HD patient ( $11.1 \pm 1.4$  g/dl vs.  $10.3 \pm 1.8$ ). No difference was found in BMI, albumin, TNF- $\alpha$ , Interleukin-6, hs-CRP and leptin levels between two groups.

The correlations among the variables are listed in Table 2. Inflammatory markers and clinical features of all patients were analyzed. The time of dialysis treatment had a negative effect on hemoglobin levels ( $r = -0.18$  and  $p = 0.017$ ). The correlation of serum leptin with TNF- $\alpha$ , Interleukin-6 and

hs-CRP levels showed significantly positive correlations ( $r = 0.44$  and  $p = 0.000$ ,  $r = 0.29$  and  $p = 0.000$ ,  $r = 0.21$  and  $p = 0.005$ , respectively). There was a positive correlation between BMI, serum CRP and TNF- $\alpha$  levels as well ( $r = 0.15$  and  $p = 0.04$ ,  $r = 0.27$  and  $p = 0.000$ , respectively). As expected, the serum leptin was significantly correlated with BMI ( $r = 0.41$  and  $p = 0.000$ ). Overweight patients ( $BMI > 24.0$  kg/m<sup>2</sup>) had higher levels of inflammatory markers than those with normal weight (data not shown).

Albumin level was positively correlated with hemoglobin and TNF- $\alpha$  levels ( $r = 0.19$  and  $p = 0.013$ ,

**TABLE 1:** Demographic and biochemical features of patients with peritoneal dialysis and hemodialysis.

Features	All (n=178) X $\pm$ SE	CAPD (n=30)	HD (n=148)	P Value
Age (years)	50 $\pm$ 3.7	43 $\pm$ 7.8	51 $\pm$ 4.2	0.01
Dialysis duration (months)	52.3 $\pm$ 3.9	31.8 $\pm$ 5.8	56.4 $\pm$ 4.6	0.001
Gender (female : male)	94 : 84	18 : 12	76 : 72	-
BMI	25.6 $\pm$ 1.9	23.8 $\pm$ 4.3	24.6 $\pm$ 2.0	NS
Hemoglobin (g/dl)	10.4 $\pm$ 0.8	11.1 $\pm$ 2.0	10.3 $\pm$ 0.8	0.02
Albumin (g/dl)	3.6 $\pm$ 0.3	3.7 $\pm$ 0.7	3.6 $\pm$ 0.3	NS
TNF- $\alpha$ (pg/mL)	52.0 $\pm$ 3.9	50.3 $\pm$ 9.2	53.7 $\pm$ 4.4	NS
IL-6 (pg/mL)	22.4 $\pm$ 1.7	20.6 $\pm$ 3.8	22.7 $\pm$ 1.9	NS
hs-CRP (ng/L)	12.4 $\pm$ 0.9	11.6 $\pm$ 2.1	12.6 $\pm$ 1.0	NS
Leptin (ng/ml)	32.6 $\pm$ 2.4	37.9 $\pm$ 19.2	31.5 $\pm$ 2.6	NS

P<0.05 (Student's t test ) CAPD vs. HD.

BMI: body mass index; hs-CRP: high sensitive C-reactive protein; IL-6: interleukin-6; TNF- $\alpha$ : tumor necrosis factor-  $\alpha$ : NS, not significant.

**TABLE 2:** Correlation values of biochemical and anthropometric variables in the study populations.

	Leptin	Age	Dd	Gender	BMI	Hb	Alb	TNF- $\alpha$	IL-6
In total patients (n =178)									
Age	-0.14								
Dd	0.40	-0.30							
Gender	-0.29*	0.20***	-0.65						
BMI	0.41*	0.05	0.06	-0.36**					
Hb	-0.10	-0.06	-0.18**	0.06	-0.15				
Alb	-0.02	0.04	-0.00	-0.04	-0.00	0.19**			
TNF- $\alpha$	0.44**	0.00	0.02	-0.20***	0.27***	-0.04	0.20***		
IL-6	0.29**	-0.10	0.10	-0.24***	-0.03	0.03	0.06	0.32*	
hs-CRP	0.21**	-0.04	-0.04	0.01	0.15***	-0.10	0.00	0.15	0.08

Dd: Dialysis duration; BMI: Body Mass Index; Hb: Hemoglobin; Alb: Albumin; TNF- $\alpha$ : tumor necrosis factor- alpha; IL-6: Interleukin-6; hs-CRP: high-sensitive C- reactive protein. All statistically significant correlations are in bold type (\*p< 0.001, \*\*p< 0.01 and \*\*\*p< 0.05).

**TABLE 3:** Multiple regression analysis of selected markers.

(a) hs-CRP*		
Independent variable	$\beta$	P
Leptin	0.21	0.005
(b) Leptin		
Independent variables		
Gender	-0.17	0.02
BMI	0.35	0.000
(c) Interleukin-6*		
Independent variables		
Leptin	0.18	0.02
TNF- $\alpha$	0.24	0.003
(d) TNF- $\alpha$ *		
Independent variables		
Leptin	0.45	0.000
Albumin	0.21	0.002

\*hs-CRP: high-sensitive C-reactive protein; tumor necrosis factor- $\alpha$ ; IL-6: Interleukin-6.

$r = 0.20$  and  $p = 0.009$ , respectively). There was a significant correlation between TNF- $\alpha$  and IL-6 levels ( $r = 0.32$  and  $p = 0.000$ ).

As shown in Table 3 (a to d), only serum leptin level was independently associated serum CRP level. Serum leptin was independently predicted by gender and BMI. IL-6 was independently predicted by serum leptin and TNF- $\alpha$  levels. Serum leptin and albumin levels were the most significant determinants for predicting TNF- $\alpha$  level.

## DISCUSSION

Chronic uremia is considered a pro-inflammatory state associated with high cardiovascular morbidity and mortality.<sup>4</sup> Leptin, IL-6 and TNF- $\alpha$  levels are suggested to be elevated in chronic renal failure, particularly in the subjects who are treated with hemodialysis and peritoneal dialysis.<sup>9-14</sup> CRP levels are increased in CRF patients and this is thought as an independent risk factor that may cause progressive atherosclerosis.<sup>12-16</sup> Our results were similar. Both groups had increased levels of leptin, CRP, IL-6 and TNF- $\alpha$ . However, there were no differences for serum leptin, CRP, IL-6 and TNF- $\alpha$  levels between the groups.

As TNF- $\alpha$  can induce production of leptin, and leptin can stimulate TNF synthesis.<sup>17</sup> In the cohort described by Hilken et al. there is a correlation between elevated TNF- $\alpha$  levels and leptin.<sup>18</sup> We found a similar finding in our study showing an association between serum levels of TNF- $\alpha$  and leptin in both HD and CAPD patients.

The relation between inflammatory cytokines and leptin seems to be much more complex and the reported results are conflicting. Pecoits-Filho et al. reported a strong positive correlation between serum leptin and IL-6 levels in dialyzed patients.<sup>19</sup> Our observations were consistent with this finding. Although we found significantly higher serum leptin in dialyzed patients with elevated IL-6 levels, Don et al. found no association between inflammatory markers and elevated leptin levels.<sup>20</sup> Shamsuzzaman et al. demonstrated that increased leptin was associated with increased CRP independently from gender and measures of adiposity in healthy subjects.<sup>21</sup> Our results found that chronic inflammation in dialyzed patients correlated with their BMI and gender. Serum CRP level was correlated with serum leptin level and BMI in our study, and multivariate regression analysis showed serum leptin level was the only predictor for the CRP level.

In pathogenesis of atherosclerosis, leptin is linked to coronary artery calcification in uremia.<sup>4</sup> Moreover, serum leptin level is an independent factor predicting cardiovascular disease.<sup>22</sup> Therefore, the positive correlation of serum leptin level with TNF- $\alpha$ , IL-6 and CRP levels in our study supports the idea that leptin is involved in the chronic inflammation observed in dialysis patients.<sup>19</sup> The uremia-associated pro-inflammatory state may contribute to the increased synthesis of leptin. On the other hand, hyperleptinemia observed in patients with CRF is at least partly due to decreased renal clearance.<sup>23</sup> With its molecular weight of 16 kDa, circulating free leptin accumulates in uremia and may exert its systemic deleterious effects as an uremic toxin. Our results also showed that the serum leptin levels were significantly higher in female patients (data not shown), and directly correlated with BMI as in general population.<sup>16,24</sup>

Our study revealed that BMI was an independent predictor of serum leptin levels. In general population, increasing BMI is associated with enhanced cardiovascular risk, and BMI has been correlated with long-term outcome of dialysis patients in a prospective study.<sup>25</sup> As there is significant correlation between BMI and inflammatory markers, this relationship implies that body fat percentage represented by BMI is a key factor contributing to chronic inflammation in dialysis patients. Therefore, this metabolic parameter should be considered an important factor in assessing cardiovas-

cular disease and survival of chronic dialyzed patients.

## CONCLUSION

In conclusion, our study demonstrated that both CAPD and HD patients were associated with chronic inflammation and serum leptin levels were elevated in dialysis patients and significantly correlated with inflammatory markers. Further studies are needed to determine their roles in the pathogenesis of cardiovascular disease in chronic dialysis patients.

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