# Peritonitis Incidence is Correlated with Increased Leptin and CD4/CD8 Ratio in Peritoneal Dialysis Patients

## *Periton Diyaliz Hastalarında Peritonit İnsidansı Artmış Leptin ve CD4/CD8 Oranı ile Korelasyon Göstermektedir*

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**ABSTRACT**

Peritonitis is one of the leading cause of hospitalization in peritoneal dialysis patients. Although inflammatory markers show an increased inflammatory response, cellular immune response is decreased in ESRD patients. Uremia has many affects on leptin and T lymphocytes that are basic elements of the cellular immune system. In this study, we aimed to demonstrate the relationship between peritonitis and the CD4, CD8 positive lymphocyte count and ratio, and serum leptin level in peritoneal dialysis patients.

Forty-six ESRD patients who had been receiving peritoneal dialysis therapy for at least 12 months were enrolled to the study. Serum leptin level, WBC count, and the CD4 and CD8 T lymphocyte count and ratio were measured. A healthy control group was also enrolled to the study.

The measured mean serum leptin level of the patient group was statistically significantly higher than the control group (1697.673±1586.081 and 478.057±601.654). The calculated peritonitis incidence was 0.49 peritonitis/year. The number of peritonitis attacks significantly correlated with the duration of peritoneal dialysis, BMI, CD4:CD8 ratio, ESR and serum leptin level.

In conclusion, although the ESRD patients have increased leptin and CD4 percentage of T lymphocytes, their immune system is not healthy to combat infections satisfactorily due to possible multifactorial reasons such as increased inflammation and decreased lymphocyte count.

**KEY WORDS:** Leptin, CD4/CD8 ratio, Peritonitis, Peritoneal dialysis

**ÖZ**

Periton diyaliz hastalarında hastane yatış nedenleri arasında peritonit ilk sırada gelmektedir. Her ne kadar SDBY (son dönem börek yetmezliği) hastalarında enflamatuvar belirteçler artmış bir enflamasyona işaret etse de bu hastaların hücresel immün yanıtı azalmıştır. Üremi leptin ve hücresel immün yanıtın temel elemanı olan T lenfositler üzerine bir çok etkiye sahiptir. Biz bu çalışmada periton diyaliz hastalarında serum leptin düzeyi, CD4, CD8 T lenfosit sayıları ile peritonit sıklığı arasındaki ilişkiyi araştırmak istedik.

SDBY olup, en az 12 aydan beri periton diyalizi yapan 46 hasta çalışmaya dahil edildi. Serum leptin düzeyleri, lökosit sayısı, CD4, CD8 pozitif T lenfosit sayısı ölçüldü. Sağlıklı kontrol grubuda çalışmaya dahil edildi.

Ölçülen ortalama serum leptin düzeyi hasta grubunda kontrol grubundan istatistiksel olarak anlamlı yüksek bulundu (1697,673±1586,081 ve 478,057±601,654). Hesaplanan peritonit insidansı 0,49 peritonit/yıl idi. Peritonti atak sayısı ile periton diyaliz süresi, vücut kitle indeksi (VKI), CD4/CD8 oranı, eritrosit sedimantasyon hızı (ESH) ve serum leptin düzeyi arasıdan istatistiksel anlamlı korelasyon tespit edildi.

Sonuç olarak; SDBY hastalarında serum leptin düzeyi ve CD4 T lenfosit oranı yüksek bulunmuş olsa da bu hasta grubunda immün sistem enfeksiyonlar ile mücadelede yeterli savunma yapamamaktadır. Bunun sebepleri çok faktörlü olup bu faktörlerin bir tanesi de azalmış lenfosit sayısı olabilir.

**ANAHTAR SÖZCÜKLER:** Leptin, CD4/CD8 oranı, Peritonit, Periton diyalizi

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## INTRODUCTION

End-stage renal disease has a high worldwide prevalence and incidence. Patients on long-term dialysis have high morbidity and mortality rates. Associated risk factors are age, gender, duration of dialysis, concomitant diseases, presence of complications and inflammatory diseases. Hospitalization requirement is more frequent and the duration is longer in dialysis patients especially in patients with comorbid infection and cardiovascular disease (1). Peritonitis is one of the leading cause of hospitalization in peritoneal dialysis patients (2).

Immune system functions are affected in uraemia. Although inflammatory markers show an increased inflammatory response, cellular immune response are decreased in ESRD patients. Decreased T cell counts have been reported in ESRD patients (3). Leptin is a regulatory protein for body composition through the control of appetite and energy expenditure. Serum leptin levels are increased in obesity, hyperglycaemia and uraemia due to decreased renal excretion (4,5). In this study, we aimed to detect the possible relationship between peritonitis incidence and the serum leptin level and peripheral blood T lymphocyte count in ESRD patients who were being treated with continuous peritoneal dialysis.

## MATERIALS and METHODS

The study design was cross-sectional. Patients were selected from the out-patient clinic of Atatürk University Medical Faculty's Nephrology Department between September 2012 and December 2012. The study was approved by the local ethics committee. Informed consent of the patient and control groups were collected before the study. 46 ESRD patients (18 males, 28 females) who had been receiving peritoneal dialysis treatment for at least 12 months were enrolled to the study. Patients with active viral or bacterial infection, malignancy, inflammatory disease, and those using immunosuppressive medication were all excluded from the study. Age- and sex-matched 36 (16 male, 20 female) healthy control subjects were also enrolled to the study. The control group was tested only for the serum leptin level.

Blood samples for leptin, CD4, CD8, and whole blood count were collected from the forearm by venipuncture in the morning. Whole blood count and CD4, CD8 measurements were done 30 min after sampling. Whole blood count was done with Beckman Coulter LH 750 USA with commercial kits.

### Flowcytometry

Collected whole blood samples were preincubated with 2% human AB serum to block non-specific binding to fc receptors. Next, 50 $\mu$l of blood sample was stained with 5 $\mu$l of an antibody containing CD4 FITC, CD8 PE and corresponding isotypic mAb controls. After 15 min incubation at room temperature in the dark, 450 $\mu$l of lysine solution was added. Data were acquired using a five-color Cytomics FC 500 (Beckman Coulter USA), and analyzed with CXP software (Beckman Coulter). CD4 and CD8 were analysed only in the patient group because it has been widely studied in the healthy population and normal levels can be found from the literature.

### Human Serum Leptin Analysis

Samples for serum leptin were centrifuged at 1000 rpm for 15 min within 30 minutes after collection. Supernetant serum samples were stored at -20 Cº until the analysis. Serum leptin levels were analysed with Boster's Human leptin ELISA kit (Lot No:156833426). 0.1 ml 4000 pg/ml leptin solution was aliquoted to each well. 0.1 ml diluted human serum sample was added and incubated at 37ºC for 90 min. Anti-human leptin antibody was added and incubated for 60 min. Plates were washed three times with washing TBS solution. 0.1 ml of Avidin-Biotin-Peroxidase Complex (ABC) was added to each well and incubated for 30 min. Plates were washed with TBS solution. 90 $\mu$l TMB color developing agent was added to each well and incubated for 15 min in the dark. 0.1 ml TMB stop solution was added. Absorbance was analysed at 450 nm 30 min after stop solution was added.

Patient characteristics, concomitant diseases, duration of peritoneal dialysis, age, gender, sex, number of peritonitis attacks, cultured microorganism, etiologic cause of ESRD, Body mas indexes, Erythrocyte sedimentation rate, C-reactive protein level, white blood cell count, lymphocyte count, blood glucose, calcium, phosphate and albumin levels were all recorded. The frequency of peritonitis attacks was calculated by dividing the number of peritonitis attacks into total follow-up times per year (attack/patient year).

### Statistical Analysis

The IBM SPSS 20.0 for Windows (SPSS Inc, Chicago, Illinois, USA) software was used in analyzing the data. Parametric tests were applied to the data with normal distribution, whereas nonparametric tests were applied to the data with non-normal distribution. Chi-square tests were used for categorical variables. One-way ANOVA test and Kruskal-Wallis One-Way Analysis of Variance on Ranks Test were applied to determine the difference between independent groups. In addition, Tukey HSD and Dunn's Post Hoc Tests were applied to check the differences. The relationships between the variables were evaluated using Pearson and Spearman's rho correlation analysis. Results were expressed as mean ± SD and median (interquartile range), and a p value $<0.05$ was considered statistically significant.

## RESULTS

Forty-six ESRD patients who had received at least 12 months of peritoneal dialysis were enrolled to the patient group and 35 healthy subjects were enrolled as the control group. Characteristic properties of the patient group and summary of the results are given in Table I.

**Table I:** Descriptive properties of the patient group and summary of the results.

| | n | Minimum | Maximum | Mean | Std. Deviation |
|---|---|---|---|---|---|
| Age (Patient) | 46 | 18 | 80 | 48.41 | 15.678 |
| Duration of Peritoneal Dialysis | 46 | 7 | 100 | 39.74 | 25.614 |
| BMI (Patient) | 46 | 18 | 37 | 24.19 | 4.403 |
| Peritonitis attack/patient (#) | 46 | 0 | 7 | 1.54 | 1.722 |
| Peritonitis incidence /year | 46 | .000 | .159 | .04130 | .045255 |
| WBC x $10^3$/ml | 46 | 3600 | 16600 | 7652.17 | 2798.550 |
| Lymphocyte count x $10^3$/ml | 46 | 300 | 2600 | 1513.04 | 531.500 |
| ESR mm/h | 27 | 9 | 89 | 38.33 | 20.424 |
| CRP mg/dl | 46 | 2.90 | 47.00 | 6.8189 | 8.06653 |
| LEPTIN ($\mu$g/ml) patient group | 45 | 62.50 | 4000.00 | 1697.6731 | 1586.08164 |
| LEPTIN ($\mu$g/ml) Control group | 35 | 62.50 | 2970.97 | 478.0574 | 601.65452 |
| CD4+ positive T cell (%) | 46 | 16 | 60 | 39.15 | 9.165 |
| CD8 positive T cell (%) | 46 | 8 | 44 | 26.07 | 8.523 |
| CD4:8 Ratio | 46 | .62 | 3.75 | 1.6966 | .75027 |
| CD4 positive T cell count x $10^3$/ml | 46 | 196 | 1260 | 596.72 | 267.924 |

**BMI:** Body mass index, **WBC:** White blood cell count, **ESR:** Eryhtrocyte sedimentation rate, **CRP:** C-Reaktive protein, **CD:** cluster fo differentiation.

The calculated peritonitis incidence was 0.0106 peritonitis attack per patient year (total number or peritonitis attacks was 71 and total follow-up period of the 46 PD patients was 1828 months). A causative bacterial organism was isolated in 58.7% of peritonitis attacks. It was detected that the number of peritonitis attack significantly correlated with the duration of peritoneal dialysis, BMI, CD4:CD8 ratio, ESR, and serum leptin level (p=0.003,p=0.006, p=0.021, p=0.024, p=0.021 respectively).

The calculated mean number of CD4 and CD8 positive T lymphocytes were 596.72±267.924 (196-1260) and 392.45±186.550 (95-900) respectively. The calculated mean CD4:CD8 ratio of the patients was 1.696 ±0.750 (range 0.62-3.75). CD4:CD8 ratio was detected to be statistically significantly correlated with the duration of peritoneal dialysis, BMI, and number of peritonitis attacks (p=0.016, p=0.024, p=0.021 respectively). The CD4 positive Lymphocyte ratio was also detected to be statistically significantly correlated with the duration of peritoneal dialysis (p=0.005), and serum albumin level (p=0.039). Such a correlation was not detected for the CD8 positive lymphocyte ratio.

The mean serum leptin level of the patient and control groups were 1697.673±1586.081 (62.5-4000.0) and 478.057±601.654 (62.5-2970.97) respectively. The mean serum leptin level of the patient group was statistically significantly higher than the control group (p<0.001). There was a statistically significant correlation between serum leptin level and duration of peritoneal dialysis, BMI, number of peritonitis attacks, and primary cause of renal failure in the patient group (p=0.014, p<0.001, p=0.021, p=0.04 respectively). The serum leptin levels of the peritoneal dialysis patients with DM and/or hypertension were significantly higher than the patients with glomerulonephritis or other primary causes of end-stage renal disease (Figure 1).

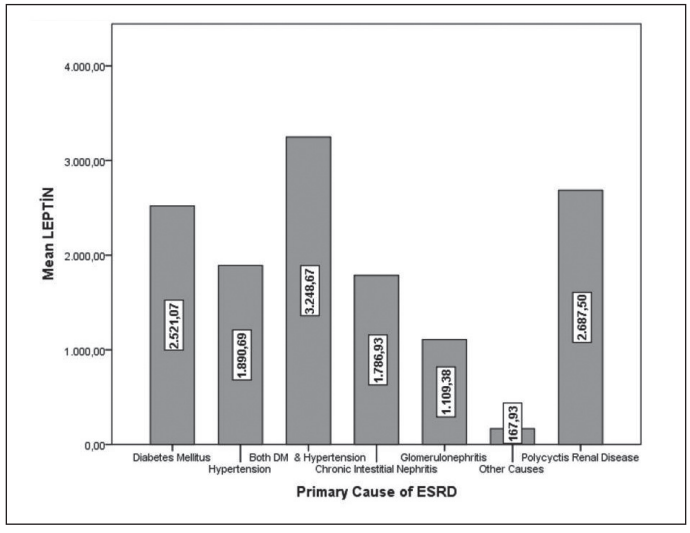


***Figure 1:*** *Mean serum leptin level of the patients according to primary causer of end stage renal disease (ESRD).*

## DISCUSSION

In this study, the number of peritonitis attack was detected to be significantly correlated with the duration of peritoneal dialysis, BMI, CD4:CD8 ratio, ESR and serum leptin level of peritoneal dialysis patients. Both CD4 and CD8 lymphocyte numbers of peritoneal dialysis patients were detected to be lower than the normal healthy population (Normal mean CD4 and CD8 positive T lymphocyte counts 727-865/$\mu$L, 539-552/$\mu$L respectively)(6-9). The CD4:CD8 ratio significantly correlated with the duration of peritoneal dialysis, BMI and number of peritonitis attacks. Peritoneal dialysis patients had significantly higher serum leptin levels compared to control group. Also, the serum leptin level of peritoneal dialysis patients was significantly correlated with the duration of peritoneal dialysis, BMI, number of peritonitis attacks and primary cause of peritonitis. In subgroup analysis, we detected that the leptin level was correlated also with the primary cause of renal failure.

Soluble factors of immune system and their ancestry cellular parents comprise a complex set of interaction to defend the host against various diseases and conditions. The basic event is inflammation that causes destruction and damage as a response to infection and tissue damage. Both immune activation and immune deficiency simultaneously present in end-stage renal disease (ESRD) patients. Increased systemic inflammation provokes cardiovascular disease, cerebrovascular disease and mortality while immune deficiency leads to impaired response to vaccination, and poor outcome of microbial infections (10,11).

Reduced T cell counts and reduced T-cell proliferation have been reported in dialysis and ESRD patients. Additionally, a significantly increased percentage of CD4 positive T cells is associated with high cytokine production in hemodialysis patients (12,13). In patients with recurrent bacterial infections, decreased CD4+ T cell counts were detected and interpreted as impaired cellular immunity that affects both T cell counts and antibody levels in case of acute infection. It was demonstrated that T-helper cells are also important in generating B cell–mediated antibody responses (14). Although the CD4 positive T cell percentage was detected to be higher in patients with recurrent peritonitis attack in our study, CD4 positive T cell counts were lower than normal levels as expected according to the literature. Decreased T cell counts are interpreted as an additional cause of immune deficiency in ESRD.

Leptin, which is a large-molecular weight protein, is an adipocyte-derived satiety hormone and is also known as a pro-inflammatory cytokine. It is also noted as a uremic toxin because serum leptin concentrations are significantly higher in hemodialysis patients and it cannot be removed by hemodiafiltration. Leptin mediates an anti-appetite role via specific receptors in the brain and peripheral tissue. Leptin has also emerged as a potential mediator of inflammatory status and a positive modulator of IL-1$\alpha$, TNF-$\alpha$ and IL-6 secretion. Leptin also increases IFN-$\gamma$, producing Th1 polarized cells and exerts its bioactivity at developmental, proliferation and activation levels (15-18). Although we did not detect a significant correlation between leptin and peritonitis incidence in our previous study (19), in the present study we detected that serum leptin levels of peritoneal dialysis patients were significantly higher than the control group. The serum leptin level was also significantly correlated with the duration of peritoneal dialysis, BMI and number of peritonitis attacks. According to subgroup analysis, we detected that patients with diabetes mellitus or hypertension as a primary cause of ESRD had significantly higher serum leptin levels compared to other causes of ESRD.

Our study has limitations regarding the factors that may affect this result. First of all, this is a cross-sectional study and it is not adequate to detect cause and response relationship accurately. Although the number of the patients is adequate for a single study, the patient group is heterogeneous for primary causes and larger study populations are needed to detect the differences between subgroups of ESRD.

In conclusion, ESRD patients have higher serum leptin levels compared to healthy adults. Increased leptin is correlated with BMI, and duration of peritonitis. Patients with ESRD have decreased CD4 and CD8 lymphocyte counts that contribute to immune system impairment. The CD4 ratio increases as the duration of peritoneal dialysis and the number of peritonitis attacks increase. We conclude that although ESRD patients have increased leptin levels and CD4 percentage of T lymphocytes, their immune system is not healthy as to combat infections satisfactorily due to multifactorial reasons. Further larger-scale prospective studies are required to reveal the relationship between lymphocyte functions and leptin in ESRD patients.

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