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# Elevated Serum Leptin Level Is Associated with Body Mass Index But Not with Serum C-reactive Protein and Erythrocyte Sedimentation Rate Values in Hemodialysis Patients

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**ABSTRACT**

**Objectives:** Aim of the present study was to investigate serum concentration of leptin and its association with values of body mass index (BMI), C-reactive protein (CRP), and erythrocyte sedimentation rate (ESR) in hemodialysis (HD) patients. **Methods:** This cross-sectional study included 60 HD patients (34 male, 26 female) and 30 age- and sex-matched (4 males, 26 females) apparently healthy subjects. Serum leptin concentration was determined by an enzyme-linked immunosorbent assay (ELISA). Serum CRP concentration was measured by means of particle-enhanced immunonephelometry. ESR value was determined by Western Green method. BMI was calculated as weight (kg) divided by height squared ($m^2$). **Results:** Results have shown that median serum leptin concentration (30.65 ng/mL; 12.48-86.40 ng/mL) was statistically significantly higher in HD patients compared to median serum leptin concentration (15.75 ng/mL; 9.15-30.65 ng/mL) in the control group of healthy subjects ($p<0.05$). Likewise, median serum CRP concentration (5.5 mg/L; 1.93-8.9 mg/L) and median ESR value (57.5 mm/h; 40.5-77.0 mm/h) were significantly higher in HD patients compared to median serum CRP concentration (0.8 mg/L; 0.38-1.43 mg/L) ($p<0.001$) and median ESR value (10.0 mm/h; 6.5-14.0 mm/h) ($p<0.001$) determined in the control group. Statistically significant positive correlation was found between BMI values and serum leptin concentration in HD patients (rho=0.434; $p<0.001$). Positive, although not significant, correlation was observed between serum CRP and leptin levels in HD patients (rho=0.171; $p>0.05$). Negative correlation between ESR values and serum leptin concentrations in HD patients was determined but it was not statistically significant (rho= -0.029; $p>0.05$). **Conclusions:** Increased serum concentration of leptin as pro-inflammatory cytokine as well as elevated serum values of CRP and ESR indicate presence of systemic micro inflammation in HD patients. Results of the present study point to possible use of serum leptin concentration as an indicator of nutritional status in HD patients based on observed significant positive correlation between serum leptin concentrations and BMI values. However, absence of significant association between serum leptin and CRP levels as well as between serum leptin concentrations and ESR values in HD patients requires further investigation and clarification.

**Key words: Leptin, C-reactive protein (CRP), Erythrocyte sedimentation rate (ESR), Body Mass Index (BMI), Hemodialysis (HD) patients.**

## 1. INTRODUCTION

Leptin is a 16-kDa protein hormone made up of 167 amino acids. It is mainly produced by adipocytes and in general population leptin is regarded as major regulator of body weight since it induces decrease of food intake and increases energy expenditure and weight loss. Furthermore, this adipocytokine is also known to have actions in the immune system, angiogenesis, and bone formation (1). Leptin is cleared from the circulation by the kidney through both glomerular filtration and metabolic degradation in the renal tubules. Several recent studies have demonstrated that serum leptin concentrations are increased in patients with chronic kidney disease (CKD) and those on hemodialysis (HD). Reports from those studies suggest that elevated leptin levels in patients with damaged kidney function

are primarily due to reduced renal filtration and metabolism (2). However, the role of leptin in CKD and in HD patients is far from being completely understood.

Leptin is considered to be a pro-inflammatory cytokine. Its synthesis is mostly dependent on the amount of body fat but it is also enhanced during acute infection and inflammation. Production of leptin is also regulated by the actions of pro-inflammatory mediators such as tumor necrosis factor (TNF)-$\alpha$, IL-6, and IL-1 (5). Moreover, leptin itself stimulates the production of pro-inflammatory cytokines from macrophages (3).

Inflammation mediated by pro-inflammatory cytokines is very common in patients with CKD and HD patients, and contributes to mortality of these patients alongside with other mortality risk factors, such as malnutrition, anemia, vascular disease, and left ventricular hypertrophy. Studies have shown that in patients with chronic renal failure TNF–$\alpha$ and IL-1 are major pro-inflammatory cytokines, whereas interleukin IL-6 appears to be key mediator of acute phase reactant synthesis, including C-reactive protein (CRP) (4).

CRP is a sensitive but non-specific marker of systemic inflammation synthesized by the liver. Studies conducted so far have shown that increased CRP levels reflect presence of chronic inflammation in HD patients (5). Earlier study by Stenvenkel (6) summarized the prevalence of elevated CRP levels from several investigations and concluded that more than 50% of HD patients had increased serum CRP concentration. Erythrocyte sedimentation rate (ESR) is another unspecific marker of systemic inflammation but insufficient data related to relevance and importance of its measurement in renal patients is present in the literature.

Inflammation in HD patients is often associated with malnutrition. Furthermore, numerous studies have demonstrated that HD patients with lower Body Mass Index (BMI) have higher relative mortality risk (7). Kara et al. (8) recently reported negative correlation between increased leptin levels and lower malnutrition inflammation score which remained significant even after adjustments for BMI. However, significance of hyperleptinemia as well as its associations with inflammatory and nutritional status in HD patients is far from being fully elucidated.

Thus, the aim of the present study was to investigate serum concentration of leptin and its association with CRP, ESR and BMI values in HD patients.

## 2. MATERIALS AND METHODS

**Patients**

The study was designed as cross-sectional and was conducted at the Clinic for hemodialysis, Clinical Centre University of Sarajevo (CCUS). The study included 60 HD patients (34 male, 26 female) and 30 age- and sex-matched (4 males, 26 females) apparently healthy subjects.

HD patients were on hemodialysis program for the period longer than six months, three times a week by four hours (bicarbonate dialysis), with low-flux dialysate and Fresenius Medical Care dialysis monitors. Patients were regarded as non-eligible for the study if any of the following criteria were met: age younger than 18 or more than 70 years, presence of malignancy, febrile disorders, acute or chronic inflammatory disease or coronary heart diseases during the study period.

Subjects of the control group had no history of inflammatory, autoimmune and rheumatic diseases, hyperlipidemia, hypertension, or coronary heart diseases. None of the control subjects had received any medication and were not current smokers or consumers of alcohol.

Upon careful explanation of the study protocol, written informed consent was obtained from all participating subjects. The study was approved by the Ethical Committee of the Clinical Centre University of Sarajevo (CCUS). Investigations were carried out in the accordance with the Declaration of Helsinki as revised in 2000.

**Methods**

Blood samples were taken after an overnight fast. In HD patients, blood was obtained from antecubital vein just before hemodialysis started, using the vacutainer technique.

Serum leptin concentration was determined with the use of commercially available quantitative sandwich enzyme-linked immunosorbent assay (ELISA) (*Mediagnost Leptin-ELISA, Germany*) using a Stat Fax-2100 microplate reader (*Awareness Technology INC, USA*) at the Department of Human Physiology, Faculty of Medicine, in Sarajevo.

Serum CRP concentration was measured by means of particle enhanced-immunonephelometry with the use of BN II analyzer at the Institute of Clinical Chemistry and Biochemistry, Clinical Centre University of Sarajevo. CardioPhase high-sensitivity CRP (DADE BEHRING) was used as a diagnostic reagent. Reference interval for CRP with the use of this method is from 0 to 5 mg/L.

ESR (in millimeters per hour) was determined by the Westergren method. Normal ESR values for subjects younger than 50 years of age were 4–25 mm/h for women and 1–15 mm/h for men, while for those older than 50 years, normal values were between 4–30 mm/h for women and 1–20 mm/h for men.

In all participating subjects height was measured with stadiometer and body weight was measured with the use of a digital scale (Soehnle-Waagen, Murrhardt, Germany) and was recorded to the nearest 100 g. Body mass index (BMI) was calculated as weight (kg) divided by the square of height in meters ($m^2$).

**Statistical analysis**

Data distribution was determined with the use of Kolmogorov-Smirnov test or Shapiro-Wilk test of normality. Normally distributed data are presented as mean value () ± standard deviation (SD) and skewed variables as median and interquartile ranges (IQR). An unpaired Student *t*-test or Mann-Whitney U-test was used to compare the difference between two groups, as appropriate. Since leptin, CRP and ESR values were not normally distributed, correlations were assessed by Spearman's test. Statistical significance was set at $p<0.05$. All statistical analyses were conducted with Statistical Package for the Social Sciences (SPSS) version 13.0 for Windows (Chicago, IL, USA).

## 3. RESULTS

The baseline characteristics of the two groups enrolled in the study are reported in Table 1. The median duration of hemodialysis at the time of the study was 5.0 years (IQR: 2.0-7.0 years). Age and BMI did not differ between the control group of healthy subjects and group of hemodialysis (HD) patients.

The median serum leptin concentration determined in HD patients was 30.65 ng/mL (IQR: 12.48-86.40 ng/mL) and it was significantly higher compared to median serum leptin concentration in the control group of healthy subjects which was 15.75 ng/mL (IQR: 9.15-30.65 ng/mL) ($p<0.05$). (Figure 1)

| Variables | CG (n=30) | HD patients (n=60) | p< |
|---|---|---|---|
| Age (yrs) | 52.43±5.49 | 55.18±12.77 | NS |
| Time on HD (yrs) | _____ | 5.0 (2.0-7.0) | _____ |
| BMI (kg/m2) | 26.66±4.38 | 26.45±4.28 | NS |

Data are presented as mean±standard deviation (SD) and median and interquartile range (IQR); BMI: body mass index; HD:hemodialysis.

*Table 1. Baseline characteristics of the control group (CG) of healthy subjects and group of hemodialysis (HD) patients*

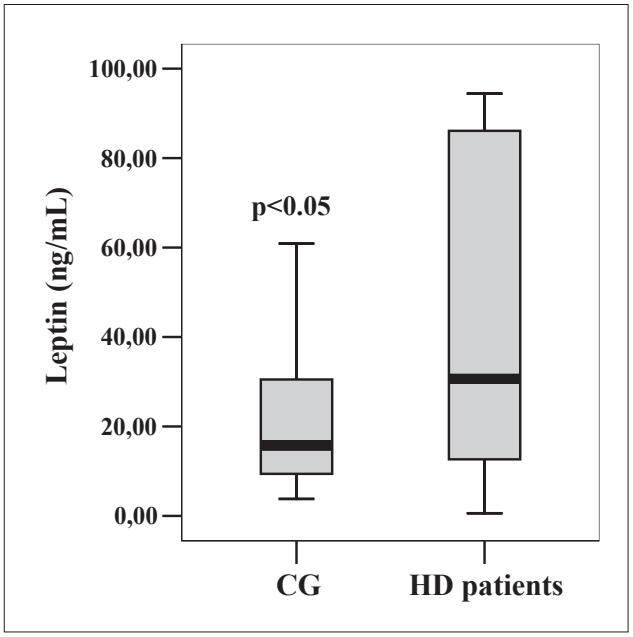


*Figure 1. Serum leptin concentration in the control group (CG) and in the group of hemodialysis (HD) patients*

Likewise, determined median serum CRP concentration of 5.5 mg/L (IQR:1.93-8.9 mg/L) was significantly higher in HD patients compared to median serum CRP concentration of 0.8 mg/L (IQR:0.38-1.43 mg/L) determined in the control group (p<0.001). (Figure 2)

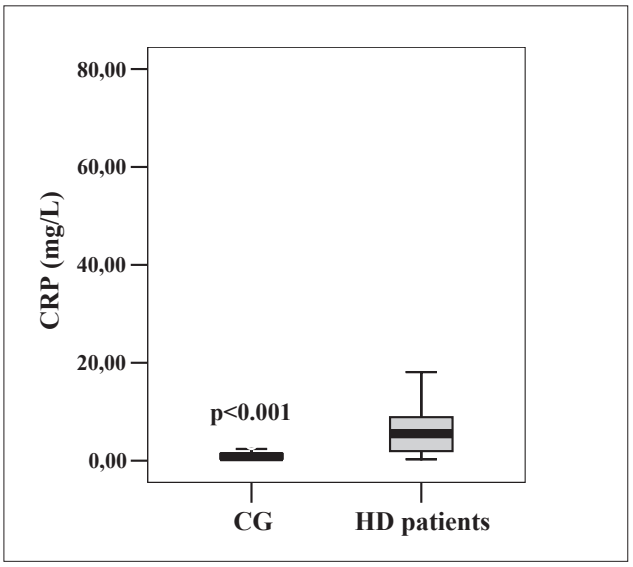


*Figure 2. Serum C-reactive protein (CRP) concentration in the control group (CG) and in the group of hemodialysis (HD) patients*

Median serum ESR value of 57.5 mm/h (IQR: 40.5-77.0 mm/h) was also significantly higher in HD patients compared to median serum ESR value of 10.0 mm/h (IQR:6.5-14.0 mm/h) determined in the control group (p<0.001). (Figure 3)

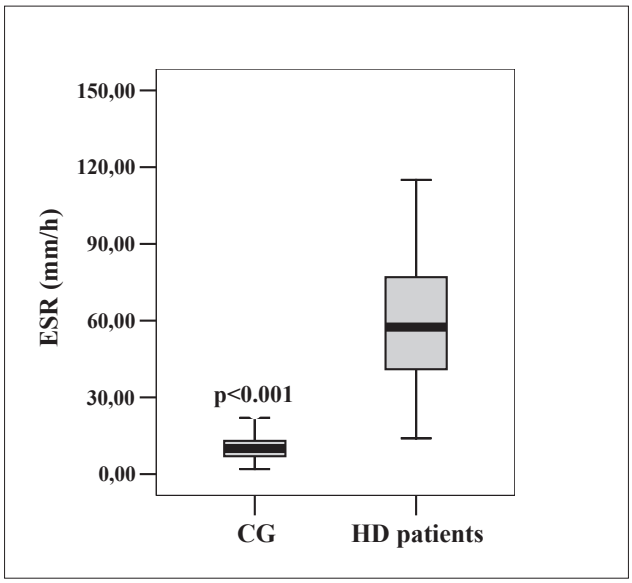


*Figure 3. Serum erythrocyte sedimentation rate (ESR) value in the control group (CG) and in the group of hemodialysis (HD) patients*

Statistically significant positive correlation was found between values of BMI and serum leptin concentrations in HD patients (rho=0.434; p<0.001).

Positive, although not significant, correlation was observed between serum CRP and leptin levels in HD patients (rho=0.171; p>0.05). Negative correlation between ESR values and serum leptin concentrations in HD patients was determined but it was not statistically significant (rho= -0.029; p>0.05).

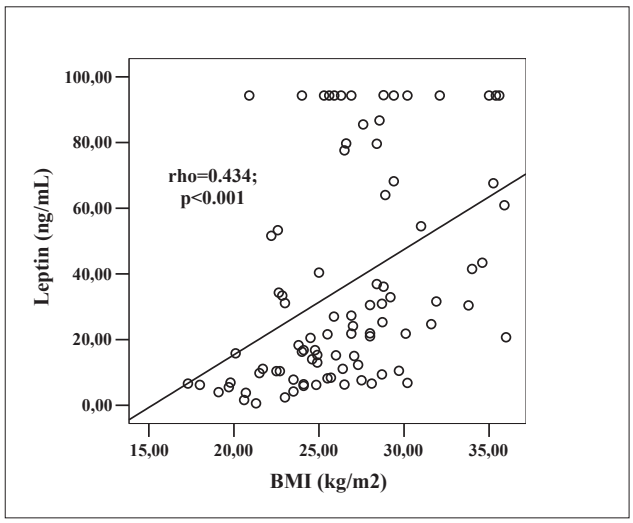


*Figure 4. Correlation between body mass index (BMI) values and serum leptin concentrations in hemodialysis (HD) patients*

## 4. DISCUSSION

Kidney diseases are one of the leading causes of death worldwide. Numerous studies have shown that in patients with chronic renal failure cardiovascular mortality is higher then in general population. According to many authors this elevated cardiovascular mortality rate is partly explained by presence of postulated 'malnutrition, inflammation, atherosclerosis' (MIA)

syndrome in end-stage renal disease patients. Furthermore, it has been shown that pro-inflammatory cytokines are pivotal in the process of inflammation that is associated with malnutrition and atherosclerosis in these patients (9). Besides well-known pro-inflammatory cytokines such as IL-6 and TNF-$\alpha$ which have been reported as strong predictors of both poor outcome and malnutrition in patients on dialysis, novel findings suggest potential role of leptin in MIA syndrome seen in HD patients (10).

Results of the present study have shown significantly higher serum leptin levels in HD patients compared to healthy subjects of the control group. These findings are in a close agreement with results reported by Beberashvili et al. (11) who have also determined increased leptin levels in HD patients. Hyperleptinemia observed in our and other studies might be result of decreased renal clearance and consequent leptin retention in HD patients.

Initially, leptin was considered as an anti-obesity hormone, but experimental data have also shown pleiotropic effects of this compound on hematopoiesis, angiogenesis, lymphoid organ homeostasis, and T-lymphocyte functions. Moreover, evidence from literature suggest possible role of leptin in the development of protein-energy malnutrition in HD population (12).

However, although some clinical studies have reported that leptin is associated with different nutritional markers and intakes, others have failed to demonstrate any relationship between them. Johansen et al. (13) showed that serum leptin levels were negatively correlated with serum albumin and protein catabolic rate (PCR), suggesting a possible negative role of leptin in nutrition.

Important component of MIA syndrome seen in HD patients is chronic low grade inflammation, or according to some authors micro inflammation, which is believed to be a consequence of pro-inflammatory compounds accumulation. Chronic micro inflammation is an important risk factor for morbidity and mortality in HD patients especially through increase of cardiovascular events incidence. The most common indicator of inflammatory status used in clinical practice is C-reactive protein. According to data from literature raised values of CRP which do not exceed 10-15 mg/L are thought to be relevant marker of micro inflammation (14).

Results of our study have shown significantly higher levels of serum CRP and ESR in HD patients compared to the control group of subjects. Obtained results point to the presence of chronic micro inflammation in our sample of HD patients and are in accordance with majority of studies which have shown increased CRP concentration in HD patients (6).

Furthermore, results of the present study have shown significant positive correlation between BMI values and serum leptin levels in HD patients. Conversely, significant correlation between serum CRP and ESR values as inflammatory markers and leptin levels was not observed in this group of patients.

Kara et al. (8) reported association of elevated serum leptin levels with good nutritional status in non-obese chronic HD patients. However, similar to our findings no correlation between leptin and inflammatory status was observed in this study. In contrast to ours and other similar reports, Yildiz et al. (15) reported significant correlation between elevated serum leptin levels and inflammatory markers in both HD and peritoneal dialysis patients.

Earlier study by Sanjay et al. (16) has shown that serum CRP levels in HD patient group were higher than the upper limit of normal for the general population, but no correlation between serum CRP and leptin levels was observed. Interestingly, results of the mentioned study have shown that some patients with higher leptin levels had lower CRP values indicating that hyperleptinemia and inflammation might be independent events in HD patients.

Erythrocyte sedimentation rate is an inexpensive and easily accessible marker of systemic inflammation. However, the significance of ESR determination in HD patients is not sufficiently validated. Increased values of ESR observed in our sample of HD patients are in accordance with results of Al-Homrany (17) who reported that dialysis patients had a general tendency for elevated ESR (>25 mm/h in 90% of patients) and almost one third of them (32%) had ESR >100 mm/h in the absence of malignancy or other clinical factors known to cause such levels.

Patients on hemodialysis are exposed to persistent low-grade inflammation which is often accompanied with malnutrition. Tomayko et al. (18) recently suggested that protein malnutrition is both a cause and a consequence of inflammation and related comorbidities in HD patients. Novel findings indicate that adipose tissue is the key regulator of serum CRP concentration, as one of the best studied markers of micro inflammation (19). Moreover, majority of studies conducted in general population have shown that serum CRP levels are significantly associated with different dietary patters and nutritional status (20).

Bearing in mind that BMI was used as a nutritional status assessment tool in HD patients, as previously described in literature (21), results of our study which have demonstrated significant positive correlation of elevated serum leptin levels with BMI values but not with values of CRP and ESR, suggest that leptin is more valuable indicator of nutritional status in HD patients than markers of micro inflammation.

## 5. CONCLUSION

Increased serum concentration of leptin as pro-inflammatory cytokine as well as elevated serum values of CRP and ESR indicate presence of systemic micro inflammation in HD patients. Results of the present study point to possible use of serum leptin concentration as an indicator of nutritional status in HD patients based on observed significant positive correlation between serum leptin concentrations and BMI values. However, absence of significant association between serum leptin and CRP levels as well as between serum leptin concentrations and ESR values in HD patients requires further investigation and clarification.

CONFLICT OF INTEREST: NONE DECLARED.

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