ACTA FAC. MED. NAISS.

UDK 616.61-78

Original article

ACTA FAC. MED. NAISS. 2005; 22 (4): 167-173

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INVERSE CORRELATION OF C-REACTIVE PROTEIN WITH ANEMIA IN MAINTENANCE HEMODIALYSIS PATIENTS

SUMMARY

The aim of the paper was to test the association between serum C-reactive protein (CRP) and serum ferritin and its role in the severity of anemia of hemodialysis patients. A cross-sectional study was conducted on the patients with an end-stage renal disease who underwent HD treatment with acetate basis dialysate and polysulfone membranes. There were 36 patients overall (f=15, m=21), 25 of whom were non-diabetic HD patients and 11 diabetic HD patients. There was an approximate significant inverse correlation between serum CRP and hemoglobin level and also a significant positive correlation between serum CRP and serum iron as well as an approximate significant inverse correlation between serum CRP and HD adequacy. A significant positive correlation between serum CRP and serum ferritin was registered. Malnutrition-inflammation complex syndrome may increase serum ferritin concentration apart from iron status, and needs more attention during iron therapy for the treatment of anemia in regular HD patients.

Key words: **End-stage renal failure, hemodialysis, anemia, C-reactive protein, hemoglobin, hematocrit, ferritin, malnutrition-inflammation complex syndrome**

INTRODUCTION

Anemia of the end-stage renal disease (ESRD) can be managed relatively successfully by recombinant human erythropoietin (EPO). Iron administration plays a central role in enhancing anaemia responsiveness to EPO. Serum ferritin concentration and iron saturation ratio are among the two most commonly used markers of iron status in maintenance dialysis patients (1). Inflammation is quite common in maintenance dialysis patients, and its prevalence among maintenance hemodialysis (HD) patients may be as high as 40–60% (1). Inflammation, which is frequently registered in dialysis patients (2), is closely related to protein-energy malnutrition in dialysis patients and the simultaneous combination of these two conditions, also referred to as "malnutrition-inflammation complex syndrome" (MICS). It was shown that high levels of serum ferritin are engendered by inflammation apart from iron stores (2). Serum ferritin is also an acute phase reactant (3-5). While MICS may play a central role in poor clinical outcome, including a high rate of mortality, hospitalization and diminished quality of life, it may also lead to hyperferritinaemia and refractory ana-

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emia including EPO hyporesponsiveness in these individuals (6). Indeed, the erythropoiesis suppressing effect of inflammation is mainly due to an increased activity of the proinflammatory cytokines (7-8). *In vivo*, the cytokines act in concert with affect precursor cells at different stages of erythropoiesis. Cytokines, TNF-*alfa* and IL-1 have been extensively studied (7-8). In experimental animal studies and in humans, administration of both cytokines causes a hypoproliferative anaemia by direct action on erythroid progenitor cells, or indirectly by stimulating interferon production (9-11). The inhibitory effect of TNF- *alfa* and IL-1 on erythropoiesis can be overcome in a dose-dependent fashion by administering EPO. It is thought that the direct inhibitory effect on erythroid precursors is primarily due to alterations in sensitivity to erythropoietin (7). C-reactive protein (CRP) is secreted by the liver and inflammation causes a rapid increase in its serum concentration. It plays a role in the host defence by interacting with the complement. Compared to measurements of other markers of inflammation and the acute-phase reaction, serum CRP has several advantages. It is a simple, reliable, readily available and inexpensive test. It is also a long-term predictor of cardiovascular risk and mortality in general population and in chronic renal failure patients (2,12). It is thought that during the acute phase response, inflammatory cytokines such as interleukin 1 beta (IL-1ß) and tumour necrosis factor alpha (TNF-*alfa*) increase the synthesis of both H and L subunits of ferritin. Hence, serum ferritin can be elevated in inflammation as mentioned above (13). In this regard, we sought to test the association between serum CRP and serum ferritin and its role in the severity of anemia of regular HD patients, including diabetic and non-diabetic patients, and to determine he association of malnutrition-inflammation complex syndrome.

MATERIAL AND METHODS

This cross-sectional study was conducted on the patients with an ESRD, who underwent hemodialysis treatment (MHD) with acetate basis dialysate and polysulfone membranes. According to severity of secondary hyperparathyroidism, each patient being treated for secondary hyperparathyroidism was given oral active vitamin D3 (Rocaltrol), calcium carbonate, and Rena-Gel capsules of various doses. According to severity of anemia, patients underwent IV iron therapy with iron sucrose (Venofer) of various doses after each dialysis session. All the patients underwent treatments with 6 mg folic acid daily, 500mg L-Carnitine daily, oral Vitamin B-complex tablet daily and also 2000U IV Eprex (recombinant human erythropoietin – (rHuEPO) after each routine dialysis session. Exclusion criteria were active or chronic infection and using NSAID or ACE inhibitor drugs. Complete blood count (hemoglobin & hematocrit level) analysis was performed using Sysmex-KX-21N Cell counter. Levels of serum iron, total iron binding capacity (TIBC) and serum ferritin, CRP and also levels of serum predialysis creatinine (Creat), post- and predialysis blood urea nitrogen (BUN), were measured using standard kits. For the efficacy of hemodialysis, the urea reduction rate (URR) was calculated from pre- and post-blood urea nitrogen (BUN) data (14). Duration and sessions of HD treatment were calculated from the patients' records. Each hemodialysis (HD) session was in duration of 4 hours. Statistical analysis was performed on total hemodialysis (HD), females, males, diabetics and non-diabetics, respectively. For statistical analysis, data are expressed as the mean ± SD. Comparison between the groups was done using the Student's t-test. Statistical correlations were assessed using partial correlation test. All statistical analyses were performed using SPSS (version 11.5.00). Statistical significance was determined at a p-value <0.05.

RESULTS

There were 36 patients overall (f=15, m=21), 25 of which were non-diabetic HD patients (f=11, m=14) and 11diabetic HD patients (f=4, m=7) (table 1).

Mean patients' age was 53 (±15.8) years. In all the patients, no significant difference of CRP between males and females was recorded as well as significant difference of CRP between diabetic and non-diabetic male patients ($p > 0.05$) . However, an approximate significant difference in CRP between

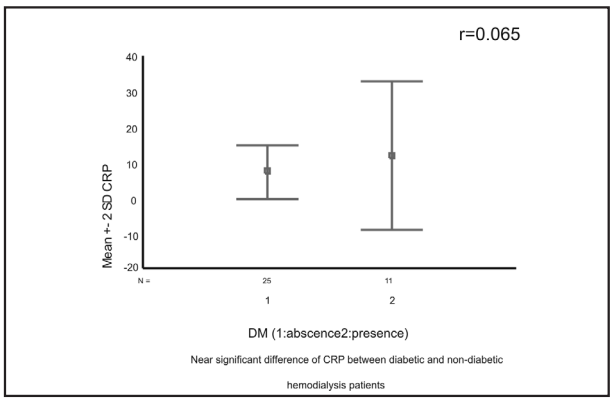

Figure 1. Approximate significant difference in CRP between diabetic and non-diabetic patients

the overall number of diabetics and non-diabetics was recorded (p=0.056) (figure 1).

Table 1. Patients' data

Total number of patients			
N=36	Minimum	Maximum	Mean±SD
Age years	27	75	53±15.8
DH* months	6	24	14.5±6
Dialysis			
dose sessions	54	216	123±54
URR %	39	75	53.5±9.8
CRP mg/l	3	40	8.7±6.7
Hgb g/dl	5	13	9±2
HCT %	14	40	28±6
Ferritin ng/dl	35	1250	519±299
Iron micg/dl	10	1515	350±454
TIBC micg/dl	200	1875	968±562
Creat mg/dl	3	18	9.5±3.6
Non-diabetics			
N=25	Minimum	Maximum	Mean±SD
Age years	16	80	44±17
DH* months	2	156	40±40.8
Dialysis			
dose sessions	36	1584	370±452
URR %	60	76	61±7.5
CRP mg/l	2	20	7.4±3.8
Hgb g/dl	5	12	8.5±2
HCT %	15	36	26.7±7.5
Ferritin ng/dl	170	1250	576±282
Iron micg/dl	10	1010	302±375
TIBC micg/dl	200	1875	862±598
Creat mg/dl	4	15	9.8±2.9
Diabetics			
n=11	Minimum	Maximum	Mean±SD
Age years	27	75	53±15.8
DH* months	6	24	14.5±6
Dialysis			
dose sessions	54	216	123±54
URR %	39	75	53.5±9.8
CRP mg/l	4	40	12±10
Hgb g/dl	0.50	13	10±2
HCT %	14	40	31.5±6.8
Ferritin ng/dl	35	1000	388±308
Iron micg/dl	11	1515	453±606
TIBC micg/dl	570	1803	1212±394
Creat mg/dl	3	18	9±4.8

*Duration of hemodialysis treatment

Also, a significant difference of CRP between female HD diabetic and non-diabetics was recorded (p<0.001) (figure 2).

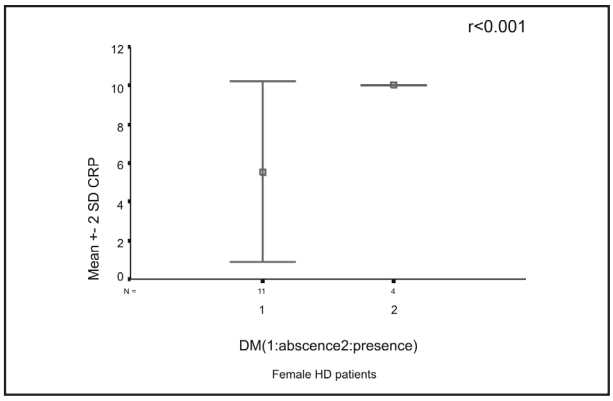

Figure 2. Significant difference in CRP between diabetic and non-diabetic female HD patients

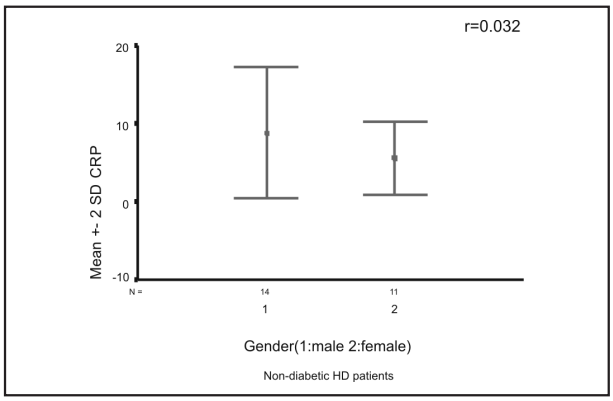

Figure 3. Significant difference in CRP between male and female non-diabetic patients

Moreover, a significant difference of CRP between males and females of non-diabetic population was registered (p= 0.032) (figure 3).

In this study, the associations between serum CRP level and variables related to age, duration of HD, HD sessions, dialysis adequacy, serum iron, serum ferritin, also hemoglobin and hematocrit (HCT) levels in all of the groups containing total, non-diabetic, diabetic, female and male HD patients were as follows: in all HD patients a significant

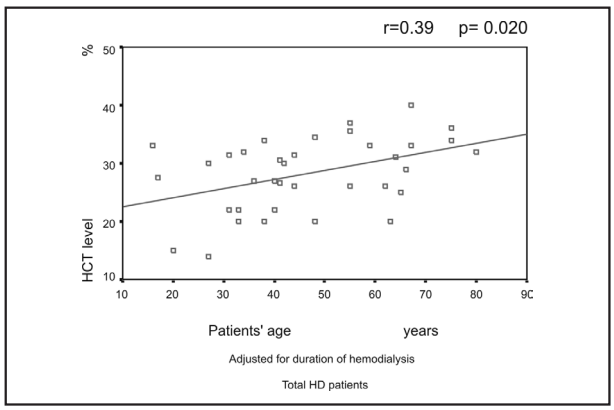

Figure 4. Significant positive correlation between HCT level and age of patients

positive correlation between HCT level and patients' age (r = 0.39, p =0.020) adjusted for duration of dialysis was registered (figure 4).

In all the patients, there was a significant positive correlations between serum ferritin and age of the patients (r = 0.51, p =0.001) , serum iron (r = 0.48, p =0.003), HD efficacy as determined by URR (r=0.33, p=0.047), (figure 5) dialysis sessions (r = 0.48, p =0.003) (figure 6) and duration of dialysis (r = 0.52, p =0.001) (figure 7).

Also, there was an approximate significant inverse correlation between serum ferritin and hemoglobin level (r = - 0.29, p =0.080) as well as significant inverse correlation between serum ferritin and hematocrite level (r = - 0.34, p =0.044) (adjusted to age in all aforementioned correlations) (figure 8).

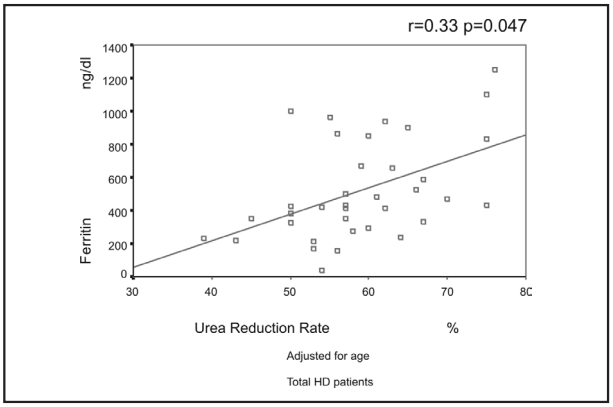

Figure 5. Significant positive correlation between serum ferritin and HD efficacy as determined by URR

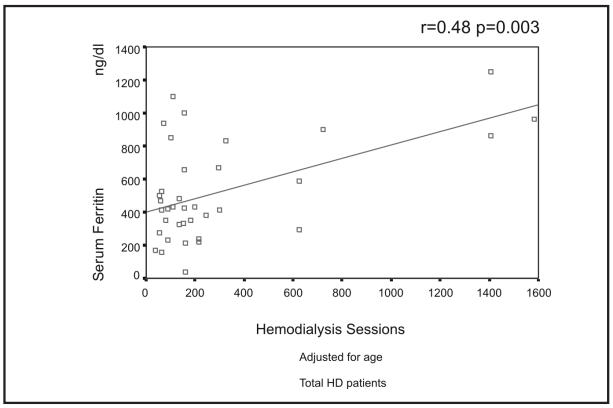

Figure 6. Significant positive correlation between serum ferritin and dialysis sessions

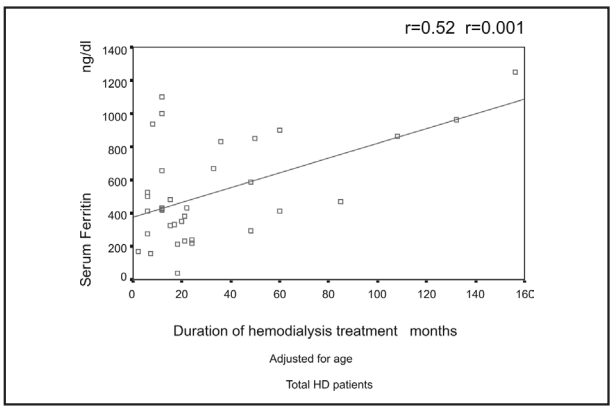

Figure 7. Significant positive correlation between serum ferritin and dialysis duration

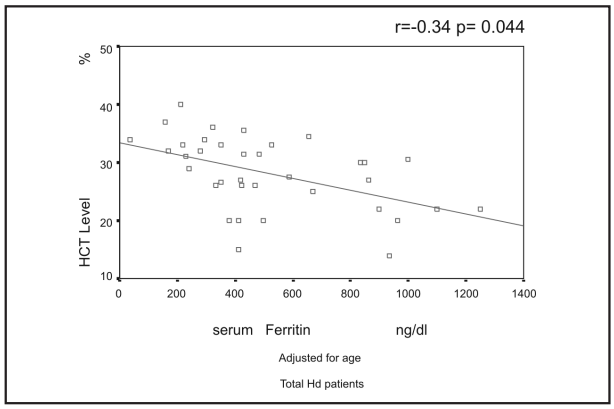

Figure 8. Significant positive correlation between serum ferritin and hematocrite level

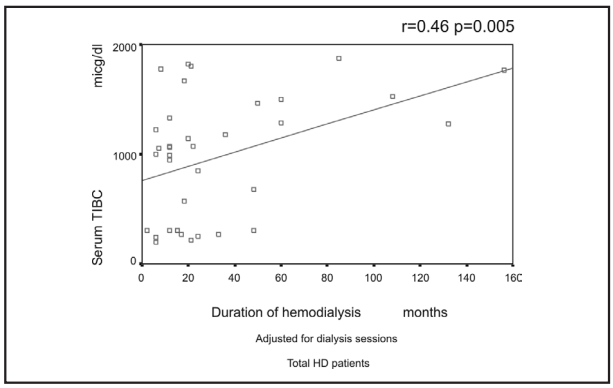

Figure 9. Significant positive correlation between serum TIBC and duration of hemodialysis treatment

A significant positive correlation between serum TIBC and duration of HD treatment was recorded in this group, too (r =0.40, p =0.017) (figure 9).

In all the patients, there was an approximate significant inverse correlation between serum CRP and hemoglobin level (r = -0.29, p =0.086) (figure 10) as well as a significant positive correlation between serum CRP and serum iron (r = 0.44, p =0.008) (figure 11) (adjusted to age in two correlations).

Moreover, in non-diabetic HD patients, an approximate significant inverse correlation between serum CRP and HD adequacy (by URR) (r =

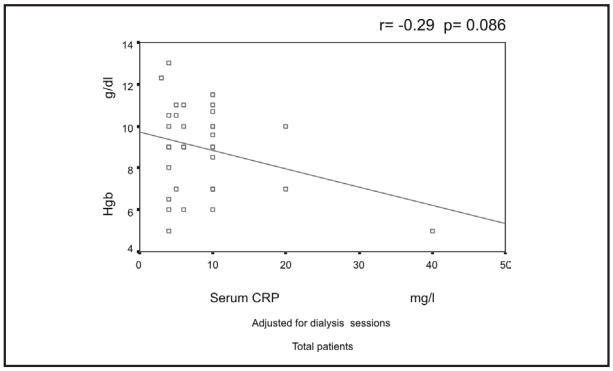

Figure 10. Approximate significant inverse correlation between serum CRP and hemoglobin level

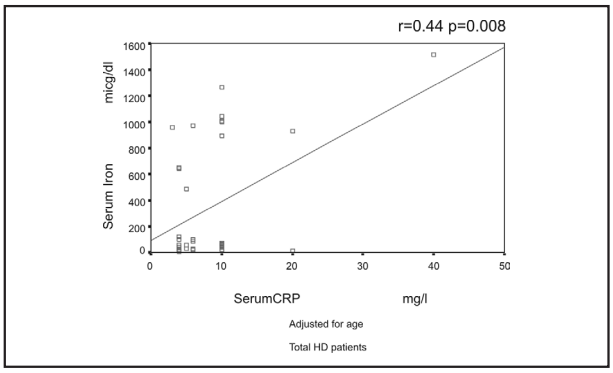

Figure 11. Significant positive correlation between serum CRP and serum Iron

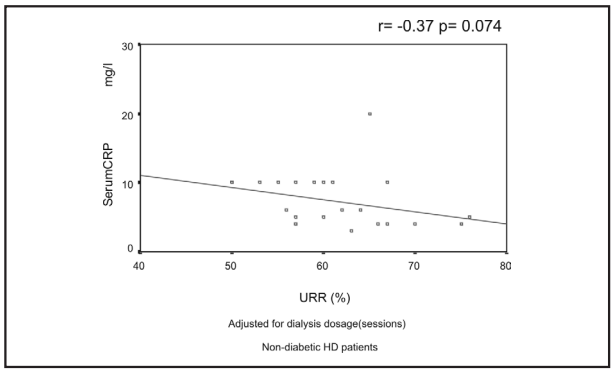

Figure 12. Approximate significant inverse correlation of serum CRP and hemodialysis adequacy

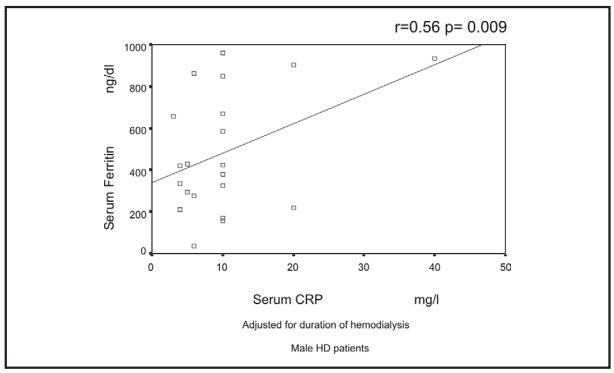

Figure 13. Significant positive correlation between serum CRP and serum ferritin

-0.37, p =0.074)(adjusted for dialysis sessions) was registered, too (figure 12).

Also, in male HD patients, a significant positive correlation between serum CRP and serum ferritin (r = 0.56, p =0.009) was registered (figure 13).

In the study, in all the patients, the correlation between serum CRP and serum ferritin was positive but insignificant (r = 0.18, p =0.29), while in the patients divided into ferritin under and above 500 ng/dl, we found tha the insignificant association between serum CRP and serum ferritin in patients with ferritin of more than 500 ng/dl was positive (r = 0.18, p =0.55), and the insignificant association of serum CRP with ferritin in the patients with ferritin below 500 ng/dl was negative(r = - 0.40, p =0.85)(adjusted to age in the three aforementioned correlations). It is interesting that when we excluded patients with ferritin below 300 ng/dl in the total group, we found that in the rest 27 patients there was a weakly positive correlation of serum CRP with serum ferritin(r = 0.33, p =0.098) (adjusted for duration of dialysis).

DISCUSSION

The important findings of our study were higher values of CRP in diabetics and male patients and significant positive correlations between serum ferritin and HD efficacy, an approximate significant inverse correlation between serum CRP and hemoglobin level, and also a significant positive correlation between serum CRP and serum iron and an approximate significant inverse correlation between serum CRP and HD adequacy. Moreover, in male patients, a significant positive correlation between serum CRP and serum ferritin was registered, as well. Anemia is a consistent finding in chronic renal disease, affecting up to 90% of patients. The central role of anemia in the development of cardiovascular dysfunction is now well-known (15). Anemia of ESRD can be managed relatively successfully by recombinant human erythropoietin. Iron administration plays a central role in enhancing anemia responsiveness to EPO. Serum ferritin concentration is a commonly used marker of iron status in maintenance dialysis patients (1). It was shown that a low serum ferritin concentration is a reliable indicator of iron deficiency among ESRD patients. However, a high serum ferritin may not be an optimal indicator of "increased" iron stores among dialysis patients, because it is an acute-phase reactant and its increase in dialysis patients may be based on the factor unrelated to iron stores such as inflammation(4). In dialysis patients, high CRP levels are associated with low Hgb levels and/or epoetin resistance

(16-18). In this regard, in the study conducted by Sirken et al. it was found that the increased levels of CRP were associated with relative EPO resistance in dialysis patients (19). Our findings support the first direct evidence that inflammation, which is closely related to protein-energy malnutrition (2) in dialysis patients, might affect anemia toward its intensification. We showed that the insignificant association of serum CRP with ferritin in all the patients who had a serum ferritin below 500 ng/dl was negative, while this insignificant association in patients with serum ferritin higher than 500 ng/dl was positive. Moreover, a weakly positive correlation between serum CRP and serum ferritin was observed when we excluded patients with serum ferritin below 300 ng/dl. This data support the evidence that inflammation may not have an effect on serum ferritin, unless there is enough iron stores in the body, so that serum ferritin is somewhat increased (13). Rogers et al. showed that IL-1ß induces ferritin gene expression by translational control of its mRNA. However, this inflammatory induction of ferritin synthesis is different from iron-dependent ferritin gene expression (13). They showed that this inflammatory regulation of ferritin requires the background presence of cellular iron (13). In other words, without adequate iron stores, serum ferritin is low and does not correlate with inflammation, but with enough iron, serum ferritin has the function of both iron and inflammation(20). Kalantar-Zadeh et al. in a study on of 82 MHD patients, showed that serum ferritin concentrations were higher in malnourished patients. They also found that a moderate correlation existed between serum ferritin and CRP concentrations when the range of serum ferritin was restricted between 200 and 2000 ng/ml (20). This finding is consistent with our clinical study and confirms that in the setting of absolute iron deficiency, serum ferritin is almost always low (20). In summary, we can conclude that MICS may increase serum ferritin concentration apart from iron status, and needs more attention during iron therapy for the treatment of anemia in regular HD patients.

Acknowledgements: *We would like to thank Dr. K. Kalantar-Zadeh, Assistant Professor of Medicine of UCLA School of Medicine for sending his articles and his comments to our center.*

REFERENCES

1. Kalantar-Zadeh K, Hoffken B, Wunsch H, Fink H, Kleiner M, Luft FC. Diagnosis of iron deficiency anemia in renal failure patients during the post-erythropoietin era. Am J Kidney Dis 1995; 26: 292-299.

2. Kalantar-Zadeh K, Ikizler A, Block G, Avram M, Kopple J. Malnutrition-inflammation complex syndrome in dialysis patients: causes and consequences. Am J Kidney Dis 2003;42:864-881.

3. Kalender B, Mutlu B, Ersoz M, Kalkan A, Yilmaz A. The effects of acute phase proteins on serum albumin, transferrin and haemoglobin in haemodialysis patients. Int J Clin Pract 2002; 56: 505-508.

4. Kalantar-Zadeh K, Don BR, Rodriguez RA, Humphreys MH.c Serum ferritin is a marker of morbidity and mortality in hemodialysis patients. Am J Kidney Dis 2001; 37: 564-572.

5. Rogers JT. Ferritin translation by interleukin-1and interleukin-6: the role of sequences upstream of the start codons of the heavy and light subunit genes. Blood 1996; 87: 2525-2537.

6. Kalantar-Zadeh K, McAllister C, Lehn R, Lee G, Nissenson A, Kopple J. Effect of malnutrition-inflammation complex syndrome on erythropoietin hyporesponsiveness in maintenance hemodialysis patients. Am J Kidney Dis 2003;42:761-773.

7. Trey JE, Kushner I. The acute phase response and the hematopoietic system: the role of cytokines. Crit Rev Oncol Hematol 1995; 21: 1-18.

8. Means RT Jr. Advances in the anemia of chronic disease. Int J Hematol 1999; 70: 7-12.

9. Feelders RA, Vreugdenhil G, Eggermont AM, Kuiper-Kramer PA, van Eijk HG, Swaak AJ. Regulation of iron metabolism in the acute-phase response: interferon gamma and tumour necrosis factor alpha induce hypoferraemia, ferritin production and a decrease in circulating transferrin receptors in cancer patients. Eur J Clin Invest 1998; 28: 520-527.

10. Allen DA, Breen C, Yaqoob MM, Macdougall IC. Inhibition of CFU-E colony formation in uremic patients with inflammatory disease: role of IFN-gamma and TNF-alpha. J Invest Med 1999; 47: 204-211.

11. Goicoechea M, Martin J, de Sequera P et al. Role of cytokines in the response to erythropoietin in hemodialysis patients. Kidney Int 1998; 54: 1337-1343.

12. Bárány P. Inflammation, serum C-reactive protein, and erythropoietin resistance. Nephrol Dial Transplant 2001; 16: 224-227.

13. Rogers JT, Bridges KR, Durmowicz GP, Glass J, Auron PE, Munro HN. Translational control during the acute phase response. Ferritin synthesis in response to interleukin-1. J Biol Chem 1990; 265: 14572-14578.

14. Boag JT. Basic Truths in Optimal Hemodialysis Dialysis & Transplantation 1994; 23:636.

15. Parfrey P. Anaemia in chronic renal disease: lessons learned since Seville 1994. Nephrol Dial Transplant 2001; 16: 41-45.

16. Owen WF, Lowrie EG. C-reactive protein as an outcome predictor for maintenance hemodialysis patients. Kidney Int 1998; 54: 627-636.

17. Barany P, Divino Filho JC, Bergström J. High C-reactive protein is a strong predictor of resistance to erythropoietin in hemodialysis patients. Am J Kidney Dis1997; 29: 565-568.

18. Gunnell J, Yeun JY, Depner TA, Kaysen GA. Acute-phase response predicts erythropoietin resistance in hemodialysis and peritoneal dialysis patients. Am J Kidney Dis1999; 33: 63-72.

19. Sirken, Gary; Kung, Shiang-Cheng; Raja, Rasib. Decreased Erythropoietin Requirements in Maintenance Hemodialysis Patients with Statin Therapy. ASAIO Journal. 2003; 49:422-425.

20. Kalantar-Zadeh K, Rodriguez RA, Humphreys MH. Association between serum ferritin and measures of inflammation, nutrition and iron in haemodialysis patients. Nephrol Dial Transplant 2004; 19:141-149.

POZITIVNA KORELACIJA C-REAKTIVNOG PROTEINA SA ANEMIJOM KOD PACIJENATA NA HEMODIJALIZI

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SAŽETAK

Cilj rada bio je da se proveri veza između seruma C-reaktivnog proteina i feritin seruma i njen značaj u ozbiljnosti anemije kod pacijenata na hemodijalizi. U studiju su bili uključeni pacijenti u završnoj fazi renalne bolesti koji su bili na acetatnoj dijalizi sa polisunfonskim dijaliznim membranama. Studija je uključila 36 pacijenata (15 žena i 21 muškarac) od kojih 25-oro nisu bili dijabetičari, a 11-oro jesu. Postojala je i aproksimativna značajna inverzna korelacija između seruma CRP-a i nivoa hemoglobina, značajna pozitivna korelacija između seruma CRP-a i seruma gvožđa, kao i aproksimativna inverzna korelacija između seruma CRP-a i adekvatnosti dijalize. Zabeležena je značajna pozitivna korelacija između seruma CRP-a i feritin seruma. Malnutriciono-inflamacioni kompleksni sindrom može da poveća koncentraciju feritin seruma nezavisno od statusa gvožđa, i zahteva veću pažnju za vreme terapije gvožđem, zbog lečenja anemije kod redovnih pacijenata na dijalizi.

***Ključne reči*: završna faza bubrežne insuficijencije, hemodijaliza, anemija, C-reaktivni protein, hemoglobin, hematokrit, feritin, malnutriciono-inflamacioni kompleksni sindrom**