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Original Article

Effect of nesfatin-1 on the nutritional status of hemodialysis patients

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Abstract

Background

Nesfatin-1 is considered an anorexigenic peptide that plays an important role in appetite regulation, weight loss, and malnutrition. Although the cause of anorexia in CKD patients is not totally understood, both anorexia and malnutrition are common presentations of chronic kidney disease (CKD) patients who are susceptible to worse outcomes.

Our aim of the work

To study the relation of serum nesfatin-1 and its relation to the nutritional status of end-stage renal disease (ESRD), who are maintained on regular hemodialysis.

Patients and methods

A total of 60 ESRD patients are maintained on regular hemodialysis, and apparently healthy thirty persons (control group) were included in this study. Those included represented 45 males and 45 females with an age range from 19 to 68 years old. Based on malnutrition inflammation score (MIS), we had three groups, the first & second group, each included 30 ESRD patients maintained on regular HD without & with malnutrition respectively. 3rd group included 30 apparently healthy control persons; all subjects were exposed to full history, clinical examination, MIS questioner, and lab. Tests in the form of Complete blood count, liver function test, Kidney Function test, s.Calcium, Phosphrus, Parathyroid hormone, iron profile, C reactive protein titer, interleukin 6, and serum nesfatin 1.

Results

There were significant increases in s. nesfatin-1 and CRP levels in groups 1, 2 more than that of the control group, with a significant increase in group 2 in comparison to other groups. Group 2 had a statistically significantly increase of IL6 and MIS when compared to group 1. In group 2. while there was a highly significant positive correlation between nesfatin-1with MIS and IL6, but a significantly negative correlation with BMI, Ph. and serum parathyroid hormone. In group 1, while there was a highly significant positive correlation between nesfatin-1with MIS and IL6, a significantly positive correlation with duration of dialysis but a significantly negative correlation with ferritin, calcium, and cholesterol. The cutoff level in group1 patients versus group 2 was more than 16.6, with a sensitivity of 56.7% and specificity of 83.3%. In conclusion, s. nesfatin-1 is specific for the detection of malnutrition in hemodialysis patients.

Keywords: Hemodialysis patient, malnutrition, nestatin-1

Introduction

Nesfatin-1, which was discovered in 2006, consists of an 82-amino acid polypeptide. It has an active core that has an anorexigenic effect, which is the mid-fragment that consists of 29 amino acids, through which it regulates food intake, gastrointestinal function, glucose and lipid

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metabolism, thermogenesis, anxiety, and depression, as well as cardiovascular and reproductive functions [1].

Hypertension and overweight obesity are considered possible factors that may increase the risk of mortality in patients with chronic renal failure (CRF). Dialysis patients with abnormal state of overhydration are linked to hypertension, left ventricular hypertrophy, and other cardiovascular adverse effects that may increase the risk of mortality, so adequate control of dry weight is an important factor that indicates dialysis adequacy [2].

Paradoxical obesity of hemodialysis (HD) patients is accompanied with better survival rates; it is discussed in some survival studies for HD patients. Is there is a different pattern considering the relation between adiposity and cardiovascular risk factors between HD patients and the normal population? [2].

Adipose tissue secretes a number of proteins including leptin, adiponectin, and interleukin 6 (IL6). Leptin is a good indicator of fat mass in both obese and nonobese patients with CRF, as it regulates the appetite and the energy catabolism. Its average molecular weight is 16 000 D. It considered a uremic toxin that can be highly eliminated with high-flux membranes and hemodiafiltration techniques in patients with CRF. Adiponectin is a hormone secreted by adipocytes and has antiatherogenic and anti-inflammatory properties, so its levels are reduced in obese patients [2].

CRF-associated malnutrition has a prevalence of 10–60% in patients on HD with high morbidity and mortality. Its main causes are uremic anorexia, comorbidities, inflammatory processes, and hypercatabolism owing to loss of nutrients during dialysis. There is no accurate method for diagnosis of malnutrition in HD patients, as even biochemical and anthropometric indicators can be affected by inflammatory and hydration status, comorbid conditions, expertise, and unavailable reference standards for HD patients [3].

Besides being a predictor of mortality, the malnutrition inflammation score (MIS) constitutes most of the main criteria for protein-energy wasting (PEW) diagnosis. MIS is used for assessment of nutritional status and diagnosis of inflammation in patients with chronic kidney disease (CKD) and also can be used in both inpatient and outpatient settings [4]. MIS includes a choice for each variable: (a) medical history, which includes dry weight changes after dialysis (3–6 months), dietary intake, gastrointestinal symptoms, functional capacity, and comorbidity according to time on dialysis; (b) the physical examination, which includes fat stores loss, for example, subcutaneous fat below the eye and in biceps and triceps and loss of lean mass in clavicle, scapula, shoulders, and quadriceps; (c) body size: the BMI; and (d) laboratory biochemical parameters: albumin, total iron-binding capacity (TIBC), or transferrin. The results can be calculated from the simple sum of each of the items, to be expressed into the following categories: normal nutritional status: 0–2 points; mild malnutrition: 3–5, moderate malnutrition: 6–8; and severe malnutrition: from 9 points [4]. The aim of this study was to explain the relation of serum nesfatin-1 and nutritional status in HD patients.

Patients and methods

A total of 90 participants were included in this study and divided into 60 patients on regular HD and 30 apparently healthy participants with normal MIS as a control group. Oral and written consents were taken from patients with approval of the ethical committee. The patients had end-stage renal disease (ESRD) and were maintained on regular HD for more than 6 months, on bicarbonate dialysate, having three sessions per week, each for 4 h with a low-flux dialyzer, with blood and dialysate flows of 300 ml/min and 500 ml/min, respectively. The main causes of ESRD were hypertension nephrosclerosis in 60%, chronic glomerulonephritis in 15%, polycystic kidney disease in 5%, and unknown causes in 20%. Full history taking, clinical examination, and evaluation of their nutritional status using MIS were performed for all participants included in this study. Predialysis collection of blood samples was performed, and the blood was subjected to the following tests: liver and renal function tests, lipid profile, calcium, phosphorus, serum iron, TIBC, and ferritin, which were detected on a dimension RxL Max analyzer (Siemens Health care GmbH-Henkester 127, Erlangen, Germany). Serum sodium and potassium were detected using ion-selective electrodes on a Hitachi 912 auto-analyzer. Serum parathyroid hormone (PTH) was performed using a sandwich ELISA kit supplied by Bio Vendor– Laboratoni Medicina, Karasek, Brno, Czech Republic [5]. Complete blood count was determined on a coulter counter T890 (Coulter Counter, Harpenden, UK). C-reactive protein (CRP) was performed using latex agglutination. Serum IL6 was performed using an ELISA kit supplied by IBL International (IBL International, Flughfenstrasse 52a, Hamburg, Germany) [6]. Nesfatin was performed using an ELISA kit supplied by Bio Vendor – Laboratoni Medicina, Karasek, Brno, Czech Republic [7].

Statistical analysis

Data analysis was performed using Statistical Package for the Social Sciences (SPSS Inc., Chicago, Illinois, USA), version 20, with expression of quantitative data as means ± SD and qualitative data as frequency and percentage. The following tests were performed: t test, analysis of variance, $\chi 2$ test, post-hoc test: least significance difference, Pearson's correlation coefficient (r) test, and receiver operating characteristic curve analysis, which was used to find out the overall productivity of a parameter. The best cutoff value with its related sensitivity and specificity were noted. *P* value less than 0.05 was considered significant and less than 0.001 was considered highly significant.

Results

This study was carried on 60 patients with ESRD on regular HD, with 30 apparently healthy participants with normal MIS as a control group. They were divided into three groups: the first and second group each included 30 patients with ESRD

Table 1: Demographic characteristics among the three studied groups

Demographic data	Group 1	Group 2	Group 3	1 vs. 2	1 vs. 3	2 vs. 3	Test
Age (years)							
Means±SD	48.6±12.6	45.07±9.01	43.67±9.30	0.194	0.321	0.243	LSD
Range	19-68	22-63	18-49				
Sex [*n* (%)]							
Female	18 (60)	14 (46.66)	13 (43.33)	0.183	0.076	0.085	
Male	12 (40)	16 (53.33)	17 (56.66)				
BMI							
Means±SD	25.20±4.68	17.48±86	24.04±3.03	<0.00	0.217	<0.001	
Range	18.3-33.7	13.33-24	19.8-32.1				
Duration of dialysis							
Means±SD	2.9±2.3	5.93±2.98	*t*		*P*		*t* test
			4.453		<0.001		
Range	1-9	2-12					
MIS score							
Means±SD	3.07±2.03	23.7±2.51	2.683		<0.001		
Range	0-8	19-27					

There was a decrease in BMI in the second group compared with other groups. There were significant increases regarding dialysis duration and a highly significant increase in MIS in group 2 compared with group 1. LSD, least significance difference; MIS, malnutrition inflammation score.

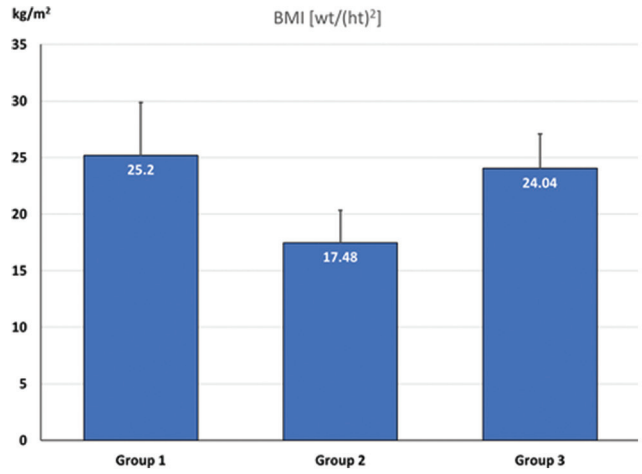

Figure 1: Comparison between groups regarding BMI.

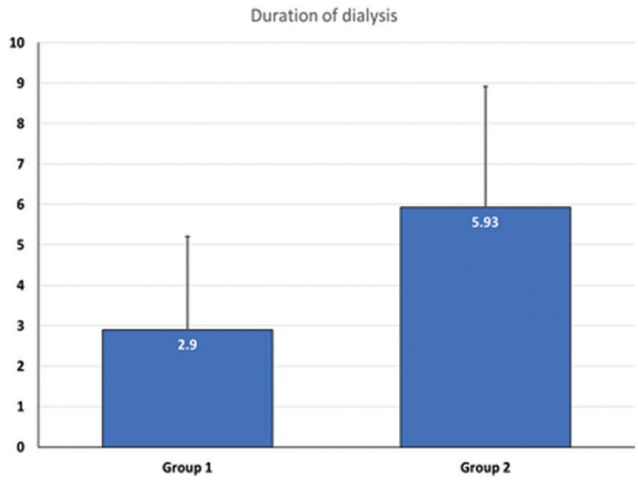

Figure 2: Comparison between groups 1and 2 regarding duration of dialysis.

maintained on regular HD without and with malnutrition, respectively, and the third group included 30 apparently healthy control persons. Their demographic characteristics are shown in Tables 1,2 and Figs. 1-3.

There were highly significant increases in mean ± SD nesfatin-1 level and IL6 in group 1 (14.65 ± 1.97 and 9.37 + 1.68, respectively) and group 2 (16.57 ± 2.28 and 16.13 + 2.23, respectively) compared with group 3 (5.11 ± 1.69 and 3.05 + 1.00, respectively) ($P < 0.001$). There were highly significant increases in nesfatin-1 level and IL6 in group 2 than in group 1 ($P < 0.001$), whereas there were highly significant increases in serum albumin and TIBC in group 1 than in group 2 ($P < 0.001$) and a significant increase in serum cholesterol in group 1 than in group 2. There were highly significant increases in serum *P*TH and ferritin in group 1 than in group 3 ($P < 0.001$), a significant increase in serum TIBC in group 1 than in group 3 ($P = 0.03$), whereas significant increases in serum iron in group 3 than in group 1 ($P = 0.01$). There were highly significant decreases in albumin, cholesterol, and serum iron ($P < 0.001$) and TIBC ($P = 0.04$) in group 2 than in group 3 whereas significant increases in serum *P*TH and ferritin in group 2 than in group 3 ($P = 0.002$ and 0.004, respectively) (Figs. 4-9 and Tables 3,4).

In group 1, nesfatin-1 has a highly significant positive correlation with MIS and IL6 ($P < 0.001$), a significant positive correlation with dialysis duration ($P = 0.004$) and aspartate aminotransferase ($P = 0.03$), and a significant negative correlation with calcium ($P = 0.007$), sodium ($P = 0.02$), ferritin ($P = 0.04$), and serum cholesterol ($P = 0.03$). In group 2, nesfatin-1 has a highly significant positive correlation with MIS and IL6 ($P < 0.001$) and a significant negative correlation with BMI ($P = 0.005$), phosphorus ($P = 0.016$), and serum *P*TH ($P = 0.047$) (Table 5).

Table 2: Comparison among the studied groups regarding mean±SD nesfatin-1 level, serum interleukin 6, serum albumin, serum parathyroid hormone, serum total cholesterol, and iron profile

Parameter	Group 1	Group 2	Group 3	LSD		
				1 vs. 2	1 vs. 3	2 vs. 3
Serum nesfatin-1 (ng/ml)						
Mean+SD	14.65±1.97	16.57±2.28	5.11±1.69	<0.001	<0.001	<0.001
Range	11-18.2	12-19.8	2.4-8.1			
Serum IL6 (pg/ml)						
Mean+SD	9.37±1.68	16.13±2.23	3.05±1.00	<0.001	<0.001	<0.001
Range	6.4-12.4	12.5-20	1.4-4.5			
Albumin (g/dl)						
Mean+SD	3.93±0.39	2.30±0.5	4.1±0.36	<0.001	0.08	<0.001
Range	3-4.8	11.5-3.4	3.5-5			
Serum PTH (µ/l)						
Mean+SD	408.1±409.3	276.6±281.6	34.04±9.35	0.079	<0.001	0.002
Range	30.36-1382	7.4-1116	18.5-54			
Cholesterol						
Mean+SD	186.4±42.45	160.8±31.25	196.0±42.72	0.01	0.345	<0.001
Range	110-262	103-217	115-284			
Serum iron (µg/dl)						
Mean+SD	81.63±49.02	63.27±36.63	105.9±27.67	0.07	0.01	<0.001
Range	21-251	19-226	69-160			
TIBC (µg/dl)						
Mean+SD	294.8±74.79	225.7±37.48	259.8±72.98	<0.001	0.03	0.04
Range	206-524	127-311	31-402			
Ferritin (ng/ml)						
Mean±SD	401.7±393.35	332.8±224.68	129.53±88.03	0.319	<0.001	0.004
Range	10.8-1983	14.3-966	25-267			

IL6, interleukin 6; LSD, least significance difference; PTH, parathyroid hormone; TIBC, total iron-binding capacity.

Table 3: Comparison of C-reactive protein among all studied groups

CRP	Group 1 [*n* (%)]	Group 2 [*n* (%)]	Group 3 [*n* (%)]	χ^2 test	
				χ^2	*P*
Negative	20 (66.7)	5 (16.7)	30 (100)	44.416	<0.001
Positive	10 (33.3)	25 (83.3)	0		
Total	30 (100)	30 (100)	30 (100)		

CRP, C-reactive protein. There was a highly significant increase in patients with positive CRP findings in group 2 than in group 1 (P<0.001).

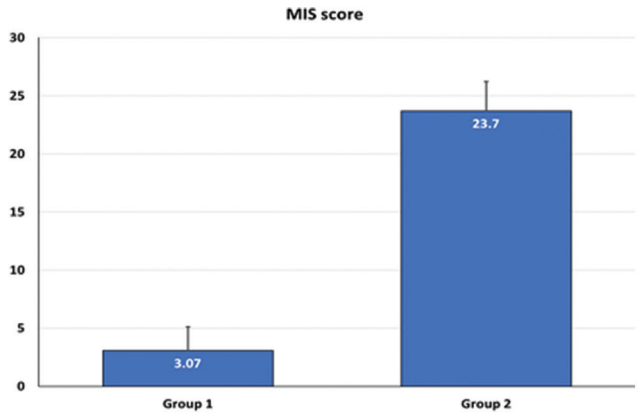

Figure 3: Comparison of MIS between groups 1 and 2. MIS, malnutrition inflammation score.

Discussion

Nesfatin-1 is considered an anorexigenic peptide that plays an important role in appetite regulation, weight loss, and malnutrition. Although the causes of anorexia in patients with CKD are not totally understood, both anorexia and malnutrition are common presentations of patients with CKD who are susceptible to worse outcomes [8]. Malnutrition prevalence varies widely between 20 and 60% in HD patients. Subjective global assessment (SGA), anthropometry, and serum albumin are the most common methods used in clinical studies to define protein-energy malnutrition in HD population. SGA is considered a rapid conduction, inexpensive, reproducible, valid, and reliable method used effectively by nursing staff, dieticians, and physicians, so it is used strongly in the clinical and research trials and recommended by many medical societies for nutritional assessment in the adult dialysis population. MIS is a quantitative tool that consists of seven original SGA components together with BMI, serum albumin, and TIBC [9]. It was found to be superior to conventional predictors of malnutrition such as serum CRP and SGA [10].

CKD is associated with immune dysregulation and inflammatory activation due to primary disease rather than to uremia per se.

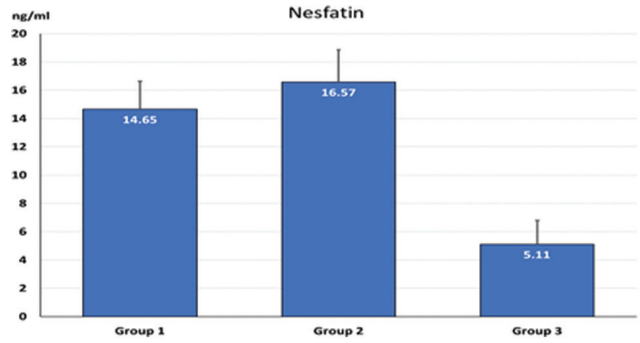

Figure 4: Comparison of serum nesfatin-1 in all study groups.

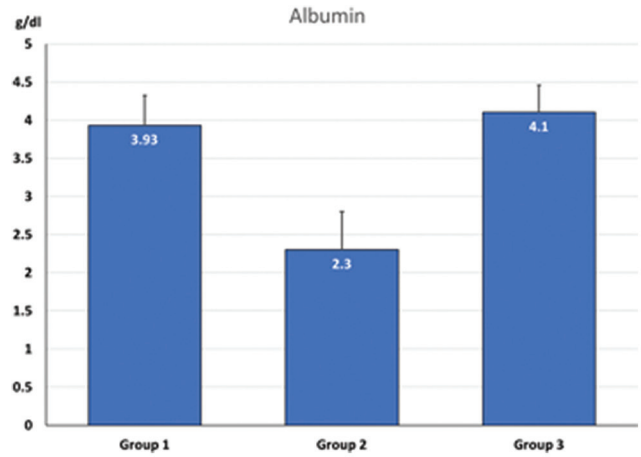

Figure 6: Comparison of serum albumin in all study groups.

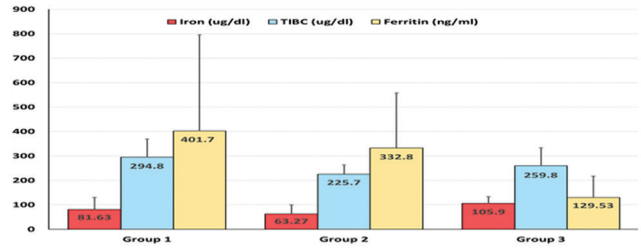

Figure 8: Comparison of iron profile between all study groups.

Genetic background and epigenetic influences such as diet, lifestyle, and environment factors may be also involved. Higher levels of circulating cytokines in CRF are attributed to decreased renal clearance and its increased production mainly from lymphocytes and adipose tissues that have a high mRNA expression of proinflammatory cytokines in ESRD. Indeed, in stages 3–5 CKD, visceral fat volume correlates with circulating IL6. High levels of leptin in pediatric CKD are owing to its overproduction rather than decrease in its clearance. Upregulation of adiponectin receptor-1 owing to high level of adiponectin in CKD, its anti-inflammatory effects may be overcome at postreceptor level by the development of adiponectin resistance [11]. Four main diagnostic criteria for diagnosis of PEW are needed: (a) biochemical measures (serum

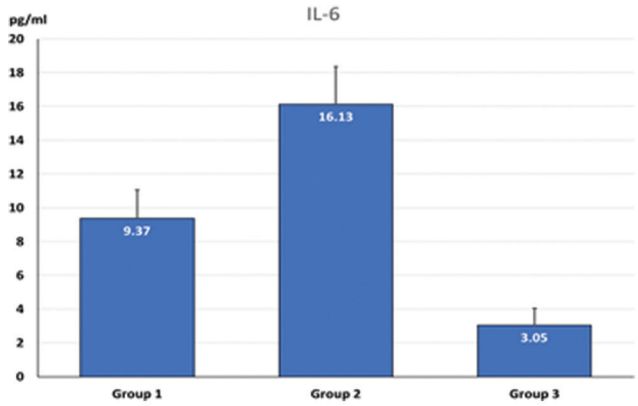

Figure 5: Comparison of IL6 in all study groups. IL6, interleukin 6.

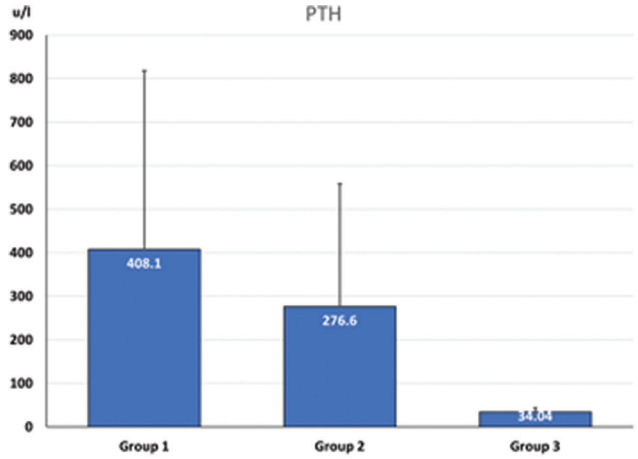

Figure 7: Comparison of serum PTH in all study groups. PTH, parathyroid hormone.

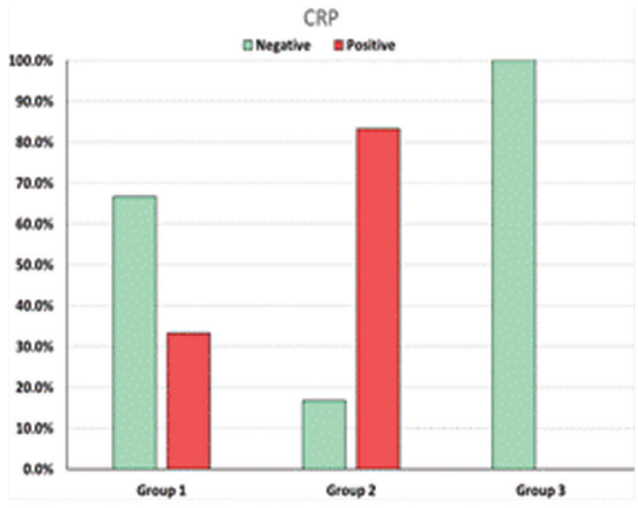

Figure 9: Comparison of CRP in all study groups. CRP, C-reactive protein.

albumin, prealbumin, transferrin, and cholesterol); (b) body mass measures (BMI, unintentional weight loss, and total body fat), (c) muscle mass measures (total muscle mass and mid-arm muscle circumference), and (d) dietary intake

measures (dietary protein and energy intake). Appetite, food intake, energy expenditure, various measures of body composition, multiple laboratory markers, and nutritional scoring systems are recognized additional measures for the diagnosis of PEW. At least three of the four diagnostic categories (and at least one test in each category) must be abnormal, and these need to be documented on at least three occasions, 2–4 weeks apart [12].

SGA, serum albumin, and prealbumin may be viable markers for nutrition assessment in HD patients as noticed in this study, evident by very low albumin in group 2 with high MIS, whereas it was normal with low MIS in group 1. This is in agreement with Kalantar-Zadeh [12], who showed that low serum albumin levels are closely related to PEW and poor survival. Moreover, Ghorbani *et al*. [13] recommended the measurement of serum albumin level to assess clinical status and nutrition of HD patients. In addition, Essadik *et al*. [14] revealed an increased incidence of PEW among HD patients. Omari *et al*. [10] concluded that there is a high prevalence of malnutrition among HD patients based on results from the MIS score, which revealed correlations between nutritional status and clinical and sociodemographic factors of the patients. So, enhancement of the nutritional status of HD patients should be increased, especially for those who have multiple comorbid diseases, chronic medications, long dialysis duration, and are elderly or live alone, through improvement of health care provider's awareness. In contrary, Gama-Axelsson *et al*. [15] reported a poor correlation of serum albumin with several markers of nutritional status, and its value in dialysis patients as a reliable marker is limited. A low serum albumin can be explained by the state of persistent inflammation rather than a nutritional marker. Our study showed a highly significant increase of CRP-positive patients in group 2 in comparison with group 1. Dragović *et al*. [16] reported a high rate of inflammation in the dialysis patients based on elevated CRP levels in the examined group. Chronic inflammation may be associated with protein-energy malnutrition; hence, there is an increased morbidity and mortality rate in these patients based on the results of negative correlations between CRP levels and serum albumin and with some other parameters of nutritional status. Indeed, Choi *et al*. [17] reported that there is a significant association of abdominal aortic calcification progression, malnutrition, and inflammation, which may be used as a predictor of all-cause mortality in HD patient. In contrary to our study, Kaysen *et al*. [18] showed variations in the levels of CRP and other acute-phase proteins considerably over time in 37 malnourished HD patients. Indeed, Hanafusa *et al*. [19], reported a poor outcome associated with malnutrition or wasting and independent of inflammation among HD patients. Our study revealed there were high levels of serum IL6 in HD patients with or without malnutrition and highly elevated in those with malnutrition (group 2) than in group 1, indicating a positive correlation between IL6 and MIS. Similarly, Den Hoedt *et al*. [20] reported that there was an increase in the level of inflammatory markers including CRP and proinflammatory cytokines such as IL6 over time with HD. Moreover, Bossola *et al*. [21] revealed that higher levels of plasma IL6 and CRP and increased frequency of

Table 4: Correlation between nesfatin-1 and other parameters using Pearson correlation coefficient in patients with chronic renal failure with and without malnutrition

Parameters	CRF without malnutrition (group 1) vs. nesfatin-1		CRF with malnutrition (group 2) vs. nesfatin-1	
	r	*P*	*r*	*P*
Age	−0.269	0.150	−0.101	0.596
BMI	−0.241	0.199	−0.497	0.005
IL6	0.983	<0.001	0.974	<0.001
MIS score	0.961	<0.001	0.982	<0.001
Serum albumin	−0.2243	0.196	−0.204	0.279
Urea	−0.163	0.388	0.036	0.851
Creatinine	−0.016	0.935	−0.046	0.807
Sodium	−0.422	0.020	−0.205	0.277
Potassium	−0.095	0.617	0.034	0.858
Calcium	−0.478	0.007	0.104	0.583
Phosphorous	−0.160	0.400	−0.437	0.016
Uric acid	−0.023	0.903	0.088	0.644
Serum PTH	0.290	0.119	−0.365	0.047
Serum iron	0.190	0.314	−0.243	0.196
TIBC	0.036	0.851	0.002	0.990
Ferritin	−0.350	0.048	−0.091	0.634
HB%	−0.153	0.419	−0.224	0.235
TLC	0.034	0.857	0.003	0.988
Platelets	−0.123	0.518	−0.244	0.194
Triglycerides	0.048	0.801	−0.171	0.365
Cholesterol	−0.384	0.036	−0.055	0.771
ALT	0.306	0.100	0.154	0.415
AST	0.379	0.039	0.291	0.118
Duration of dialysis	0.506	0.004	0.234	0.214

ALT, alanine aminotransferase; AST, aspartate aminotransferase; CRF, chronic renal failure; HB, hemoglobin; IL6, interleukin 6; MIS, malnutrition inflammation score; PTH, parathyroid hormone; TIBC, total iron-binding capacity; TLC, total leukocyte count.

Table 5: Diagnostic performance of nesfatin-1 in discrimination of groups

Groups	Cut-off	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	AUC (%)
CRF without malnutrition vs. CRF with malnutrition	16.6	56.7	83.3	77.3	65.8	73.3
CRF without malnutrition vs. control	8.1	100	100	100	100	100
CRF with malnutrition vs. control	8.1	100	100	100	100	100

AUC, area under the curve; CRF, chronic renal failure; NPV, negative predictive value; PPV, positive predictive value. Receiver operating characteristics (ROC) curve was used to define the best cutoff value of nesfatin-1.

comorbidities correlate with the presence of both anorexia and fatigue in chronic HD patients. Conversely, Beberashilli *et al*. [22] reported that higher IL6 levels were associated with higher mortality without inducing measurable PEW in clinically stable and well-nourished HD patients during years of longitudinal observation. In contrary, those longitudinal studies results were equivocal. Patients on maintenance HD have to be periodically monitored for MIS score instead of IL6 level measurement, which is considered a cost-effective methods as a short-term tool to risk stratify patients [22].

Nesfatin-1 level was significantly increased in group 2 compared with group 1 and in both groups 1 and 2 rather than the control group in this study. Similarly, nesfatin-1 was significantly higher in malnourished children rather than in healthy control, playing a role in the pathogenesis of anorexia and food intake regulation in children [23]. Conversely, its level was nonsignificantly different when compared between matched (age, % body fat mass, and BMI) HD patients and healthy participants [24,25].

In our study, nesfatin-1 had significant positive correlations with MIS score and IL6 and a significant negative correlation with BMI in groups 1 and 2. Similarly, Kim *et al*. [26] observed that nesfatin-1 has a negative correlation with BMI in a prospective study that included 42 obese/overweight group and 36 healthy control group of Korean children and adolescents; hence, it might regulate food intake in obese children and adolescents. Moreover, Guo *et al*. [27] revealed that nesfatin-1 level decreased in obese adults, as it had negative correlations with BMI and waist and hip circumferences, whereas it was not correlated with women with anorexia nervosa. In contrary, plasma nesfatin-1 has a positive significant correlation with BMI (r = 0.596, P = 0.004) [28,29]. In our study, we found that the cutoff level in group 1 patients versus group 2 was more than 16.6, with a sensitivity of 56.7% and a specificity of 83.3%.

Conclusion

Nesfatin-1 is specific for detection of malnutrition in HD patients. Newer studies should be performed on a larger scale for more accurate results.

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Conflicts of interest

There are no conflicts of interest.

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