



## Plasma Ghrelin Level and Nutritional Status in End Stage Kidney Diseased Patient Maintained on Hemodialysis

Authors

Mohammad Kamal Ghitany<sup>1,\*</sup>, Iman Ezzat El Gohary<sup>2</sup>, Eman Zaki Azzam<sup>3</sup>,  
Yasmine Saad Ahmed Mahmoud<sup>4</sup>

<sup>1</sup>Internal Medicine, Internal Medicine Department, Endocrinology Unit, Faculty of Medicine, Alexandria University, Alexandria, Egypt  
Email: [kghitany@hotmail.com](mailto:kghitany@hotmail.com)

<sup>2</sup>Internal Medicine, Internal Medicine Department, Nephrology Unit; Faculty of Medicine, Alexandria University, Alexandria, Egypt  
Email: [Imanalgohary@yahoo.com](mailto:Imanalgohary@yahoo.com)

<sup>3</sup>Internal Medicine, Internal Medicine Department, Endocrinology Unit Department, Alexandria University, Alexandria, Egypt  
Email: [Azzam\\_eman@yahoo.com](mailto:Azzam_eman@yahoo.com)

<sup>4</sup>Nephrology Unit, Alexandria Fever Hospital, Alexandria, Egypt  
Email: [jasminesaad58@yahoo.com](mailto:jasminesaad58@yahoo.com)

### ABSTRACT

Protein energy malnutrition is often present in patients with chronic kidney disease with or without ongoing renal replacement therapy Ghrelin is peptide hormone that consists of 28 amino acids with a molecular weight of about 3.3kDa. Ghrelin might have a role in the regulation of long term energy balance. So we conducted this study with the aim to investigate the changes in levels of serum ghrelin in HD patients and its relationship to nutritional status and inflammatory markers compared with healthy control. The study was conducted on 25 CKD patients maintained on hemodialysis and 20 healthy control subjects; the mean age of both groups was  $47.92 \pm 5.19$  years. Ghrelin concentration was significantly higher in patients on hemodialysis compared with healthy control subjects ( $p$  value = 0.002\*) CRP level was significantly higher in hemodialysis patients compared with healthy control ( $p= 0.001$ ). Conclusion :there was significant increase in both CRP and ghrelin levels in malnourished hemodialysis patients than healthy control subjects.

**Keywords:** Ghrelin, CRP, Nutritional status.

## INTRODUCTION

Protein energy malnutrition (PEM) is often present in patients with chronic kidney disease with or without ongoing renal replacement therapy but it is more profound in hemodialysis patients. The cachexia syndrome in patients with CKD consists of muscle wasting, anorexia, and increased energy expenditure. Cachexia is an important risk factor for mortality in patients with ESKD, which is 100-fold to 200-fold higher than in the general population. <sup>(1)</sup>

Ghrelin is peptide hormone that consists of 28 amino acids with a molecular weight of about 3.3 kDa. It was identified in 1999 in a study which was designed to search for an endogenous ligand for an orphan receptor the type1a growth hormone secretagogue receptor (GHS-R1a). <sup>(2)</sup>

Two forms of circulating ghrelin have been described: acylated ghrelin (AG) (<10% of circulating ghrelin) and unacylated ghrelin (UAG). <sup>(3)</sup>

Some suggestions have been made that ghrelin might have a role in the regulation of long term energy balance. This is supported by the findings showing that ghrelin concentrations are inversely correlated with certain obesity related parameters such as BMI, waist circumference and percentage of body fat. <sup>(4)</sup>

Ghrelin regulates fat distribution and energy metabolism in lean tissues such as liver and muscles. In liver, ghrelin induced a lipogenic and glucogenic effect and increased triglyceride content while reducing activated (phosphorylated) stimulator of fatty acid oxidation. <sup>(5)</sup>

Increased circulating levels of inflammatory markers, such as C-reactive protein (CRP) and the pro inflammatory cytokines interleukin (IL)-6, and tumor necrosis factor-alpha (TNF-a), <sup>(6)</sup> The reason(s) for the increased prevalence of persistent low-grade inflammation in ESRD patients are complex and include a variety of factors related to uremia (such as increased susceptibility to infections and endotoxin exposure that stimulate the inflammatory response

by activating the production of IL-1, IL-6, TNF-a, and interferon-gamma by the macrophages. In addition, the impaired immune response characterized by hypo responsiveness neutrophils and T-cells present in CKD patients also contributes to the low-grade inflammation seen in ESRD. <sup>(7)</sup>

So we conducted this study with the aim investigate the changes in levels of serum ghrelin in HD patients and its relationship to nutritional status and inflammatory markers compared with healthy control.

## MATERIALS AND METHODS

The study was conducted on forty five subjects classified into: twenty healthy subjects as control group (Group I) and twenty five patients with end stage renal disease (ESRD) (group II) maintained on regular hemodialysis three times per week, four hours per session for at least six month using bicarbonate as a buffer, polysulphon membrane to achieve URR (Urea Reduction Ratio) of 0.7-2.0. <sup>(8)</sup>

All the procedures followed were in accordance with the ethical standards of the responsible committee and with the Helsinki declaration of 1975, as revised in 2000 and informed consent was obtained from each patient. All patients and control group were at the same age range from (39-57 years)

### Nutritional assessment: By

a. Anthropometric measurements were obtained immediately after the HD session by a trained researcher. tricep skin folds and upper midarm circumference (MAC) were measured in the arm free of fistula, using standard techniques Three sets of measurements at each site were averaged and used in the analyses. Midarm muscle area (MMA) was calculated using the following equation:  $MMA = \frac{[MAC (cm) - \text{tricep skin fold (cm)}]^2}{4} - n$ , where, n = 10 for male and 6.5 for female. <sup>(9)</sup>

b. Subjective global assessment (SGA). <sup>(10)</sup>

**Biochemical variables:**

Serum Ghrelin level by ELIZA kit, CRP (high sensitivity), Complete blood picture, serum albumin, urea, creatinine, albumin, fasting blood glucose, calcium and phosphorus.

Blood samples were obtained from the "arterial line" of the hemodialysis before the start of session, after the patients had fasted overnight, and plasma was immediately frozen at  $-80^{\circ}\text{C}$  until analyzed. Plasma total ghrelin levels were measured by a commercial ELISA kit.

**Statistical Analysis:**

Results were expressed as mean  $\pm$  SD (standard deviation) or percentage change, as needed. The Student t test or the Mann-Whitney test was used according to normality test. The correlation coefficients between the variables were calculated using either Spearman or Pearson correlations. Statistical significance was accepted as  $p < 0.05$ . The statistical analyses were performed through the SPSS 16.0 program

**RESULTS**

In this study there was significant difference between both patient and control group in hemoglobin level ( $p < 0.001$ ) with median 8.090 in patient group and 11.50 in control one. There is no significant difference between both groups as regards WBCs ( $p = 0.601$ ), platelets ( $p = 0.244$ ) (table I) and fasting blood glucose ( $p = 0.084$ ). As regards urea ( $p < 0.001$ ), creatinine ( $p < 0.001$ ) (table II), albumin ( $p = 0.002$ ), calcium ( $p < 0.001$ ) and phosphorus ( $p < 0.001$ ) (table III) levels there was significant difference between both groups.

As regards CRP there was significant difference between both healthy and patient group with increasing values in the last one ( $p = 0.001$ ) (table III)

According to our results there is decrease in mid arm circumference and increase in the triceps skin

fold in patients' group than the control on ( $p = 0.001$  and  $p < 0.001$  respectively). (table IV)

found that ghrelin levels were much higher in patients group more than control healthy ones with ( $p = 0.002$ ) (table V).

We found that patients with severe malnutrition have higher ghrelin and CRP levels than patients with mild and moderate malnutrition (table VI).

**Table (1)** Comparison between two studied groups according to CBC

	Patient (n = 25)	Control (n = 20)	t	p
HB				
Min. – Max.	7.60 – 13.30	10.0 – 13.50		
Mean $\pm$ SD.	9.31 $\pm$ 1.50	11.57 $\pm$ 0.98	5.787*	<0.001*
Median	8.90	11.50		
WBCs ( $\times 10^3$ )				
Min. – Max.	5.0 – 12.0	6.50 – 9.80		
Mean $\pm$ SD.	8.02 $\pm$ 1.64	8.23 $\pm$ 1.01	0.527	0.601
Median	8.0	8.0		
Plat ( $\times 10^3$ )				
Min. – Max.	175.0 – 450.0	180.0 400.0	–	
Mean $\pm$ SD.	309.20 $\pm$ 85.0	280.0 78.94	$\pm$ 1.182	0.244
Median	320.0	255.0		

**Table (II):** comparison between two studied groups according to renal function.

	Patient (n = 25)	Control (n = 20)	Test of Sig.	p
Urea				
Min. – Max.	42.0 – 120.0	12.0 – 19.0		
Mean $\pm$ SD.	73.80 $\pm$ 22.97	15.80 $\pm$ 2.26	t = 12.549*	<0.001*
Median	68.0	16.0		
Creatinine				
Min. – Max.	8.0 – 12.0	0.10 – 0.80		
Mean $\pm$ SD.	9.54 $\pm$ 0.95	0.34 $\pm$ 0.21	Z = 5.649*	<0.001*
Median	9.50	0.30		

**Table (III)** Comparison between two studied groups according to different parameters

	Patient (n = 25)	Control (n = 20)	Test of Sig.	P
FBG				
Min. – Max.	60.0 – 115.0	60.0- 130.0		
Mean ± SD.	88.88 ± 14.05	83.10 ± 16.87	Z= 1.729	0.084
Median	90.0	80.0		
Albumin				
Min. – Max.	2.0 – 5.30	3.80 – 5.0		
Mean ± SD.	3.79 ± 1.0	4.53 ± 0.43	t= 3.337*	0.002*
Median	4.0	4.50		
Ca				
Min. – Max.	4.30 – 9.40	8.50 – 10.50		
Mean ± SD.	7.62 ± 1.34	9.82 ± 0.55	t= 7.440*	<0.001*
Median	7.80	9.95		
Ph				
Min. – Max.	3.20 – 9.10	2.50 – 4.0		
Mean ± SD.	5.93 ± 1.74	3.21 ± 0.44	t= 7.492*	<0.001*
Median	6.0	3.10		
CRP				
Min. – Max.	2.0 – 21.0	1.0 – 6.0		
Mean ± SD.	6.88 ± 4.69	3.15 ± 1.66	Z= 3.176*	0.001*
Median	6.0	3.0		

**Table (IV)** Comparison between two studied groups according to mid arm cir and triceps skin fold

	Patient (n = 25)	Control (n = 20)	Test of Sig.	P
Mid arm cir				
Min. – Max.	17.0 – 36.0	28.0 – 35.0		
Mean ± SD.	26.50 ± 5.24	30.85 ± 2.27	t= 3.735*	0.001*
Median	26.0	30.75		
Triceps skin fold				
Min. – Max.	11.0 – 30.0	8.0 – 22.0		
Mean ± SD.	22.08 ± 6.09	15.05 ± 5.20	Z= 3.515*	<0.001*
Median	24.0	18.0		

**Table (V)** Comparison between two studied groups according to ghrelin

	Patient (n = 25)	Control (n = 20)	Z	p
Ghrelin (pg/ml)				
Min. – Max.	200.0 – 2000.0	80.0 – 1000.0		
Mean ± SD.	691.60 ± 434.24	339.0 ± 225.29	3.164*	0.002*
Median	600.0	260.0		

**Table (VI)** Relation between SGA with ghrelin and CRP in patients group

	Well nourished (n = 8)	Mild malnourished (n = 4)	Mod malnourished (n = 8)	Severe malnourished (n = 5)
Ghrelin (pg/ml)				
Min. – Max.	260.0 – 1000.0	200.0 - 1040.0	240.0 - 800.0	620.0 – 2000.0
Mean ± SD.	583.75 ± 293.16	480.0 ± 384.71	540.0 ± 216.20	1276.0 ± 497.27
Median	500.0	340.0	580.0	1200.0
r <sub>s</sub> (p)	0.402*(0.046*)			
CRP				
Min. – Max.	2.0 – 9.0	2.0 – 8.0	3.0 – 10.0	10.0 – 21.0
Mean ± SD.	4.50 ± 2.62	4.50 ± 2.65	6.0 ± 2.67	14.0 ± 4.53
Median	4.0	4.0	5.50	12.0
r <sub>s</sub> (p)	0.636*(0.001*)			

**DISCUSSION**

Increased total ghrelin levels in CKD are primarily due to the decreased degradation of ghrelin in the kidney<sup>(11)</sup>. Nutritional status of CKD patients may also influence the metabolism

of ghrelin. Markedly elevated plasma ghrelin levels are found in advanced renal failure and correlate with fat mass.

The present study demonstrates markedly elevated plasma ghrelin levels in ESRD patients. So, our

results can be explained by the work done by Yoshimoto et al. <sup>(12)</sup> suggesting that the kidney is an important site for clearance and/or degradation of ghrelin. It could be speculated that the increased plasma ghrelin concentration observed in the present study may rather represent a physiological adaptation to a long-term negative energy balance associated with wasting in ESRD patients. Indeed, Masaoka et al. <sup>(13)</sup> recently demonstrated in streptozotocin-induced diabetes in rats that a negative energy balance may enhance ghrelin secretion into the blood stream, and we found a significant relation between plasma ghrelin levels in ESRD patients with signs of wasting (as indicated by SGA and anthropometric measurements), and plasma ghrelin level was significantly higher in patients with cachexia and muscle wasting.

Altered levels of ghrelin, one of the peptides that affect appetite and nutritional status, have been described in patients with CKD, some authors have found an inverse relation between total ghrelin and BMI in CKD patients with cachexia. In view of these findings, this profile has been suggested as a mechanism to maintain energy balance, avoiding weight loss in these patients <sup>(14,15)</sup> According to Chen *et al.*, <sup>(16)</sup> reductions in body weight increase ghrelin concentrations, while increases in body weight reduce ghrelin concentrations. Thin patients appear to have some form of ghrelin resistance, for which reason they have a tendency to develop anorexia. This fact is consistent with our findings. According to Carrero & Stenvinkel, <sup>(17)</sup> pro-inflammatory cytokines, particularly IL-6, play an important role in muscle catabolism and contribute to the onset of wasting, a condition seen in 23% to 76% of patients on hemodialysis characterized by caloric-protein depletion. This nutritional status deterioration is characterized by anorexia, high energy expenditure, low protein serum levels, loss of weight and muscle tissue. <sup>(18,19,20)</sup>

## CONCLUSION

Elevated plasma ghrelin in HD patients may represent a state of resistance to the action of this hormone at receptor level.

Elevated serum ghrelin levels and inflammation may cause diminished appetite and malnutrition in patients with ESKD maintained on hemodialysis.

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