



# Protein Energy Wasting in a Sample of Egyptian Children on Regular Hemodialysis: Relation to Anorexigenic Hormones

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## Abstract

**Edited by:** Ksenija Bogoeva-Kostovska  
**Citation:** Said RM, El Wakeel MA, El-Kassas GM, Kamhawy AH, Mostafa HH, Galal EM, Nassar MS, El-Zayat SR, Fadi NN. Protein Energy Wasting in a Sample of Egyptian Children on Regular Hemodialysis: Relation to Anorexigenic Hormones. Open Access Maced J Med Sci. 2020 Aug 25; 8(B):658-665. https://doi.org/10.3889/oamjms.2020.4981

**Keywords:** ESRD; Hemodialysis; Anthropometric parameters; Unacylated ghrelin; Obestatin.  
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**Received:** 23-May-2020

**Revised:** 01-Jul-2020

**Accepted:** 05-Jul-2020

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**Funding:** This research did not receive any financial support

**Competing Interests:** The authors have declared that no competing interests exist

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**BACKGROUND:** Increased incidence of pediatric end-stage renal disease (ESRD) with associated serious consequences indicating a major public health problem. Malnutrition and uremic wasting are leading causes of growth impairment and increasing morbidity and mortality of pediatric ESRD patients, predominantly those on regular hemodialysis (HD). Ghrelin and obestatin, which are known appetite regulatory hormones, might have a pivotal role in uremic wasting and growth impairment in hemodialyzed children.

**AIM:** The aim of the present study was to measure serum unacylated ghrelin (UAG) and obestatin and to investigate their roles in the growth impairment of Egyptian hemodialyzed children.

**SUBJECTS AND METHODS:** The study included 50 hemodialyzed and 40 healthy children recruited from the Department of Nephrology, Pediatric Hospital, Ain Shams University. Full clinical examination and measurement of anthropometric indices were done. Routine labs were done as well, with an assessment of serum levels of obestatin, UAG, and insulin by enzyme linked immunosorbent assay. Furthermore, we determined fasting serum glucose and lipid profile with the calculation of homeostasis model assessment for insulin resistance (HOMA-IR).

**RESULTS:** Anthropometric measurements were statistically significantly decreased in the hemodialyzed group than that of the control group ( $p < 0.05$ ). Weight z-score was the most affected anthropometric parameter (37 patients = 74% with underweight and 13 patients = 26% with normal weight). The hemodialyzed children showed a significant increase of UAG, obestatin, insulin, glucose, HOMA-IR, and TG, while a significant decrease of HDL-cholesterol and albumin ( $p < 0.01$ ). UAG had a negative correlation with Wt-z score, Ht z-score, fat mass %, albumin, and TG while obestatin was inversely correlated to Wt-z score, BMI z-score, waist circumference, and waist-height ratio (W/H).

**CONCLUSION:** UAG and obestatin hormones were elevated in a group of Egyptian children on regular HD. These hormones were strongly related to the impairment of renal functions, and anthropometric parameters, dyslipidemia, hypoalbuminemia, and insulin resistance in these pediatric hemodialyzed patients.

## Introduction

Chronic kidney disease (CKD) in children is a serious public health problem [1]. The overall number of pediatric patients with end-stage renal disease (ESRD) needing renal replacement therapy has increased significantly [2].

Nutritional status is particularly important in children as it influences growth, sexual, and neurocognitive development. Its accurate and routine assessment is strongly recommended in pediatric patients undergoing regular hemodialysis (HD) [3].

Protein-energy wasting (PEW) and growth retardation are typical issues in pediatric CKD associated with lower quality of life and robustly predict morbidity and mortality [4], in particular, ESRD imposing maintenance dialysis treatment [5], [6], [7].

Malnutrition contends a pivotal role in the development of stunted growth, commonly observed in

children with CKD. The pathophysiology of malnutrition and wasting in pediatric CKD is complex, multifaceted, and not yet fully established. Various factors are potentially elaborated, embracing; uremic toxins retention, metabolic acidosis, elevated circulating pro-inflammatory cytokines, insulin resistance, besides the occurrence of depression secondary to the disease per se [8], [9].

Negative energy balance is one of the virtually important interposed mechanisms of PEW, imposed by increasing energy expenditure and reduced food intake [10], [11]. That may be consequential to anorexia due to variable mechanisms, including nausea, early satiety due to delayed gastric emptying, altered food taste, and smell, in some patients, the need to drink plenty of fluid to keep up with high urine output. Perturbations in anorexigenic/orexigenic hormonal balance may be a pivotal key in the pathogenesis of PEW in children with CKD [5], [12].

Ghrelin and obestatin are appetite regulatory hormones, originated from the same ghrelin gene

(GHRL), and produced by the stomach [13]. Their potential roles in uremic PEW have been investigated in adults [14], [15], [16] and, to a lesser extent, in pediatric CKD [17], [18], [19], with contradictory issues.

Ghrelin is a 28-amino acid peptide hormone secreted primarily by the oxyntic cells of the stomach. It is principally metabolized and excreted through the kidneys. Ghrelin is known as one of the most important appetite-regulating hormones. Two major circulating ghrelin forms detected; acyl ghrelin (AG), the active form, with orexigenic effect and the unacylated ghrelin (UAG), the main circulating ghrelin form, representing >90%, which appears to have the ability of increasing energy expenditure by declining food intake through the hypothalamus [20].

Obestatin (ghrelin-associated peptide) produced by the stomach from the same ghrelin gene, also recognized to have a homologous anorexigenic effect, fullness sensation, as well as slowing peristalsis [21].

To our knowledge, up till now, no studies have been done assessing the role of the hormones regulating energy homeostasis in Egyptian children with CKD, so in this study, we aimed to measure serum levels of UAG and obestatin in a group of Egyptian children with CKD on regular HD and to investigate their association with the anthropometric parameters in these hemodialyzed children.

## Patients and Methods

This cross-sectional case-control study had been conducted on fifty children with an age range between 5 and 16 years, suffering from chronic renal failure, of variable durations on regular HD therapy, they have been recruited from the Department of Nephrology, Pediatric Hospital, Ain Shams University. Forty age- and sex-matched healthy children had been included as a control group. Exclusion criteria were treatment with growth hormone and/or the presence of neurologic disability or syndromes affecting per se food intake, patients with severe conditions such as generalized inflammation or end-stage malignant diseases.

Full history had been taken from the parents, including the original renal disease, previous interventions for renal problems, concomitant medications, child's previous growth and development, precise timing, and sequence of the physical milestones. Clinical examination and anthropometric indices have been evaluated. Height was measured by the Harpenden stadiometer in centimeters. Weight was recorded in kilograms using an electronic weight scale. Body mass index (BMI) had been calculated by weight in kg/(height in meters)<sup>2</sup>. Z scores of height for

age, weight for age, and BMI were calculated using AnthroPlus Program for personal computers based on the WHO growth standards [22]. Waist circumference (at the smallest point between the rib cage and the iliac crest) has been measured in centimeters using a flexible inextensible tape, and then waist/height (WHR) had been calculated. Biceps, triceps, subscapular and suprailliac skinfold thicknesses were measured by Holtain skinfold calipers. The percentage of fat mass (FM %) was calculated according to the Siri equation [23]. The parents of all participating children signed a written informed consent before taking part in the study, after explanation of the study's objectives.

About 3 ml of fasting 12 hours venous blood samples were withdrawn from all subjects, centrifuged at 3000 r.p.m. for 10 min and sera were separated then, stored at -20°C until laboratory analysis. Serum creatinine was determined also on Hitachi AutoAnalyzer, Hitachi 736 (Roche Diagnostics GmbH, D-68298 Mannheim, USA) using colorimetric techniques. Serum albumin was determined using the bromocresol green method (Ortho-clinical Diagnostics Inc., Rochester, NY, USA). Serum levels of obestatin and UAG were measured using a commercial enzyme-linked immunosorbent assay (ELISA) kit, produced by Glory Science Co., Ltd. 2400 Veterans Blvd. Suite 16-101, Del Rio, TX 78840, USA. The detection range of the obestatin kit was 20 ng/L–400 ng/L, catalog number #:17526. The detection range of the ghrelin kit was 3 pg/ml–200 pg/ml catalog number #: 95622. The serum concentration of insulin was quantitatively determined by ELISA kit produced by (Chemux Bioscience, Inc, USA), a commercial kit of insulin was processed according to the manufacturer's instructions. The assay sensitivity was found to be 2.0 µIU/ml. Fasting serum glucose, total cholesterol, and triglycerides (TG) were measured on BioSystems BTS-302 photometer by an enzymatic colorimetric method using the Bio-Diagnostic kit (Egypt) [24]. HDL-cholesterol (HDL-C) was measured by the precipitation endpoint method, the supernatant was separated, and HDL-C was determined using the same method for total cholesterol described above. Serum LDL-cholesterol (LDL-C) levels were calculated using the Friedewald formula (LDL-C = Total cholesterol–HDL-C–[TG/5]) [25]. Insulin resistance was calculated by the homeostasis model assessment for insulin resistance (HOMA-IR) by the formula (fasting insulin [mIU/ml] × fasting glucose [mg/dl]/405). Considering insulin resistance if HOMA-IR index exceeding 3.16 [26].

### Statistical analysis

Data were analyzed using IBM SPSS Statistics v. 22. Independent t-test was used for comparison between quantitative data of the two studied groups; normally distributed variables have been expressed as mean ± SD. The correlation between two quantitative parameters in the same group was assessed by

Pearson correlation analysis. Statistical significance was considered when  $p < 0.05$ .

## Results

The current study included 50 hemodialyzed children and 40 age- and sex-matched healthy Egyptian children as a control group, their mean age was ( $12.8 \pm 3.01$  and  $13.5 \pm 2.6$  years), respectively. According to primary renal disease, 12 (24%) patients had post renal cause, while 38 (76%) patients had intrinsic renal disease. Their anthropometric measurements as shown in Table 1 indicating statistically significant decrease in the hemodialyzed group than that of the control group (all  $p < 0.05$ ), except for waist-height ratio.

**Table 1: Comparison between cases and controls as regards anthropometric measures**

Variables	Hemodialyzed children (n=50) Mean $\pm$ SD	Control (n=40) Mean $\pm$ SD	p-value
Age (years)	12.8 $\pm$ 3.01	13.5 $\pm$ 2.6	0.306
Wt-z score	-3.00 $\pm$ 1.72	0.05 $\pm$ 1.11	<0.001**
Ht z-score	-2.87 $\pm$ 1.60	1.38 $\pm$ 0.42	<0.001**
BMI z-score	-1.41 $\pm$ 1.25	1.38 $\pm$ 0.42	<0.001**
Waist circumference	62.78 $\pm$ 8.0	79.8 $\pm$ 11.10	0.003**
Waist-height ratio (W/H)	0.48 $\pm$ 0.07	0.45 $\pm$ 0.04	0.3
Biceps-SFT (mm)	5.11 $\pm$ 1.61	8.42 $\pm$ 2.45	0.004**
Triceps-SFT (mm)	7.05 $\pm$ 2.20	10.10 $\pm$ 2.58	0.01*
Subscapular-SFT (mm)	6.38 $\pm$ 2.50	8.95 $\pm$ 2.78	0.02*
Suprailiac-SFT (mm)	5.68 $\pm$ 2.72	8.89 $\pm$ 2.95	0.005**

\*( $p < 0.05$ ): Hemodialyzed cases significantly different from controls. \*\*( $p < 0.01$ ) Hemodialyzed cases highly significant difference from controls. SD: Standard deviation, Wt-z score: Weight for age z score, Ht z-score: Height for age z score, BMI: Body mass index, SF: Skinfold.

Biochemical features of the studied groups shown in Table 2 with a statistically significant increase of the levels of UAG, obestatin, insulin, glucose, HOMA-IR, and TG, while a significant decrease of HDL-C and albumin in the studied hemodialyzed children than that of the control group ( $p < 0.05$ ).

**Table 2: Comparison between cases and controls as regards biochemical features**

Variables	Hemodialyzed children (n=50) Mean $\pm$ SD	Control (n=40) Mean $\pm$ SD	p-value
Unacylated ghrelin (pg/ml)	111.45 $\pm$ 31.15	87.59 $\pm$ 28.47	0.002**
Obestatin (ng/L)	223.73 $\pm$ 88.72	145.31 $\pm$ 19.78	<0.001**
Insulin ( $\mu$ U/ml)	22.45 $\pm$ 12.76	9.46 $\pm$ 12.56	<0.001**
Glucose (mg/dl)	112.99 $\pm$ 31.84	71.2 $\pm$ 12.85	<0.001**
HOMA-IR	6.08 $\pm$ 4.53	1.73 $\pm$ 2.39	<0.001**
Total cholesterol (mg/dl)	97.57 $\pm$ 47.47	95.00 $\pm$ 11.87	0.744
TG (mg/dl)	162.4 $\pm$ 74.54	86.6 $\pm$ 22.2	<0.001**
HDL-cholesterol (mg/dl)	53.91 $\pm$ 8.97	71.00 $\pm$ 22.2	<0.001**
LDL-cholesterol (mg/dl)	45.27 $\pm$ 36.1	38.27 $\pm$ 7.51	0.239
Albumin (g/dl)	3.28 $\pm$ 0.54	4.89 $\pm$ 0.9	0.03*

\*( $p < 0.05$ ): Hemodialyzed cases significantly different from controls. \*\*( $p < 0.01$ ) Hemodialyzed cases highly significant difference from controls.

There were inverse statistically significant correlations between UAG with Wt-z score (Figure 1), Ht z-score and FM %, and also between obestatin with Wt-z score, BMI z-score (Figure 2), waist circumference, and waist-height ratio (W/H). A statistically significant negative correlation between UAG and albumin was detected. Both UAG and obestatin showed a statistically significant positive correlation with predialysis creatinine (Table 3).

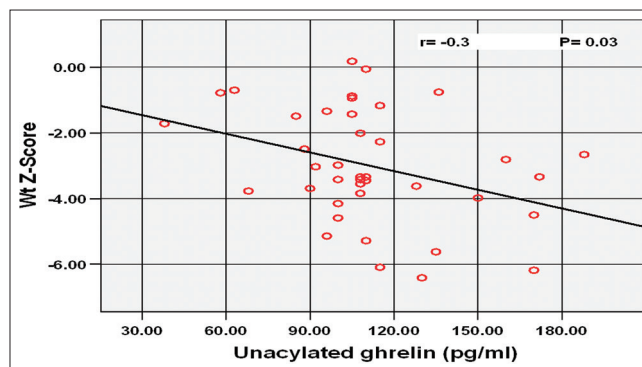


Figure 1: Correlation between unacylated ghrelin and Wt-z score in hemodialyzed group

## Discussion

The current study assessed the relationship between anthropometric parameters and serum levels of UAG and obestatin in a group of Egyptian children with ESRD on regular HD to investigate the association between these anorexigenic hormones with growth retardation and wasting of these pediatric hemodialyzed patients.

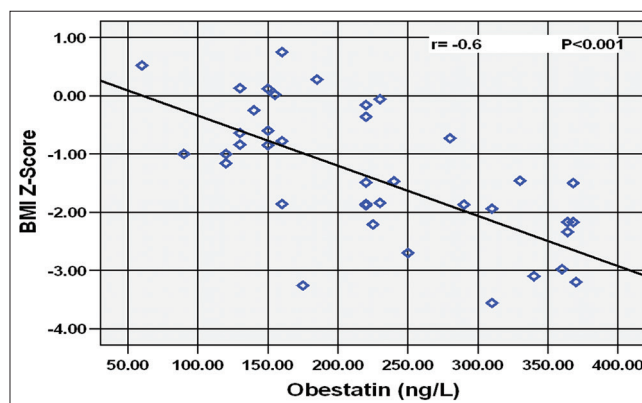


Figure 2: Correlation between obestatin and BMI z-score in hemodialyzed group

As regarding the anthropometric measurements, a statistically significant decline of all anthropometric indices was observed in hemodialyzed children when compared with their healthy peers,

**Table 3: Correlations between serum unacylated ghrelin, obestatin, and other variables**

Variables	Unacylated ghrelin (pg/ml)		Obestatin (ng/L)	
	r	p	r	p
Wt-z score	-0.343	0.03 (*)	-0.434	0.005 (**)
Ht z-score	-0.452	0.003 (***)	-0.140	0.389
BMI z-score	-0.008	0.959	-0.612	0.000 (**)
Waist circumference	-0.053	0.746	-0.328	0.039 (*)
Waist-height ratio (W/H)	0.025	0.878	-0.456	0.003 (**)
Fat mass (FM %)	-0.511	0.002 (*)	-0.295	0.06
Creatinine	0.402	0.01 (*)	0.373	0.02 (*)
HOMA-IR	-0.066	0.725	-0.110	0.554
Total cholesterol (mg/dl)	0.077	0.637	-0.005	0.975
TG (mg/dl)	-0.335	0.035 (*)	0.002	0.989
HDL-cholesterol (mg/dl)	-0.003	0.986	-0.023	0.890
LDL-cholesterol (mg/dl)	-0.180	0.267	0.037	0.820
Albumin (g/dl)	-0.462	0.03 (*)	-0.136	0.354

\*\*Correlation is significant at the 0.01 level (2-tailed). \*Correlation is significant at the 0.05 level (2-tailed).

except for waist-height ratio that showed an increase, the ratio might be influenced by a severe decline in the height parameter than waist circumference of the hemodialyzed group. Weight z-score was the most affected anthropometric parameter (37 patients = 74% with underweight and 13 patients = 26% with normal weight). Many studies came in agreement with our global finding of impaired anthropometrics but with some differences about the details due to different CKD series, and age groups with different development stages. In the same context of our findings, receiving dialysis treatment was associated with being underweight [27]; similarly, underweight was predominant in the hemodialyzed group [19], while percentages of overweight increased in CKD with renal transplant (maybe a drug effect due to corticosteroids administration, besides, appetite recovery after renal transplant therapy) [27]. In the contrast, the height was the most affected anthropometric parameter than weight in some studies [28], [29], [30].

In the present study, obestatin and UAG were statistically significantly higher in hemodialyzed children than control ( $223.73 \pm 88.72$  vs.  $145.31 \pm 19.78$ ,  $p < 0.001$ ) and ( $111.45 \pm 31.15$  vs.  $87.59 \pm 28.47$ ,  $p = 0.002$ ), respectively. On the same context, Monzani *et al.* [19] who measured ghrelin (acylated and unacylated) and obestatin in 110 pediatric CKD (42 on conservative treatment, 20 on HD, and 48 transplantation recipients) and 43 controls, recording elevated UAG and obestatin concentrations in uremic children, notably in hemodialyzed than non-dialyzed CKD patients and controls. Furthermore, increased total ghrelin with alternated AG/total ghrelin ratio (denoting elevation of UAG) in patients with ESRD undergoing dialysis was previously reported in many studies [31], [32], [33], [34], [35].

Both UAG and obestatin hormones showed a statistically significant positive correlation with predialysis levels of serum creatinine in the present study, confirming preceding findings have been reported by many researchers, with a suggested trend to increase of these anorexigenic hormones with the progression of renal impairment [19], [31], [36], [37].

Some researchers suggested that elevated levels of anorexigenic hormones such as obestatin and UAG observed in children with CKD on HD could be part of a defensive strategy to prevent the accumulation of toxic metabolites, which are normally cleared through the kidneys [19], prolonged half-life due to reduced renal clearance of these hormones under uremic conditions [38]. In addition, alterations in the activity of the enzymes involved in ghrelin deacylation, as uremic dyslipidemia may impair their function [39]. It may be ascribed to the inflammatory and substandard nutritional status of uremic patients [35].

Some studies were controversial, by their revealed normal or even subnormal serum levels of ghrelin in CKD patients on dialysis [14], [15], a possible clarification for this controvert might be that only UAG

levels were elevated in the CKD patients. For instance, Gupta *et al.* [37] measured serum levels of AG and UAG levels in 51 CKD and 15 hemodialyzed patients, then demonstrated increased UAG levels with declining estimated glomerular filtration rate, while serum levels of AG kept unchanged.

On the other side, the study of Mafra *et al.* [40] has revealed low levels of obestatin and high levels of des-AG in hemodialyzed patients. However, lean patients had elevated levels of obestatin and des-AG. Despite, obscured by the unchanged obestatin levels after a meal in patients on maintenance HD, the relationship between sense of fullness and obestatin detected by Beberashvili *et al.* [41] might stipulate obestatin to behave as an anorexigenic. Up till now, there is controversy about the level of obestatin and its potential role, as an anorexigenic hormone, in the regulation of appetite and long-term food intake besides, its influence on the nutritional status and anthropometry in the hemodialyzed population [21], [40], [41], [42].

Despite, the variability of obestatin and UAG levels in CKD [9], [19], [34], [43], it has been postulated that disparity of these appetite-regulating hormones might assist in the defective appetite and PEW in uremic patients [44], [45], by an anorexigenic effect [33], as a compensatory response reflecting imbalanced energy status [46].

The current study revealed statistically significant negative correlations between UAG with Wt-z score, Ht z-score, and FM %, also between obestatin with Wt-z score, BMI z-score, waist circumference, and waist-height ratio (W/H), so both anorexigenic hormones are found to be inversely related to the anthropometric indices, besides, the statistically significant inverse relationship between UAG and albumin in these uremic pediatric patients.

To some extent, the findings of previous researches came in accordance with our conclusion about the negative correlation between the ghrelin system and the anthropometric parameters in CKD patients [35], [47], and healthy children [48]. Similarly, the inverse correlations between obestatin, BMI ( $r = -0.40$ ,  $p = 0.007$ ), and waist circumference ( $r = -0.38$ ,  $p = 0.024$ ) reported by Mafra *et al.* [40].

Furthermore, Monzani *et al.* [19] gave accentual evidence concerning the negative correlation between obestatin, UAG, and the anthropometric parameters (negative association between UAG with weight-SDS and BMI-SDS, also between obestatin with weight-SDS). These results help them to consider the hormones of UAG and to less extent, obestatin as promising inverse biomarkers of nutritional state in pediatric CKD patients, while on the contrast of our findings, they did not detect any relations between these two hormones and waist circumference, waist-height ratio, or serum albumin.

In another respect, the reported results of the studies done by Eftekhari *et al.* [18] and Monzani *et al.* [19]

had confirmed our findings concerning the negative correlation between UAG and FM %. They suspected UAG to be considered as a promising biomarker of muscle-wasting, known to be associated with deleterious long-term consequences in uremic subjects, even in conditions with normal BMI records [49].

In the present study, hypoalbuminemia (serum albumin <3.5 mg/dl) have been detected in CKD patients on HD. Serum albumin was considered as the most common nutritional marker in HD patients, strongly predicting mortality [50]. However, this was criticized by Stenvinkel *et al.* [51] due to its close link to other non-nutritional factors, mostly infection and inflammation [52].

There are strong existed data regarding dyslipidemia as a common finding in CKD patients, particularly those on maintenance HD, and its contribution to the risk of the high prevalence of cardiovascular disorders in these patients [53], [54].

Principally, dyslipidemia of CKD incorporated increased TG and decreased HDL-C levels [55], [56], this came in agreement with our findings of statistically significant increase of TG levels while a significant decrease of HDL-cholesterol in the studied hemodialyzed children than that of the control group ( $p < 0.01$ ), with a non-significant increase of total cholesterol and LDL-C in the hemodialyzed group. Furthermore, previous studies reported similar findings [57], [58].

In the current study, no correlations have been detected between measured obestatin or UAG hormones and any of the lipid profile parameters, except for, the statistically significant negative correlation found between UAG and TG in the hemodialyzed group ( $r = -0.335$ ,  $p = 0.035$ ), otherwise, Monzani *et al.* [19] reported a statistically significant positive correlation between obestatin and TG ( $r = 0.195$ ,  $p = 0.02$ ).

On the other hand, no correlations between ghrelin and lipid profile have been detected by some researchers [35], [47], [59]. Otherwise, some experimental studies supposed the ability of obestatin to reduce serum lipids through reduction of food intake and intestinal absorption of TG, besides stimulating leptin secretion and fatty acid uptake [60], [61], [62].

In the current study, HOMA-IR was statistically elevated in hemodialyzed patients with a non-significant negative correlation with obestatin and UAG. Moreover, insulin resistance was found to be a common finding in patients on maintenance HD even without diabetes [41], [63], Barazzoni *et al.* [64], [65] reported a strong negative association between UAG and HOMA-IR in their studies. In addition, Cappellari *et al.* [66] suggested an improvement of insulin activity due to overexpression of UAG in obesity-induced insulin resistance through reducing reactive oxygen species and increasing autophagy, with concomitant improvement of tissue inflammation.

Despite, the strong positive association between UAG and obestatin detected by Monzani *et al.* [19]

and previously by Mafra *et al.* [40] suggesting clear association of the two hormones with common post-translation regulation and a strong appetite management mechanism. On the contrast, this strong association could not be validated by our results due to the positive correlation between the two hormones was statistically non-significant. Ongoing the relationship between UAG and obestatin and their effect on the nutritional status and growth of CKD patients, especially those on maintenance HD still under debate and need more research.

## Conclusion

The present study detected elevated serum levels of UAG and obestatin in hemodialyzed children compared to controls. Both hormones were negatively related to the anthropometric parameters, suggesting their link to the nutritional and growth impairment of these growing patients.

Further studies are needed to detect the role of the appetite regulators in CKD patients and their link to nutritional and growth impairment. This may help to provide reliable innovatory biomarkers for the diagnostic and therapeutic presumption of nutritional and growth defects in pediatric patients with CKD.

We recommend regular assessment of anthropometric and biochemical nutritional parameters in pediatric CKD, especially those on HD with regular follow-up by specialized dietitians.

## Acknowledgment

The authors want to give thanks to all participants in the study (children and their families, lab, and clinic staff members) for their assistance and efforts.

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