Original Article

Ghrelin and its Association with Nutritional and Inflammatory Status of Patients on Maintenance Hemodialysis in a South Indian Tertiary Care Hospital

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Abstract

Background: Malnutrition and inflammation are associated with morbidity and mortality in patients on maintenance hemodialysis (MHD). Ghrelin, an orexigenic peptide hormone, is speculated to be associated with nutritional and inflammatory status in MHD. **Aim:** To assess the serum total ghrelin levels and its possible relationship with inflammation and nutritional status in patients on MHD. **Subjects and Methods:** The study was conducted on 90 patients on MHD for 6 months and above (56 males, 34 females, mean age 52.6 [11.7] years; mean dialysis vintage 20.9 [12.1] months) and 70 healthy volunteers as control (5 males, 25 females, mean age 50.6 [9.7] years). Demographics were obtained for the study population, and dialysis-related data were collected for cases. Anthropometry, biochemical parameters, serum total ghrelin and inflammatory markers tumor necrosis factor-alpha (TNF-α), and high-sensitivityC-reactive protein (hsCRP) were assessed for cases and control. Self-reported appetite (five questions of appetite and diet assessment tool) and nutritional status (subjective global assessment-dialysis malnutrition score) were assessed for cases. **Results:** Ghrelin (242.5 [62.3] pg/mL vs. 80.2 [19.6] pg/mL; $P < 0.001$), TNF-α (39.8 [15.2] pg/mL vs. 6.5 [1.2] pg/mL; $P < 0.001$), hsCRP (10.2 [2.8] mg/L vs. 2.7 [0.54] mg/L; $P < 0.001$) were significantly elevated in cases versus control, anthropometry, and biochemical parameters were significantly decreased in hemodialysis patient. Of 90 cases, (13/90 [14.4%]) were well-nourished, (28/90 [31%]) mild to moderately malnourished, and (49/90 [54.4%]) were moderate to severely malnourished. Appetite was very good for14.4%, good and fair for 47.8%, poor and very poor for 37.8% patients. There was a significant difference in appetite with respect to nutritional status ($P < 0.001$). Ghrelin had positive correlation with inflammatory markers and negative correlation with nutritional status ($P < 0.001$). **Conclusion:** The study identified the association of ghrelin with appetite, nutritional, and inflammatory status of the patients on MHD.

Keywords: Appetite, Ghrelin, Hemodialysis, Inflammation, Nutritional status

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Introduction

Protein-energy malnutrition (PEM) is the state of decreased body pools of protein with or without fat depletion or decline in functional capacity, induced partly by inadequate nutrient intake relative to nutrient demand and/or which is improved by nutritional repletion. PEM is a common phenomenon in maintenance dialysis patients, a majorrisk factor for poor quality of life andincreased morbidity and mortality inclusive of cardiovascular death.[1] Various studies have established the prevalence of PEM between 18% and 75% in patients undergoing chronic hemodialysis.[2,3]

The causes of malnutrition in dialysis patients are multifactorial. Appetite, i.e., the subjective desire to ingest food, is diminished in many dialysis patients.[3] Anorexia or loss of desire to eat contributes largely to malnutrition and affects thequality of life in hemodialysis patients.[4] As the disease progresses, anorexia also increases and lead to metabolic disturbances, protein-energy wasting, cachexia all resulting in high rates of morbidity and mortality. The pathogenesis of anorexia in hemodialysis is essentially unknown. It was proposed that uremic toxins (e.g., middle molecules, proinflammatory cytokines, altered amino acids, hormones (e.g., leptin and ghrelin), and neuropeptides (e.g., neuropeptide Y [NPY], peptide [P]) are involved.[5]

PEM often overlaps with inflammation leading to malnutrition-inflammation syndrome. The key role in these events is played by proinflammatory cytokines such as interleukins-1 (IL-1), IL-6, and tumor necrosis factor-alpha (TNF-α). A strong and consistent association between anorexia and high levels of inflammatory markers has been shown in previous studies. Cytokines may inhibit feeding by causing not only nausea and vomiting but also decreased gastric motility and emptying or by modifying gastric acid secretion.[6,7]

Ghrelin is a recently identified 28 amino acid peptide hormone which is produced by the endocrine cells of the stomach. It was the first peripheral orexigenic hormone identified.[8,9] Ghrelin is presently regarded as the only known circulating orexigen and exerts antagonistic effects on the leptin-induced decrease in food intake through activation of the hypothalamic NPY-Y1 pathway.Ghrelin is released under conditions of negative energy balance and is an initiating signal for food intake. Ghrelin and its receptor are expressed in the arcuate nucleus of the hypothalamus, a potent appetite controlling center. A recent study reported the presence of ghrelin in previously uncharacterized hypothalamic neurons adjacent to the third ventricle. These ghrelin-containing neurons send efferent fibers to neurons that contain NPY and agouti-related peptide and may stimulate the release of these orexigenic peptides and suppress the release of pro-opiomelanocortin, which inhibits food intake.[10-12]

Ghrelin increases food intake and was reported to be elevated in patients with chronic kidney disease (CKD). Conflicting results of circulating ghrelin levels in CKD have been presented. Elevated plasma ghrelin levels were observed in adult dialysis patients than those of age-matched controls.[13-15] Yoshimoto *et al*. reported 2.8-fold higher ghrelin and desacyl ghrelin levels in patients with renal failure, but data on the association between ghrelin levels and body composition in end-stage renal disease (ESRD) patients has not been much reported in the literature.[16]

Likewise, though some studies have reported a strong association between ghrelin and inflammatory markers in endotoxemic dogs,[17] little is known about the function and levels of ghrelin during inflammation. Studies of ghrelin levels appear to be important in maintenance hemodialysis (MHD) patients because ghrelin may be implicated in the appetite dysregulation, inflammation, and malnutrition, which are interlinked and are frequently observed in these patients. Hence, a study was conducted to characterize the serum total ghrelin levels and its relation with nutritional and inflammatory status in patients undergoing chronic hemodialysis.

Subjects and Methods

A case–control study was conducted for a period of 1 year (September 2013 to August 2014), after obtaining the approval of the Institutional Ethics Committee and the consent of the study participants.

Study population

Cases

Patients undergoing MHD were continuously screened for eligibility as per the following criterion. About 90 patients with eligibility criteria and consented to participate in the study were included as case population.

Inclusion criteria

- Patients above 18 years of age, undergoing hemodialysis for at least 6 months
- Patients on oral diet
- Patients undergoing twice/thrice weekly MHD.

Exclusion criteria

- Patients with chronic systemic inflammatory diseases, smoking history, and known malignancies
- Patients with acute illness and overt infectious complications
- Patients on concurrent use of glucocorticoids and other immunosuppressive agents
- Patients on enteral or parenteral nutrition.

Control

A population-based group of 70 subjects of either sex, aged above 18 years, with no known significant health problems or illness and are confirmed to be medically fit, who accepted to

participate as volunteers were randomly selected and included as controls for comparative reasons.

Sample size was calculated based on the literature.[18,19] The expected alpha error probability to detect the difference in mean serum ghrelin levels between hemodialysis patients and healthy controls was estimated to be about 0.05, and the assumed odds ratio was 5. Assuming 95% confidence level and 81% power, the minimum sample size required was 70 per arm (70 cases and 70 controls, total = 140). The calculated sample size of the case population was 90 (15% increase in the sample size than the control) in order to evade attrition.

Data collection

A detailed history elucidation including the demographic data (age, sex, weight in kg, height in cm), were obtained for both cases and control. Further details on comorbidities, pre-and post-dialytic blood pressure, duration of dialysis, total number of dialysis sessions, interdialytic weight gain, and ultrafiltration (L) were obtained from the patients' medical records. The dialysis adequacy (Kt/V) was determined from pre-and post-dialytic blood urea nitrogen (BUN) levels and the pre-and post-dialysis weights as described byDaugirdas.[20] The normalized protein catabolic rate (nPCR) was estimated using a simple formula:[7] nPCR (g/kg/day) = (0.0136 × [Kt/V × ([predialysis BUN + postdialysis BUN]/2)]) +0.251. The urea reduction rate (URR) was calculated using the formula:[21] 100 (1post-BUN/pre-BUN).

Blood sample collection

To execute the study specific investigations, 5 mL of venous blood samples were collected from the case and the control groups after overnight fast, placed in appropriate tubes for separation of serum, and then stored at −70°C until analyzed for serum ghrelin and inflammatory cytokines TNF-α and high-sensitivity C-reactive protein (hsCRP) levels. For the case group, the blood samples were obtained before the initiation of the dialysis session.Estimation of the following was done using commercial kits according to the manufacturer's instructions:

- Serum ghrelin by enzyme-linked immunosorbent assay (ELISA) using DRG International, Inc., USA, human ghrelin (total) ELISA Kit. The minimum detectable dose of ghrelin was 30 pg/mL
- Serum TNF-α by ELISA using DRG International, Inc., USA. Human TNF-α ELISA Kit. The minimum detectable dose of TNF-α is typically <12 pg/mL
- Serum hsCRP by immunoturbidimetric method using Daiichi kit (Daiichi pure Chemicals Co. Ltd., Tokyo, Japan).

The other biochemical parameters estimated for the case and the control groups included hemoglobin, packed cell volume, BUN (pre- and post-dialytic levels for cases), creatinine, albumin, total protein, and globulin. Further for the case group alone, serum iron, ferritin, transferrin, and total iron binding capacity (TIBC) were also obtained and all done by routine laboratory methods.

The anthropometric measurements were performed for both case and the control groups (between 10 and 20 min after termination of dialysis session for the cases), and the following assessments were done to obtain the body composition. The assessments were made by the same individual with the guidance of the clinical nutritionist using the same equipment to avoid errors during measurement:

- Body mass index (BMI)[22] – calculated using the formula body weight in kg/height in m^2. In cases, dry body weight (the weight obtained by the end of dialysis without causing hypotension and/or cramps) was considered for calculation of BMI
- Mid-arm circumference (MAC)[23] – measured with a plastic tape (on the nondialysis access arm for cases) for 3 times and average result of the three measurements was taken as final result
- Triceps skin fold thickness (TSF)[23] – measured with a conventional skinfold caliper (Harpenden calipers) using standard techniques (on the nondialysis access arm for cases) for 3 times and average result of the three measurements was taken as final result
- Mid-arm muscle circumference (MAMC)[23] was calculated from the formula:
  MAMC = MAC −(3.1416 × TSF)
- Mid-arm muscle area (MAMA)[23] is an estimation of the area of the bone and muscle portions of the upper arm. It was calculated using the formula:
  MAMA = (MAC [cm] –[30.14 × TSFcm])^2 – 10 (males) or – 6.5 (females)/4π
- Mid-arm fat area (MAFA):[24]
  MAFA = (MAC × TSF)/2− π (TSF)^2/4
- Lean body mass (LBM)[25] is an estimation of difference between the total body mass (weight in kg) and weight of the body fat. LBM was obtained using the formula:
  LBM in kg (men) = (1.10 × weight [kg]) −128 × (weight^2/[100 × height (m)^2])
  LBM in kg (women) = (1.07 × weight [kg] −148 × (weight^2/[100 × height (m)]^2)
- Ideal body weight (IBW) was calculated using Devine formula:[26]
  IBW in kg (men) =50 kg + 2.3 kg × (height [in] −60)
  IBW in kg (women) =45.5 kg + 2.3 kg × (height [in] −60)
- Total body water (TBW) gives the urea volume of distribution. It was calculated from the formula by Watson *et al*.:[27]
  Male TBW (Liters) =2.447− (0.09156 × age) + (0.1074 × height) + (0.3362 × weight)
  Female TBW (Liters) = −2.097+ (0.1069 × height) + (0.2466 × weight)
- Fat-free mass (FFM): Calculated as follows:[28]
  FFM = TBW/0.72.

- Total body fat (TBF): Calculated as follows:[28]
  TBF (kg) = Weight − FFM

Nutritional and appetite assessments in case population

The case group alone was subjected to five questions of appetite and diet assessment tool (ADAT)[29] for the assessment of self-reported appetite and subjective global assessment-dialysis malnutrition score (SGA-DMS)[30] for the assessment of nutritional status. These questionnaires were answered by the cases either at the time of or within a week of blood sample collection. The questionnaires were answered independently by the patients after explanation of those tools by the investigator. Those who required assistance were assisted by the investigator.

ADAT is a 44 item, self-administered questionnaire divided into three sections. Questions are about the patient's general level of appetite, recent changes in dry weight, dietary compliance, need for assistance with food shopping and meal preparation, common food practices, and the patient's perceptions of food enjoyment and diet satisfaction. The answers to the appetite questions are scored as (1) very good, (2) good, (3) fair, (4) poor, and (5) very poor, based on Likert five-point grading scale. Five questions of ADAT are directly related to appetite. The first question focuses on self-rating of the appetite during the past week. The second and third questions focus on the change in appetite in the past week and if so, had appetite increased, remained the same or decreased. The fourth and the fifth questions focus on the appetite levels of the patients on dialysis and non-dialysis days respectively. The study patients were made to answer the five questions of the ADAT, and the appetite levels of the study patients were graded based on their response. Based on their appetite status, patients were categorized into three groups as very good, good, and fair, poor and very poor.

As the ADAT questionnaire was not used in the Indian population to our knowledge, the consistency and the internal reliability of the ADAT questionnaire in the study population was assessed by Cronbach's alpha, and a value of 0.732 was obtained (a value of above 0.6 indicated high internal reliability of the scales).

SGA-DMS is a fully quantitative scoring system consisting of seven features: Weight change, dietary intake, gastrointestinal symptoms, functional capacity, comorbidity, subcutaneous fat, and signs of muscle wasting. Each component has a score from 1(normal) to 5 (very severe). Thus, the malnutrition score (sum of all seven components) is a number between 7 (normal) and 35 (severely malnourished). Therefore, a lower score denotes tendency toward normal nutritional status. A higher score, however, is considered to be an indicator of the presence of malnutrition elements that is the higher the nutritional score the stronger the tendency toward protein calorie malnutrition. In the present study, patients with a score of 7–10 were considered as well-nourished, 11–20 as mild to moderately malnourished, and 21–35 as moderate to severely malnourished.

Statistical tests

The analysis was performed using Statistical Package for Social Sciences, (SPSS, Version 16.0, Chicago, IL, USA). Categorical variables were expressed as frequency and percentage and continuous variables were expressed as mean (standard deviation). Independent *t*-test was used to analyze the statistical difference in serum levels of ghrelin and inflammatory markers TNF-α, hsCRP, anthropometry, and other biochemical parameters between case and control population. The statistical analysis of differences in anthropometric and biochemical data, serum levels of ghrelin, and inflammatory markers TNF-α and hsCRP in case population with respect to their appetite status as per ADAT and nutritional status as per SGA-DMS were done using one-way analysis of variance with Tukey's *post hoc* test. Pearson's correlation was used to analyze the association between serum ghrelin concentrations, inflammatory markers, and nutritional status. A $P < 0.05$ was considered statistically significant.

Results

Characteristics of the study population

The study was conducted in 90 patients (55/90 [61%] males and 35/90 [39%] females) undergoing twice/thrice weekly MHD and 70 healthy volunteers as control (45/70 [64%] males and 23/70 [36%] females). The age range of the cases was 18–73 years and the mean age was 52.6 (11.7) years. Similarly, the age range of the control was 19–67 years and the mean age was 50.4 (10.3) years. Majority of the study subjects were in the age range of 46–65 years (65/90 [68.9%] in cases and 45/70 [66.8%] in control). There was no significant difference in the age ($P = 0.74$) and gender distribution ($P = 0.22$) of the case and the control population [Table 1]. The primary causes of renal disease cases were diabetic nephropathy in (39/90 [43.3%]) patients, hypertensive nephropathy in (27/90 [30%]), polycystic kidney disease in (11/90 [12.2%]), glomerulonephritis in (9/90 [10%]), pyelonephritis in (3/90 [3.4%]), and neurogenic bladder in (1/90 [1.1%]) patients.

Table 1: Demographics of cases and control

Characteristics	Cases (*n*=90) (%)	Control (*n*=70) (%)	Total (*n*=160)	*t*-test (*P*)
Males	55 (61)	45 (64)	100	
Females	35 (39)	25 (36)	60	0.74 (NS)
Age range (years)				
≤35	8 (8.9)	7 (10)	15 (9.4)	0.22 (NS)
36-45	9 (10)	14 (20)	23 (14.4)	
46-55	35 (38.9)	20 (28.6)	55 (34.4)	
56-65	27 (30)	25 (35.7)	52 (32.4)	
>65	11 (12.2)	4 (5.7)	15 (9.4)	
Mean age in years (SD)	52.6 (11.7)	50.43 (10.3)	-	

n: Number of subjects, NS: Nonsignificant, SD: Standard deviation

Dialysis data of the case group

The mean dialysis vintage of the cases was found to be 23 (12.1) months. The mean interdialytic weight gain was found to be 3.1 (1.2) kg. The mean pre- and post-dialytic systolic blood pressure was 149.2 (12.1) and 134.8 (11.5) mm Hg, and the mean pre- and post-dialytic diastolic blood pressure was 88.7 (6.1) and 82.2 (6.4) mmHg, respectively. The pre- and post-dialytic BUN was 120.2 (36) and 40.2 (12.2) mg/dL, respectively. The mean dialysis adequacy (Kt/V) was found to be 1.38 (0.09) and mean (nPCR g/kg/day) was 1.76 (0.5). The mean URR was found to be 66.4 (2.4).

Anthropometric parameters of cases and control

Table 2 depicts the anthropometric parameters of cases and control. The mean values of the anthropometric indices were decreased in the case group when compared to the control, and the difference was statistically significant ($P < 0.001$). The IBW was also lesser for the cases when compared to the control, but the difference was not statistically different ($P = 0.19$).

Biochemical parameters of cases and control

Table 3 depicts the biochemical parameters of the study population. The levels of serum hemoglobin, packed cell volume, creatinine, estimated glomerular filtration rate (eGFR), albumin, and total protein were found to be significantly higher in the cases when compared to the control, whereas the hemoglobin, packed cell volume, albumin, and the total protein levels were significantly lower in the cases than the control ($P < 0.001$). There was no difference in the serum globulin levels between the case and the control population.

Serum levels of total ghrelin and inflammatory markers TNF-α and hsCRP in cases and control is given in Table 4. The levels of serum total ghrelin, TNF–α, and hsCRP were significantly elevated in the cases than the control ($P < 0.001$). The cases were further subjected to the following assessments.

Nutritional status based on subjective global assessment-dialysis malnutrition score and appetite based on appetite and diet assessment tool

The SGA-DMS scores showed that 49/90 (54.4%) patients were moderate to severely malnourished with a score range of 21–35 (mean score 25.6 [3.8]), 28/90 (31%) patients were mild to moderately nourished with a score range of 11–200 (mean score 14.5 [3.2]) and 13/90 (14.4%) patients were well-nourished with a score range of 7–10 (mean score 8.9 [1.2]) [Table 5].

Based on the ADAT scores, the appetite was found to be very good for13/90 (14.4%) patients, of which 3 were severely malnourished, 5 patients were moderately nourished, and 5 were well-malnourished. There were 43/90 (47.8%) patients with good and fair appetite, of which 16 were severely malnourished, 19 were moderately malnourished, and 8 were

Table 2: Anthropometric data of cases and control

Anthropometric indices	Cases (*n*=90)	Control (*n*=70)	*t*-test (*P*)
Height (cm)	157.9 (9.04)	161.7 (6.8)	<0.01*
Body weight (kg)	53.7 (10.7)	64.2 (8.1)	<0.001**
BMI (kg/m^2)	21.5 (3.6)	24.54 (2.9)	<0.001**
Mid arm circumference (cm)	20.06 (3.8)	24.82 (1.3)	<0.001**
Mid arm muscle circumference (cm)	16.9 (2.8)	21.97 (1.5)	<0.001**
Triceps skin fold thickness (mm)	9.54 (1.5)	10.20 (3.1)	<0.001**
Mid arm muscle area (m^2)	23.3 (7.3)	38.6 (5.9)	<0.001**
Mid arm fat area (cm^2)	9.9 (4.7)	11.25 (2.3)	<0.001**
Lean body mass (kg)	43.8 (8.9)	49.1 (5.6)	<0.001**
Ideal body mass (kg)	54.5 (7.9)	56.2 (8.3)	0.19 (NS)
Total body water (L)	31.1 (4.9)	34.7 (4.8)	<0.001**
Fat free mass (kg/m^2)	43.2 (6.9)	48.4 (6.7)	<0.001**
Total body fat (kg)	10.5 (6.7)	16.3 (5.5)	<0.01*

*Level of significance at *P*<0.05, **Level of significance at *P*<0.001, Values are expressed as means (SD). SD: Standard deviation, BMI: Body mass index, NS: Nonsignificant, *n*: Number of subjects

Table 3: Biochemical parameters of cases and control

Variables	Cases (*n*=90)	Control (*n*=70)	*t*-test (*P*)
Hemoglobin (g%)	9.1 (1.4)	13.6 (2.6)	<0.001**
Packed cell volume (%)	32.7 (4.9)	40.2 (4.3)	<0.001**
Albumin (g/dL)	3.4 (0.5)	3.9 (0.3)	<0.001**
Globulin (g/dL)	3.2 (0.4)	3.3 (0.4)	0.78 (NS)
Total protein (g/dL)	6.3 (0.7)	7.1 (0.5)	<0.001**
Creatinine (mg/dL)	6.6 (2.9)	0.8 (0.1)	<0.001**
eGFR (ml/min/1.73 m^2)	9.9 (4.9)	103.7 (9.5)	<0.001**

**Level of significance at *P*<0.001, Values are expressed as means (SD). SD: Standard deviation, NS: Nonsignificant, *n*: Number of subjects, eGFR: Estimated growth factor receptor

Table 4: Ghrelin and inflammatory markers in cases and control

Variables	Cases (*n*=90)	Control (*n*=70)	*t*-test (*P*)
Ghrelin (pg/ml)	242.5 (62.3)	80.7 (19.6)	<0.001**
TNF-α (pg/ml)	39.8 (15.2)	6.54 (1.2)	<0.001**
hsCRP (mg/L)	10.2 (2.8)	2.7 (0.5)	<0.001**

**Level of significance at *P*<0.001, Values are expressed as means (SD). SD: Standard deviation, *n*: Number of subjects, hsCRP: High-sensitivity C-reactive protein, TNF-α: Tumor necrosis factor-alpha

Table 5: Nutritional status assessment of cases based on subjective global assessment-dialysis malnutrition score

Nutritional status	SGA-DMS score range	Mean SGA-DMS score (SD)	Number of patients (*n*=90) (%)
Well nourished	7-10	8.9 (1.2)	13 (14.4)
Mild to moderately malnourished	11-20	14.5 (3.2)	28 (31.1)
Moderate to severely malnourished	21-35	25.6 (3.8)	49 (54.4)

SGA: Subjective global assessment, DMS: Dialysis malnutrition score, SD: Standard deviation

well-nourished. The appetite was found to be poor and very poor for 34/90 (37.8%) patients, of which 30 were severely malnourished and 4 patients were moderately malnourished. There was a statistically significant difference in the appetite of the hemodialysis patients based on their nutritional status ($P < 0.001$) as shown in Table 6. Poorer the nutritional status significantly poorer was the appetite.

Dialysis data and nutritional status

Table 7 explains the dialysis data of the case population with respect to their nutritional status. There was no significant difference in the dialysis vintage ($P = 0.77$), interdialytic weight gain ($P = 0.93$), dialysis adequacy ($P = 0.48$), nPCR ($P = 0.6$), URR ($P = 0.53$), and ultrafiltration ($P = 0.75$) in the case group based on their nutritional status.

The anthropometric indices of the study population based on their nutritional status are described in Table 8. There were no difference between the mean height ($P = 0.92$) of the case population based on their nutritional status. The mean dry body weight ($P = 0.16$), LBM ($P = 0.14$), ideal body mass ($P = 0.79$), TBW ($P = 0.38$), FFM (0.39), and the TBF ($P = 0.26$) were found to be higher in well-nourished patients than the moderately and severely malnourished patients but the differences were not statistically significant. The mean BMI ($P = 0.02$), TSF ($P < 0.01$), MAC ($P < 0.001$), MAMC ($P < 0.01$), MAMA ($P < 0.001$), and MAFA ($P < 0.01$) were also higher in well-nourished patients than the malnourished patients and the difference was statistically significant.

The biochemical parameters, serum total ghrelin, TNF-α, and hsCRP levels of the case population based on their nutritional status is explained in Table 9. There was a statistically significant decrease in the serum hemoglobin ($P < 0.01$), packed cell volume ($P < 0.01$), serum iron ($P < 0.01$), transferrin ($P < 0.001$), TIBC ($P < 0.01$), and the albumin ($P < 0.001$) levels with respect to decline in the nutritional status. However, serum ferritin ($P < 0.01$) levels were found to be significantly increased with thedecline in nutritional status since ferritin is an acute phase reactant which tends to increase in inflammation. There was no significant difference in the parameters such as serum globulin ($P = 0.87$), total protein ($P = 0.12$), creatinine (0.18), eGFR ($P = 0.09$), pre- and post-dialytic BUN levels ($P = 0.28$ and 0.23, respectively) based on the nutritional status. The total ghrelin ($P < 0.001$), TNF-α ($P < 0.001$), and hsCRP levels ($P = 0.02$) were significantly increased in patients who were moderate to severely malnourished when compared to patients who were mild to moderately nourished and well-nourished. There was a positive correlation between serum ghrelin and TNF-α ($r = 0.72$; $P < 0.001$), ghrelin and hsCRP ($r = 0.55$; $P < 0.001$), ghrelin and the SGA-DMS ($r = 0.72$; $P < 0.001$). Similarly, there was a positive correlation between TNF-α and SGA-DMS ($r = 0.63$; $P < 0.001$) and hsCRP and SGA-DMS ($r = 0.38$; $P < 0.001$). The results indicated an increase in the levels of total ghrelin and inflammatory markers with an increase in the SGA-DMS scores which reflected a decline in the nutritional status.

Discussion

Protein energy malnutrition PEM is widespread in patients with ESRD, adversely affecting their quality of life and also associated with increased mortality and morbidity. In light of the discovery of ghrelin, the orexigenic peptide and its role on appetite regulation and nutritional status, the present study had assessed whether the nutritional status and inflammation in ESRD patients on MHD was associated with altered regulation of serum levels of total ghrelin.

Table 6: Appetite and nutritional status of cases

Appetite	Nutritional status			Total (*n*=90) (%)	ANOVA (*P*)
	Well nourished (*n*=13)	Mild to moderately malnourished (*n*=28)	Moderate to severely malnourished (*n*=49)		
Very good	5	5	3	13 (14.4)	<0.001**
Good and fair	8	19	16	43 (47.8)	
Poor and very poor	0	4	30	34 (37.8)	

**Level of significance at *P*<0.001. *n*: Number of subjects, ANOVA: Analysis of variance

Table 7: Dialysis data versus nutritional status of cases

Dialysis data	Well nourished (*n*=13)	Mild to moderately malnourished (*n*=28)	Moderate to severely malnourished (*n*=49)	ANOVA (*P*)
Dialysis vintage (months)	20.1 (13.2)	19.9 (11.1)	21.8 (12.5)	0.77 (NS)
Interdialytic weight gain (kg)	3.1 (1.3)	3.1 (1.1)	3.2 (1.2)	0.93 (NS)
Dialysis adequacy (Kt/V)	1.4 (0.1)	1.4 (0.1)	1.4 (0.9)	0.48 (NS)
nPCR (g/kg/day)	1.8 (0.4)	1.8 (0.5)	1.7 (0.4)	0.60 (NS)
URR	66.2 (2.2)	66.8 (2.7)	66.2 (2.3)	0.53 (NS)
Ultrafiltration (L)	3.6 (0.7)	3.7 (0.6)	3.6 (0.7)	0.75 (NS)

Values are expressed as means (SD). *n*: Number of subjects, NS: Nonsignificant, ANOVA: Analysis of variance, nPCR: Normalized protein catabolic rate, URR: Urea reduction rate, SD: Standard deviation

Table 8: Anthropometry and nutritional status of cases

Anthropometric indices	Well nourished (*n*=13)	Mild to moderately malnourished (*n*=28)	Moderate to severely malnourished (*n*=49)	ANOVA (*P*)
Height (cm)	157.2 (10.3)	157.7 (7.7)	158.3 (9.6)	0.92 (NS)
Dry body weight (kg)	57.7 (10.0)	55.1 (8.0)	51.9 (11.9)	0.16 (NS)
BMI (kg/m^2)	23.3 (3.4)	22.1 (2.6)	20.6 (3.6)	0.02*
Mid arm circumference (cm)	22.3 (2.3)	21.5 (1.9)	18.7 (4.3)	< 0.001**
Mid arm muscle circumference (cm)	18.5 (1.5)	18.1 (1.5)	15.8 (3.2)	<0.001**
Triceps skin fold thickness (mm)	12.0 (2.67)	10.9 (1.9)	9.3 (3.5)	<0.01*
Mid arm muscle area (cm^2)	27.4 (4.5)	26.1 (4.2)	20.59 (8.2)	<0.001**
Mid arm fat area (cm^2)	12.4 (3.9)	10.9 (2.8)	8.6 (4.8)	<0.01*
Lean body mass (kg)	46.9 (6.3)	45.7 (6.9)	42.2 (9.2)	0.14 (NS)
Ideal body mass (kg)	55.0 (8.4)	54.4 (9.3)	53.7 (6.5)	0.78 (NS)
Total body water (L)	31.9 (3.9)	31.9 (4.4)	30.4 (5.5)	0.38 (NS)
Fat free mass (kg/m^2)	44.3 (5.4)	44.29 (6.2)	42.3 (7.6)	0.39 (NS)
Total body fat (kg)	10.4 (3.4)	9.5 (2.2)	8.2 (2.4)	0.26 (NS)

*Level of significance at *P*<0.05, **Level of significance at *P*<0.001, Values are expressed as means (SD). *n*: Number of subjects, NS: Nonsignificant, ANOVA: Analysis of variance, BMI: Body mass index, SD: Standard deviation

Table 9: Biochemical parameters of cases versus nutritional status

Biochemical parameters	Well nourished (*n*=13)	Mild to moderately malnourished (*n*=28)	Moderate to severely malnourished (*n*=49)	ANOVA (*P*)
Hemoglobin (g%)	10.2 (0.9)	9.9 (1.1)	9.02 (1.4)	<0.01*
Packed cell volume (%)	35 (5.0)	34.3 (4.7)	31.1 (4.6)	<0.01*
Serum iron	88.9 (32.7)	75.43 (24.4)	64.8 (17.8)	<0.01*
Serum ferritin	261.4 (72.9)	277.5 (70.3)	374.2 (186.1)	<0.01*
Serum transferrin	237.7 (35.2)	202.3 (35.9)	170.2 (39.1)	<0.001**
TIBC	183.3 (11.2)	219.9 (81.8)	174.1 (37.5)	<0.01*
Albumin (mg/dL)	3.4 (0.6)	3.7 (0.3)	3.3 (0.4)	<0.001**
Globulin (mg/dL)	3.2 (0.2)	3.3 (0.3)	3.2 (0.4)	0.87 (NS)
Total protein (mg/dL)	6.3 (0.6)	6.5 (0.7)	6.2 (0.7)	0.12 (NS)
Creatinine (mg/dL)	6.3 (2.3)	6.6 (3.4)	7.9 (3.9)	0.18 (NS)
eGFR (ml/min/1.73 m^2)	7.2 (2.8)	10.1 (4.4)	10.8 (6.1)	0.09 (NS)
Predialytic BUN	116 (33.9)	121.2 (40.7)	133.9 (31.8)	0.28 (NS)
Postdialytic BUN	38.7 (11.0)	40.5 (14.4)	45.15 (10.9)	0.23 (NS)
Ghrelin (pg/ml)	112.3 (27.5)	182.57 (31.9)	256.45 (38.9)	<0.001**
TNF-α (pg/ml)	30.7 (11.1)	33.6 (11.7)	45.8 (15.5)	<0.001**
hsCRP (mg/L)	9.12 (2.3)	9.8 (2.7)	10.6 (2.9)	0.02*

*Level of significance at *P*<0.05, **Level of significance at *P*<0.001. Values are expressed as means (SD). *n*: Number of subjects, NS: Nonsignificant, ANOVA: Analysis of variance, TIBC: Total iron binding capacity, SD: Standard deviation, BUN: Blood urea nitrogen, hsCRP: High-sensitivity C-reactive protein, TNF-α: Tumor necrosis factor-alpha, eGFR: Estimated growth factor receptor

Nutritional status assessment could be done by several methods ranging from anthropometric measurements to more elaborate techniques such as dual energy X-ray absorptiometry and biochemical parameters such as albumin, serum iron, transferrin, and ferritin. Though these biochemical parameters are extensively used to assess the nutritional status, they do not necessarily correlate with changes in other nutritional parameters and can be influenced by nonnutrition-related factors.

The subjective methods for nutritional status assessment can be clinical history and physical examination, and objective methods include anthropometry, biochemical exams, and bioelectrical impedance.[31] SGA is a useful and reproducible instrument for assessing the nutritional status of dialysis patients. The National Kidney Foundation – Kidney Disease and Dialysis Outcomes Quality Initiative recommended the SGA-DMS as an appropriate nutritional assessment tool for dialysis patients as it correlates the subjective and objective aspects of medical history and physical examination.[32]

Anthropometry provides a semi-quantitative estimate of the components of body mass, particularly the bone, muscle, and fat compartments, and thus gives us information concerning nutritional status. In the present study, BMI, skinfold thickness, and the arm circumferences were the significant predictors of nutritional status when compared to the other anthropometric indices. Chen *et al*. demonstrated that BMI and MAC indicated the strong relationship with nutritional status, and they had better predictive value for nutritional status evaluation in comparison with the other anthropometric measurements.[33] Detection of malnutrition in patients on MHD should be done as early as possible for prevention of severe consequences. Anthropometric measurements and SGA-DMS could have

great value in early detection especially in HD units with limited financial recourses.

The presence of anorexia and its degree can be evaluated through the ADAT. In the ADAT designed by Burrowes *et al*. and used in the HEMO trial, the initial three questions relate to appetite. In the HEMO trial, patients who ranked their appetite as poor or very poor had serum albumin levels, nPCR, and serum creatinine concentrations that were significantly lower than those in patients who ranked their appetite as very good, good, or fair. The study also demonstrated that ADAT can be used in clinical practice to assess the effects of changes in the patient's medical condition, which may have an impact on appetite and nutritional status.[34]

To our knowledge, the ADAT was not used in Indian population. Hence, in the present study, only five questions of the ADAT were used to assess the self-reported appetite of the patients. Unlike other items in the ADAT, these five questions directly reflect on the appetite changes and may not be affected by cultural and ethnic variations of the population. Further studies are needed to validate the applicability of entire ADAT in Indian population.

The serum total ghrelin, TNF-α, and hsCRP levels were found to be significantly high in the MHD patients when compared to the healthy individuals. There was a positive correlation between total ghrelin and the inflammatory markers. Mafra *et al*.[35,36] in their studies have also reported a high levels of fasting serum total ghrelin levels in MHD patients and its positive correlation with the inflammatory markers TNF-α and IL-6.

A study done by Kandil *et al*.[37] also reported a marked increase in plasma total ghrelin levels in chronic hemodialysis patients and a moderate increase in predialysis patients compared to healthy control group. Carrero *et al*.[6] reported markedly elevated plasma total ghrelin levels in ESRD patients treated by HD when compared with normal control. Rodriguez Ayala *etal*.[38] have also reported a significantly higher total ghrelin levels in MHD Patients. In contrast to the strong tendency for anorexia in MHD, the increase in the total ghrelin levels in ESRD patients may result from decreased degradation or elimination of ghrelin in the kidney or may be overproduction of ghrelin in organs other than the stomach and also may be attributable to different basal levels in different races.Chang *et al*.[39] have reported an increase in the plasma levels of ghrelin in MHD patients than the healthy control suggesting ghrelin resistance in MHD either peripheral, central, or both.

In the present study, the anthropometric indices and the biochemical markers hemoglobin, packed cell volume, and albumin were also decreased in the case group when compared to the control group thus reflecting the compromised nutritional status of the MHD patients. These observations are similar to other research.[40]

Based on SGA-DMS scores of the 90 patients on MHD, 54.4% patients were moderate to severely malnourished and 31.1% were mild to moderately malnourished. Studies done by Tapiawala *et al*.[41] and Prakash *et al*.[42] have also reported a 50–65% prevalence of mild to severe malnutrition in Indian population with ESRD. The prevalence is slightly higher in Indian patients than the Western world (10–54%). The reasons for the higher prevalence of malnutrition in Indian population could be possibly due to poor socioeconomic status and related consequences such as the inability to afford to adequate renal nutrition.

In the present study, the self-reported appetite had a strong association with nutritional status. Patients who were well-nourished had a very good appetite when compared to patients with moderate to severe malnutrition. Considering that lack of appetite is one of the manifestations of uremia, it seems plausible to expect that this symptom may be improved by increasing the dialysis adequacy.[34] In the present study, the dialysis adequacy (Kt/V) was found to be at a correct dose, but it did not have a significant effect on the appetite and the nutritional status of the MHD patients. Similarly, other dialysis-dependent parameters such as nPCR, URR, ultrafiltration, and dialysis vintage had no significant effect on the nutritional status. Lopes *et al*.[43] have also reported the lack of association between lack of appetite, Kt/V, dialysis vintage, and other dialysis-related factors. In contrast to this, studies have also reported a strong association between loss of appetite, malnutrition, and dialysis-related factors such as dialysis vintage, dialysis dose (Kt/V), URR, and protein catabolic ratio.[44]

The present study had also identified a strong positive correlation with serum total ghrelin levels, and the SGA-DMS scores indicating that the ghrelin levels were significantly high in moderate to severely malnourished patients than the mild to moderate and well-nourished patients. Similarly, higher was the total ghrelin levels higher were the inflammatory markers TNF-α and hsCRP. This finding suggests a strong correlation between serum total ghrelin, inflammation, and the nutritional status. Given the fact that ghrelin is an orexigenic hormone and inflammation is associated with renal anorexia, it is reasonable to discussPharmacovigilance reporting databases, Signal detection and Management the role of desacylghrelin, which was previously reported to be a prime factor in renal patients on inflammatory anorexia. Since desacyl ghrelin is the major form of total circulating ghrelin and this form could significantly reduce appetite in these patients, the assumption that inflammation causes elevation of total ghrelin may be viable.Muscaritoli *et al*.[45] also suggests that the desacyl form of ghrelin could be involved in the pathogenesis of anorexia in MHD patients.

Study evaluating inflammation and ghrelin in non-CKD patients has shown that ghrelin levels seem to be elevated

in inflammation. In addition, endotoxins and cytokines modulate serum ghrelin levels in a different manner depending on the time course.[46] Vila *et al*.[47] reported an acute surge in the circulating total ghrelin levels at 120 min following the administration of *Escherichia coli* endotoxin (lipopolysaccharide) in humans, along with the release of inflammatory mediators TNF-α and IL-6. Hence, they suggested that ghrelin was one of the hormones secreted initially in response to bacterial endotoxic shock in humans. Though there are presently conflicting information regarding the association between total ghrelin and markers of chronic inflammation, these reports hypothesized that ghrelin could have anti-inflammatory effects, and it is likely that the elevation of serum ghrelin concentrations in inflammation is one of the defense mechanisms against the overshooting of inflammation. These authors concluded that increased ghrelin may represent a compensatory mechanism under catabolic–anabolic imbalance in the ESRD patients.

In the present study, patients with high TNF-α and hsCRP levels had poor nutritional status and a poor appetite suggesting that systemic inflammation in MHD patients could lead to reduced appetite, which is also contributed by high total ghrelin levels (predominantly due to desacyl ghrelin which forms a major portion of total ghrelin).

Conclusion

The present study substantiates the prevalence of malnutrition and anorexia in patients on MHD and had shed light on the association of total ghrelin with their nutritional and inflammatory status. Since malnutrition and inflammation has a significant impact on the prognostic outcome of dialysis patients, nutritional assessment, and management of malnutrition-inflammation complex appears to be of prime importance. Ghrelin emerges as a promising biomarker of cachexic malnutrition-inflammation complex in patients on MHD. Further studies are warranted in larger population to corroborate the aforementioned role of ghrelin.

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Conflicts of interest

There are no conflicts of interest.

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