Original Article

Evaluation of Proteolytic Enzyme on Hemodialysis Patients with Protein–Energy Wasting and Associated Variables

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Abstract

Introduction: Protein–energy wasting (PEW) is very commonly seen among hemodialysis (HD) patients. This study was carried out to estimate the nutrition status of HD patients receiving aminase tablets, a proteolytic enzyme with a high-protein diet, for reducing PEW. **Methods and Materials:** The outpatients who visited the HD unit were nutritionally assessed using the Subjective Global Assessment (SGA). Malnourished patients were selected for the study. Diet advice was given on a high-protein diet as per the standard renal guidelines. They were nutritionally assessed pre- and postsupplementation at 0–6 months gap using SGA, malnutrition-inflammation score (MIS), bioelectrical impedance analysis, hand grip dynamometer, C-reactive protein (CRP), serum albumin, and total iron-binding capacity. Patients were given proteolytic enzyme 2 capsules daily after food (morning and night) for 6 months and high-protein diet. **Results:** A significant improvement ($P < 0.05$) was seen in the intracellular water, body cell mass, skeletal muscle mass, and protein mass. An improvement in the mean hand grip strength and mean reduction of CRP, SGA, and MIS scores were observed. **Conclusion:** Proteolytic enzyme along with a high-protein diet could be recommended for better clinical outcomes in dialysis patients.

Keywords: Body cell mass, intracellular water, malnutrition inflammation score, protein–energy wasting, skeletal muscle mass

Introduction

Protein–energy wasting (PEW) occurs between 18% and 75% among hemodialysis (HD) patients. PEW is exhibited as a loss of muscle mass and energy store. A wide range of clinical complications is associated with PEW, augmenting a decrease in the quality of life, onset of infections, increased hospitalizations, poor quality of life, and decreased survival. Increased nutrient losses during dialysis accompanied with low food intake is one of the primary factors that worsens PEW.[1] A loss of 11.95 ± 0.69 g amino acid through the dialysate was observed in a study.[2] To offset the protein losses, it becomes very essential to provide a high-protein diet to prevent PEW.[3] HD patients apart from the systemic derangement in protein metabolism, there is a derangement in the gut metabolism of proteins causing poor protein assimilation and perpetuating PEW.[4] The protein once ingested is broken down by the endogenous peptidases into dipeptides and tripeptides. Protein losses occur at minimal amounts during normal conditions and can be lost in more amounts during an impaired digestion process or when there is high protein intake but improper digestion. Unbroken proteins pass unabsorbed through the gastrointestinal tract and are excreted from the body. These unabsorbed proteins can be acted upon by microbes causing a release of harmful metabolites such as ammonia, causing dysbiosis of the gut flora.[5]

A high-protein diet is usually taken by the bodybuilders as well as in the health-care setting by the malnourished patients to cope up with the critical illness. However, it becomes even more very essential that these proteins get digested as well, for better absorption and utility. It has shown that

Access this article online

Quick Response Code:

Website: www.jrnm.in

DOI: 10.4103/JRNM.JRNM_7_23

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Received: 08-12-2023 **Revised:** 12-12-2023
Accepted: 15-12-2023 **Published:** 09-02-2024

How to cite this article: Joseph KM, Anandhi D, Abraham G, Shankar B, Rajagopal A. Evaluation of proteolytic enzyme on hemodialysis patients with protein–energy wasting and associated variables. J Renal Nutr Metab 2023;8:43-8.

supplementing a proteolytic enzyme had improved digestion and absorption of protein in normal healthy subjects.[6] Consuming a high-protein diet by dialysis patients along with proteolytic enzymes had shown an improvement in the protein digestion and nutrition status of HD patients.[7] This study was carried out to study the nutrition status of dialysis patients receiving proteolytic enzymes with a high-protein diet using bioelectrical impedance analysis (BIA) and other nutrition assessment variables.

BIA is a very useful tool to assess body composition. A minute voltage is passed through the body and based on the resistance, the body compartments are measured.[8] BIA indexes reflected nutritional state. Phase angle and body cell mass (BCM) seem to be less influenced by changes in volume and can be used for nutritional assessments of dialysis patients. Its results are highly comparable with DEXA scan reports.[9]

Aim

The aim of this study was to explore novel methods of nutritional intervention among HD patients.

Objective

Estimating the efficacy of consuming oral proteolytic enzyme with high-protein diet in reducing PEW among HD patients.

Materials and Methods

Place of study

This study was conducted at Outpatients of the Ambattur Tamil Nadu Kidney Research Foundation Nephrology Department.

Period of study

The period of study was from August 2021 to March 2022.

Tools used in the study

a. In body S10, BIA a measuring device, was calibrated and verified for accuracy and precision in the study center. The BIA was measured after the dialysis session. It was measures as per the specification mentioned in the BIA manual
b. Height and calibrated weighing scale
c. Handgrip dynamometer
d. Laboratory test: C-reactive protein (CRP), total iron-binding capacity (TIBC), and serum albumin
e. Subjective Global Assessment (SGA) score (SGA-Annexture 2) and malnutrition-inflammation score.

Selection of samples

Inclusion criteria

a. The HD patients were nutritionally assessed using the SGA. SGA scores of 15 and above, that is denoting malnutrition, were selected for the study. A score of 7–14 was considered well nourished, 15–35 was considered mild-to-moderate malnourished, and 36–49 was considered severely malnourished
b. Patients on dialysis for more than 2 months.

Exclusion criteria

a. Normal nourished HD patients
b. Critically ill and patients with amputated legs.

The gender distribution of the study group was 18 male and 2 female. Patients were explained about the study and those who were willing to participate in the study were asked to confirm their participation in the consent form. The patients were counseled on their diet as per the Kidney Disease Outcomes Quality Initiative guidelines. Patients were given proteolytic enzyme aminase (obtained from Bacillus Subtilis) 2 capsules daily after food (morning and night) for 6 months along with a high-protein diet. Patients were nutritionally assessed using BIA, handgrip dynamometer, MIS, SGA, laboratory test CRP, serum albumin, and TIBC pre- and postsupplementation from 0 to 180 days gap.

Research design

This was a single-arm evidence-based observational study.

Statistical analysis

Univariate regression done between 0 and 180 days.

Sample size using factorial design

The sample size was decided based on the power to detect the difference of body fat % in the confidence interval of 95%, power of detection of 80% based on the characteristics of protein mass, BCM (measurement of PEW), using analysis of variance techniques. In body mass index (BMI), soft lean mass and body fat % to detect the effect with the variation of 2.93, 5.537, and 9.863 units (±standard deviation [SD]) for patients day 0, and for 2 factors of case and control, each at day 0 and day 180-therefore, with four corner points, at level of significance 5% and power at 80%, the runs required in terms of patients are minimum 12 [explained in Figure 1 and Table 1]. We have taken 20 in a group, which will detect BMI, soft lean mass, and body fat % difference of 5.4, 10, and 18% units. Accordingly, it was decided to observe on 20 patients.

Two-level factorial design, $\alpha = 0.05$ assumed SD = 2.937, 5.537, and 9.863.

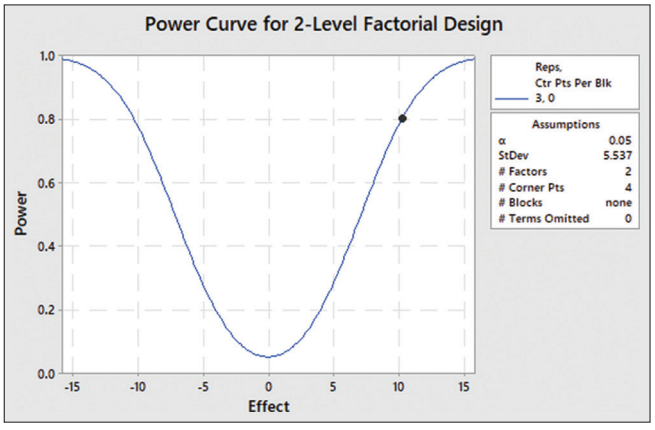

Figure 1: Power curve

Results

There were totally of 108 study variables observed. The variables that had shown a significant improvement were intracellular water (ICW), BCM, skeletal muscle mass (SMM), and protein mass.

Table 1: Sample size calculation

Center points	Reps	Total runs	Power	Effect
0	3	12	0.8	5.4
0	3	12	0.8	10.2
0	3	12	0.8	18.2

Table 2: Analysis of variance in Intracellular water

ICW model summary		
S	***R*²**	***R*² (adj)**
0.735465	94.09%	93.76%

Analysis of variance					
Source	**DF**	**SS**	**MS**	***F***	***P***
Regression	1	154.982	154.982	286.52	0.000
Error	18	9.736	0.541		
Total	19	164.718			

ICW: Intracellular water, DF: Degrees of freedom, SS: Sum of square, MS: Mean square

ICW, BCM, SMM, and protein mass at day 180 paired with day 0 were observed to find a significant linear relationship, regression coefficient (Reg. Coeff) at 1.108 ($P = 0.000$) for ICW, 1.100 ($P = 0.000$) for BCM, 1.097 ($P = 0.000$) for SMM, and 1.088 ($P = 0.000$) for protein mass. The increase was 10.8% for ICW, 10% for BCM, 9.7% for SMM, and 8.8% for protein mass on day 0. This explains ICW 93.76%, BCM 93.85%, SMM 93.65%, and protein mass 94.15% measured by coefficient of determination R square [Tables 2-5 and Figures 2-5]. Percent body fat (PBF) at day 180 paired with day 0 was observed to find a significant linear relationship regression coefficient at 0.836 ($P = 0.000$). The increase was 16.4% less on day 0. This explains why 79.76% are measured by the coefficient of determination R square [Table 6 and Figure 6].

The body composition variables SMM, BCM, ICW, and protein mass that changed during the study (0–180 days) are represented in Table 7. At the beginning of the study, out of 20 patients, 2–3 patients (10%–15%) were only having normal BCM, SMM, protein mass, and ICW values. After the end of the treatment (after 6 months), 55%–65% of subjects, the baseline values increased, 5%–15% of the subjects were

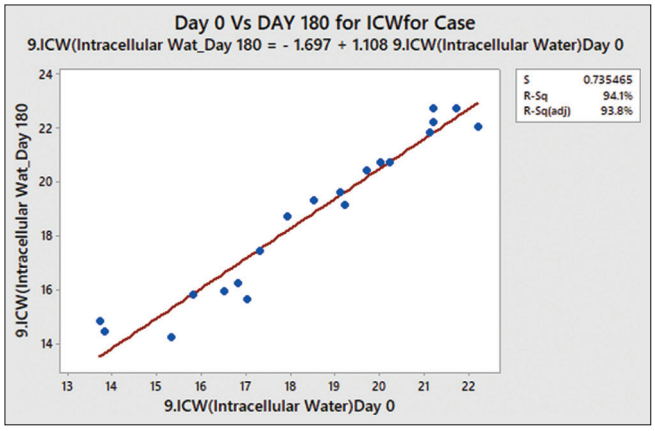

Figure 2: Intracellular water coefficient of variance. ICW: Intracellular water

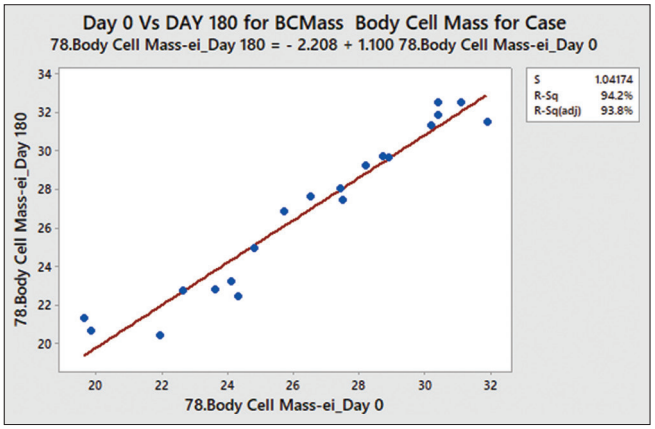

Figure 3: Body cell mass coefficient of variance. BCM: Body cell mass

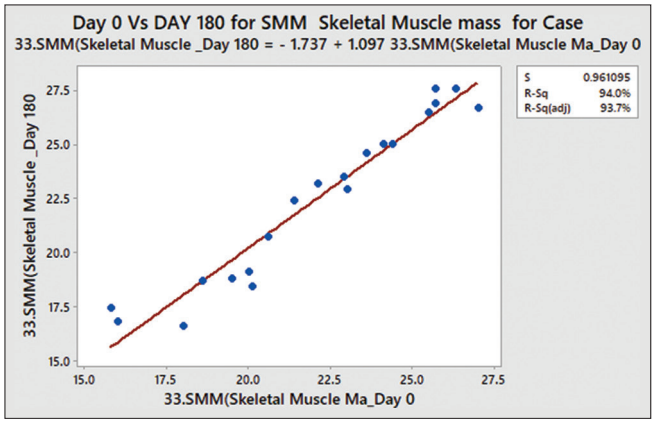

Figure 4: Skeletal muscle mass coefficient of variance. SMM: Skeletal muscle mass

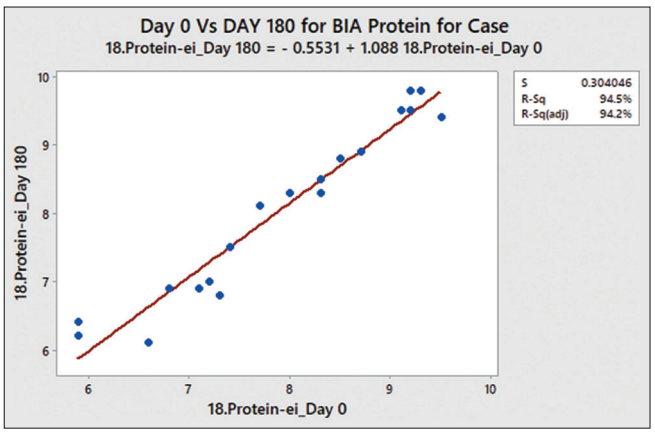

Figure 5: Protein coefficient of variance

Table 3: Analysis of variance in Body cell mass

BCM model summary					
S		R^2		R^2 (adj)	
1.04174		94.17%		93.85%	
Analysis of variance					
Source	**DF**	**SS**	**MS**	***F***	***P***
Regression	1	315.624	315.624	290.84	0.000
Error	18	19.534	1.085		
Total	19	335.158			

BCM: Body cell mass, DF: Degrees of freedom, SS: Sum of squares, MS: Mean square

Table 4: Analysis of variance in Skeletal Muscle Mass

SMM model summary					
S		R^2		R^2 (adj)	
0.961095		93.99%		93.65%	
Analysis of variance					
Source	**DF**	**SS**	**MS**	***F***	***P***
Regression	1	259.885	259.885	281.35	0.000
Error	18	16.627	0.924		
Total	19	276.512			

SMM: Skeletal muscle mass, DF: Degrees of freedom, SS: Sum of square, MS: Mean square

Table 5: Analysis of variance in Protein Analysis of variance

Protein model summary					
S	R^2			R^2 (adj)	
0.304046	94.46%			94.15%	
Analysis of variance					
Source	**DF**	**SS**	**MS**	***F***	***P***
Regression	1	28.3680	28.3680	306.87	0.000
Error	18	1.6640	0.0924		
Total	19	30.0320			

DF: Degrees of freedom, SS: Sum of square, MS: Mean square

Table 6: Analysis of variance in Percent Body fat

PBF model summary					
S		R^2		R^2 (adj)	
3.21497		80.82%		79.76%	
Analysis of variance					
Source	**DF**	**SS**	**MS**	***F***	***P***
Regression	1	784.209	784.209	75.87	0.000
Error	18	186.048	10.336		
Total	19	970.258			

PBF: Percent body fat, DF: Degrees of freedom, SS: Sum of square, MS: Mean square

maintained at normal values, while 25%–30% of the subjects, the values decreased. It was observed 30% of the subjects the fat mass decreased from the baseline values. Ten percentage

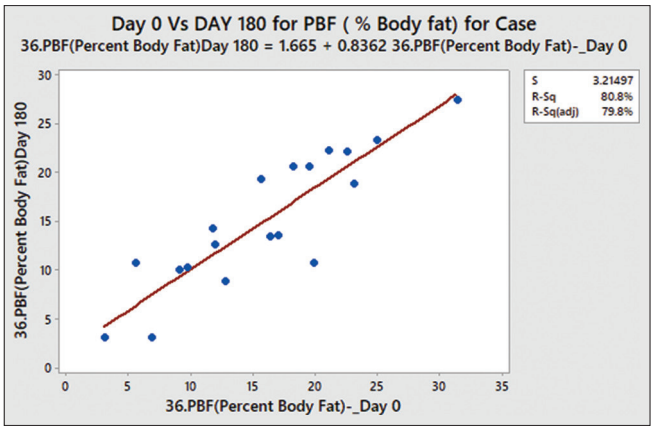

Figure 6: Percent body fat coefficient of variance. PBF: Percent body fat

of the patients did not show any changes. While 30% of the patients showed an increase in fat mass and 30% of the subjects maintained body fat in the normal range.

The nutritional assessment, SGA score (day 0) mean was 18.95 (SD 2.28). It was reduced after aminase treatment to a mean of 15.25 (SD 3.64) at day 180. A reduction of 19.5% of the SGA score from 18.95 was observed. The nutritional assessment, MIS score (day 0) mean was 14.05 (SD 2.62). It was reduced after aminase treatment to a mean of 10.50 (SD 4.26) at day 180. A reduction of 25% of MIS score from 14.05 was observed [Table 8]. There was a 14% increase in the mean handgrip strength measurements and 31.5% decrease in the mean CRP values in the aminase treatment group.

Discussion

This study was conducted to observe the effect of HD patients taking a proteolytic enzyme and a high-protein diet. Among the variables that showed a significant improvement during the treatment period were ICW, protein mass, BCM, SMM, and PBF. These parameters are the very important variables that represent the protein and energy status of a patient.

Extracellular water/total body water (ECW/TBW) (Total) helps to evaluate edema more objectively and is an indicator of the nutrition status of the cell, which makes it extremely important when checked along with muscle mass. A significant improvement in the protein mass with ECW/TBW maintained at normal limits would show a better survival and nutrition status.[10]

Among 20 patients, 80% (16 patients) of the patients maintained a normal ECW/TBW ratio (0.360–0.390) with a mean average ratio of 0.383 with SD of 0.010 at the end of the study.

A mean improvement in handgrip strength measurements, SGA, and MIS scores was also observed. Among the 20 malnourished patients, at the end of the study, the nutrition status improved, a decrease in SGA score for 17 patients (85%), one patient (5%), the SGA score remain the same while two

Table 7: Body composition changes from 0 to 180 days

Body composition variables	Number of patients the values increased to normal values (%)	Number of patients the values increased but still at lower range (%)	Maintained at normal/ no changes, *n* (%)	Values decreased, *n* (%)
ICW	3 (15)	8 (40)	3 (15)	6 (30)
Protein	3 (15)	9 (45)	3 (15)	5 (25)
SMM	4 (20)	9 (45)	1 (5)	6 (30)
BCM	3 (15)	9 (45)	2 (10)	6 (30)

ICW: Intracellular water, SMM: Skeletal muscle mass, BCM: Body cell mass

Table 8: Body composition changes 0-180 days - Mean and SD

Variables	Day 0	Day 180	Variables	Day 0	Day 180	Variables	Day 0	Day 180
BMI mean	18.03	18.19	SGA mean	18.95	15.25	HG mean	20.5	23.37
SE mean	0.505	0.553	SE mean	0.51	0.81	SE mean	1.53	1.94
StDev	2.25	2.47	StDev	2.28	3.64	StDev	6.84	8.69
CoefVar	12.52	13.60	CoefVar	12.04	23.87	CoefVar	33.37	37.17
Protein mean	7.93	8.08	MIS mean	14.05	10.50	PBF mean	15.14	14.32
SE mean	0.25	0.28	SE mean	0.58	0.95	SE mean	1.72	1.6
StDev	1.12	1.25	StDev	2.62	4.26	StDev	7.68	7.15
CoefVar	14.15	15.56	CoefVar	18.69	40.58	CoefVar	50.75	49.89
SMM mean	22.01	22.42	ICW mean	18.41	18.71	BCM mean	26.38	26.81
SE mean	0.75	0.85	SE mean	0.57	0.65	SE mean	0.82	0.93
StDev	3.37	3.81	StDev	2.57	2.94	StDev	3.7	4.2
CoefVar	15.31	17.02	CoefVar	14.00	15.74	CoefVar	14.05	15.67
CRP mean	9.18	6.28	ECW/TBW (total) mean	0.382	0.383	AMC mean	22.46	22.75
SE mean	2.73	1.18	SE mean	0.002	0.002	SE mean	0.397	0.479
StDev	12.20	5.28	StDev	0.010	0.010	StDev	1.77	2.14
CoefVar	132.86	84.21	CoefVar	2.86	2.80	CoefVar	7.91	9.42

AMC: Arm muscle circumference, ECW: Extracellular water, TCW: Total body water, ICW: Intracellular water, SMM: Skeletal muscle mass, BCM: Body cell mass, CRP: C-reactive protein, PBF: Percent body fat, MIS: Malnutrition inflammation score, BMI: Body mass index, SGA: Subjective Global Assessment, StDev: Standard deviation, SE: Standard error, CoefVar: Coefficient of variance, HG: Hand Grip

patients (10%), the SGA score increased, denoting becoming more malnourished. At the end of the study, patients with normal SGA scores (score: 7–14) were 9 and moderate malnourished SGA scores (15–35) were 11.

Among the 20 severely malnourished patients (MIS score 9 and above), at the end of the study, the nutrition status improved, a decrease in MIS score for 15 patients (75%), three patients (15%), the MIS score remains the same while two patients (10%), the MIS score increased, denoting becoming more malnourished. Patients with mild malnutrition MIS scores (score: 3, 4, and 5) were 2, moderate malnourished MIS scores (6, 7, and 8) were 7, severely malnourished MIS scores (9 and above) were 11, at the end of the study.

A reduction of 31.5% in mean CR*P* values observed indicates a reduction in inflammation.

Conclusion

Supplementing aminase (a proteolytic enzyme) along with a high-protein diet would be an effective way for dialysis patients to reduce the PEW and its associated inflammations. It would reduce protein wastage due to improper digestion.

Acknowledgment

I would like to acknowledge Mylin Biotech India Pvt Ltd for supporting this study by giving the aminase tablets. I express my sincere gratitude to my guide and coguides for giving expert guidance and their valuable suggestions to this project.

Financial support and sponsorship

Nil.

Conflicts of interest

This study was carried out as part of the Ph.D. requirements, under the Meenakshi Academy for Higher Education and Research in Chennai, India.

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