Ghrelin as a biomarker for nutritional and inflammatory status in patients on maintenance hemodialysis

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Background of the Study

ESRD & MIS

- **Protein energy malnutrition (PEM) - a severe complication that increases morbidity and mortality in ESRD patients on Maintenance hemodialysis (MHD).**
- **Major causes of PEM - uremic anorexia, Inflammation & misbalance between anorexigenic and orexigenic mediators - leading to Malnutrition Inflammation Syndrome (MIS) in patients on MHD**

Ghrelin

- **A novel orexigenic peptide – released from the stomach**
- **Two major molecular forms- short lived acyl ghrelin (orexigenic) & long acting des acyl ghrelin (anorexigenic) accounting for 90% of total ghrelin**
- **appears of interest as it is implicated in the control of appetite and nutritional status in CKD**

Literature .....

A significantly higher total ghrelin levels in MHD Patients- Ayala et al

One fifth increase in the plasma levels of ghrelin in MHD patients than the healthy control in contrast to the strong tendency for anorexia in MHD. This suggests ghrelin resistance in MHD either peripheral, central or both. -Chang et al

A strong and consistent association between anorexia and high levels of inflammatory mediators in MHD. Pro inflammatory cytokines TNF –alpha and interleukin 6 are uremic toxins, act on CNS and inhibit appetite, increase leptin levels – Kalantar Zadeh et al.

Elevated plasma ghrelin in PD, the anorexic patients showing a lower levels than that of the obese and patients with normal appetite.- Aguilera et al

Ghrelin is degraded in the kidney. Elevated total ghrelin levels in CKD is due to decreased degradation of ghrelin. Increase in the levels of des acyl ghrelin and decrease in acyl ghrelin – Muscaritoli et al

Mafra et al reported a similar finding and suggest a potential association between this finding and inflammation

Objectives

To assess the nutritional status by anthropomery, Modified Subjective Global Assessment Score and biochemical markers of nutrition (serum hemoglobin & albumin levels) in CKD patients on MHD

To assess the inflammatory status of the study population (serum TNF -alpha and hs CRP levels)

To correlate the serum levels of total ghrelin with serum inflammatory markers and with the nutritional status in study population

Methodology

Study design	• Cross sectional Case control study
Study site	• Dialysis Unit, Sri Ramachandra Medical Center and hospital
Study population	• **Cases** - 90 patients of either sex, aged above 18 yrs, with stage V CKD, on twice/thrice weekly MHD for > 6 months • **Exclusion criteria**: Patients with inflammatory diseases, uncontrolled DM, smoking history, acute illness, long term therapy with steroids and immunosuppressants, known malignancies and patients on once weekly MHD • **Control** – 45 healthy volunteers

Approval of IEC

Consent of the study participants

Data collection (case and control) :
demographics (age, sex, height , weight , Body mass index [wt in kg/ht in m2]), Comorbidities

Blood sample (case and control) :

After an overnight fast, 5ml of venous blood samples were collected in the morning at a similar baseline time, (before initiation of dialysis for cases), serum seperated and analyzed for serum Ghrelin levels and pro inflammatory cytokines TNF-α, hs CRP, BUN creatinine and biochemical markers of nutrition hemoglobin and albumin

PARAMETERS ASSESSED (case and control)

I. Anthropometric Indices - measurement was performed 10-20 minutes after termination of dialysis session.

Body mass index - body weight in kg (dry weight for cases)/ height in m^2)

Mid arm circumference (MAC) - measured with a plastic tape on the arm(for cases - non dialysis access arm)

Triceps skin fold thickness (TSF) - measured with a conventional skinfold caliper (Herpenden calipers) on the arm (non-dialysis access arm for cases) using standard techniques

(Both were done for three times and average result of the three measurements taken as final result)

Mid arm muscle circumference (MAMC) calculated from the formula :

MAMC=MAC - (3.1416 x TSF)

Lean Body mass calculated from the formula **(Hume R, 1966**):

For men : LBM = (0.32810 * W) + (0.33929 * H) - 29.5336

For women : LBM =(0.29569 * W) + (0.41813 * H) - 43.2933

where W is body weight in kg and H is body height in cm

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PARAMETERS ASSESSED (case and control)

2. Analysis of Serum samples:

- Serum total Ghrelin by DRG Elisa kit (**DRG International, Inc., USA).**

The minimum detectable dose of Ghrelin is < 30pg/mL

- Serum Tumor necrosis factor (TNF) alpha by DRG TNF-alpha ELISA Kit-

The minimum detectable dose of TNF-alpha is < 15 pg/ml

- Serum concentrations of hs-CRP were measured using Daiichi kit with a linear range of 0.2 to 20 mg/L, (Daiichi Pure Chemicals Co. Ltd., Tokyo, Japan) by immuno-turbidimetric method as mentioned in the kit.
- Serum levels of BUN, creatinine, hemoglobin and albumin were assessed as per standard laboratory procedures.

PARAMETERS ASSESSED (cases)

3. SUBJECTIVE GLOBAL ASSESSMENT –DIALYSIS MALNUTRITION SCORE (SGA-DMS) (Kalantar Zedeh, et al) - a fully quantitative scoring system consisting of seven features: weight change, dietary intake, gastrointestinal symptoms, functional capacity, co morbidity, 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) of 7 – 11 is (well nourished) , 12-25 is moderately malnourished, 26-35 is severely malnourished. This questionnaire was answered by the case population either at the time of or within a week of blood sample collection.

Statistical analysis was done by SPSS 15.0 version and results were expressed as mean ± Standard Deviation. Independent t test was used to correlate the mean values of the case and control groups.

The correlation between the nutritional status, inflammation and serum ghrelin levels was done by Analysis of variance. A P value of <0.05 was considered statistically significant.

Results

1. Characteristics of study population

Characteristics	Cases (N=90) Mean ± SD	Control (N=45) Mean ± SD	P
Sex Male Female	56 34	29 16	-
Age (Years)	52.62 ± 11.7	50.6 ± 9.7	0.206
BuN (mg/dL)	40.16 ± 6.32	11.66 ± 2.6	<0.001**
Sr. Creatinine (mg/dL)	6.6 ± 3.1	0.9 ± 0.2	<0.001**
eGFR (ml/min/ 1.73m2)	9.86 ± 4.52	102.2 ± 3.6	<0.001**
Dialysis vintage (months)	20.8 ± 5. 2	-	-
Kt/V	1.38 ± 0.05	-	-
nPCR	1.76 ± 0.65	--	-

2. Nutritional markers of study population

Characteristics	Cases (N=90) Mean ± SD	Control (N=45) Mean ± SD	P
BMI (wt in kg/ht in m2)	21.48 ± 3.6	23. 97 ± 2.8	< 0.001**
TSF (mm)	9.20 ± 1.31	10.20 ± 3.08	0.009*
MAC (cm)	20.06 ± 3.76	24.90 ± 1.27	< 0.001**
MAMC (cm2)	16.9 ± 2.8	22.01 ± 0.98	< 0.001**
LBM (kg)	43.84 ± 8.27	48.24 ± 5.41	0.002*
Hb (g/dL)	9.27 ± 1.4	13.61 ± 1.41	< 0.001**
Alb (mg/dL)	2.9 ± 1.2	3.8 ± 1 .5	< 0.001**
SGA – DMS	19.7 1 ± 7.45		

3. Ghrelin and Infllammatory markers of study population

Characteristics	Cases (N=90) Mean ± SD	Control (N=45) Mean ± SD	P
Hs CRP mg/dL)	10.22 ± 2.87	2.58 ± 0.51	< 0.001**
TNF α (pg/mL)	39.65 ± 15.7	6.167 ± 1.10	< 0.001**
Ghrelin (pg/mL)	132.48 ± 42.26	75.54 ± 17.02	< 0.001**

4. SGA – DMS Vs Inflammatory markers & Ghrelin in MHD patients

Markers	Well nourished (n -13)	Moderately malnourished (n-51)	Severely malnourished (n-26)	P
Hs CRP mg/dL)	9.40	9.64	12.14	<0.001**
TNF α (pg/mL)	32.98	34.46	55.48	<0.001**
Ghrelin (pg/mL)	100.62	115.95	180.67	< 0.001**

Discussion

Findings of the present study

- Statistically significant low levels of anthropometry, albumin and hemoglobin in cases. Patients on MHD had a compromised nutritional status than healthy control.
- A significant increase in the inflammatory markers and ghrelin in MHD patients than control
- Of the 90 patients on MHD, 14% were well nourished, 57% were moderately nourished and 29% were severely malnourished as per SGA- DMS. The levels of inflammatory markers and ghrelin were significantly higher in severely malnourished patients when compared to moderately and well nourished patients.
- There was a strong positive correlation between ghrelin, inflammatory markers (hs CRP & TNF □), dialysis vintage, serum BUN and creatinine in hemodialysis patients; where as a negative correlation was observed between ghrelin , eGFR and nutritional status in MHD patients

Discussion

Reports have emphasized increased fasting total ghrelin in MHD patients (Mafra et al, Muscaritoli et al). Given the fact that ghrelin is a potent orexigenic hormone, and inflammation is related to loss of appetite, the role of des acyl ghrelin in CKD patients needs to be explored.

Since the majority of total circulating ghrelin is des acyl ghrelin, this could reduce appetite in CKD patients, hence needs more focus.

Ghrelin and its forms acyl and des acyl ghrelin could be effective markers for malnutrition and anorexia in ESRD patients on MHD .

Limitations:

- Small sample size
- Only total ghrelin levels were estimated
- Role of acylated and desacyl ghrelin in MIS in CKD need to be assessed

Future scope:

There is a dire need for appetite stimulatory therapies in ESRD. Ghrelin is more potent than other orexigenic factors in stimulating appetite and it also possess anti-inflammatory effect, hence could be considered as an ideal therapeutic agent for uremic cachexia and also to combat ghrelin resistance observed in these patients.

Subcutaneous ghrelin administration has shown to enhance short term food intake in MHD patients (Wynne et al)..

References

- Aguilera A, Codoceo R, Selgas R et al. Anorexigen (TNF-a, cholecystokinin) and orexigen (neuropeptide Y) plasma levels in peritoneal dialysis (PD)patients: their relationship with nutritional parameters. Nephrol Dial Transplant 1998; 13: 1476–1483. J Renal Nutr 2004; 14: 143–148.
- Ayala ER, Pecoits-Filho R, Heimburger O, Lindholm B, Nordfors L, Stenvinkel P. Associations between plasma ghrelin levels and body composition in end-stage renaldisease: a longitudinal study. Nephrol Dial Transplant 2004; 19:421–6
- Chang CC, Hung CH, Yen CS, et al: The relationship of plasma ghrelin level to energy regulation, feeding and left ventricular function in non-diabetic haemodialysis patients. Nephrol Dial Transplant 2005;20:2172-2177
- Hume, R (Jul 1966). "Prediction of lean body mass from height and weight.". *Journal of clinical pathology* **19** (4): 389–91. doi:10.1136/jcp.19.4.389. PMC 473290. PMID 5929341
- Kalantar-Zadeh K. Kleiner M, Dunne E, Lee GH, Luft FC; A modified quantitative subjective global assessment of nutrition for dialysis patients. Nephrol Dial Transplant 1999;14: 1732-38
- Mafra D, NE Farage, JC Lobo, et al. Relationship between total grhelin and inflammation in hemodialysis patients. Peptides 2011 (32):358-61
- M. Muscaritoli, A. Molfino, M. G. Chiappini, et al., "Anorexia in hemodialysis patients: the possible role of des-acyl ghrelin," American Journal of Nephrology , 2007; vol. 27, no. 4, pp. 360–365
- Wynne K, Stanley S, McGowan B, et al: Appetite control. J Endocrinol 2005; 184:291-318.

SGA-DMS

(A) PATIENT RELATED MEDICAL HISTORY

1) Weight change (overall change in past 6 months)

1	2	3	4	5
No weight change or gain	Minor wt loss(<5%)	Wt loss 5 to 10%	Wt loss 10 to 15%	Wt loss > 15%

1) Dietary intake

1	2	3	4	5
No change	sub-optimal Change	full liquid diet or moderate overall decrease	hypo-caloric liquid	starvation

1) Gastrointestinal symptoms

1	2	3	4	5
No symptoms	nausea	vomiting or moderate GI symptoms	Diarrhoea	severe anorexia

1) Functional capacity (nutritionally related functional impairment)

1	2	3	4	5
None (improved)	Difficulty with ambulation	Difficulty with normal activity	Light activity	Bed chair ridden ridden with no or little activity

1) Co-morbidity

1	2	3	4	5
MDH* < 12 months and healthy otherwise	MDH* 1-2 years or mild co-morbidity	MDH* 2-4 years or age > 75 or moderate co-morbidity	MDH* > 4 years or severe co-morbidity	very severe multiple co-morbidity

(A) PHYSICAL EXAM

1) Decreased fat stores or loss of subcutaneous fat (below eyes, triceps, biceps, chest)

1	2	3	4	5
None (no change)		Moderate		Severe

1) Signs of muscle wasting (temple, clavicle, scapula, ribs, quadriceps, knee, interosseous

1	2	3	4	5
None (no change)		Moderate		Severe