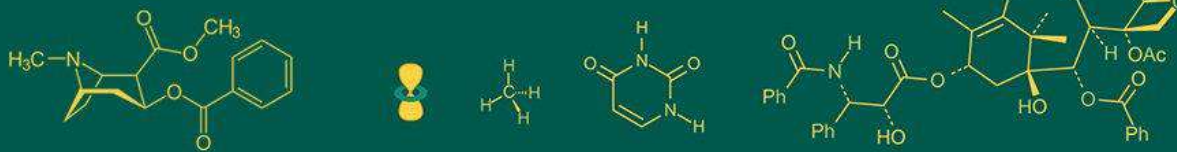


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## Study of serum leptin levels in patients with chronic kidney disease

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### Abstract

**Background and objectives:** Chronic kidney disease is a global threat to health in general and for developing countries. It is characterized by progressive deterioration of kidney function which develops eventually into a terminal stage of chronic kidney failure. Leptin is an adipose tissue-derived hormone shown to be related to several metabolic, inflammatory, and hemostatic factors related to chronic kidney disease. The objective of the study is to understand the role of serum Leptin in chronic kidney disease.

**Material and Methods:** A case control study was done with 87 patients divided into 2 groups. 43 healthy individuals were served as controls and 44 chronic kidney disease patients were served as cases. Serum Leptin was estimated by ELISA method. Blood urea was estimated by urease method and serum creatinine by modified Jaffe's method. BMI was calculated. Data was analysed by using graph pad prism 7.0.

**Results:** In the present study, patients with chronic kidney disease had significant higher levels of serum Leptin ( $55.88 \pm 31.59$ ), blood urea ( $75.23 \pm 30.91$ ), serum creatinine is ( $4.09 \pm 1.90$ ) and BMI is ( $24.66 \pm 2.667$ ) when compared to healthy individuals serum Leptin levels is ( $7.91 \pm 8.24$ ), blood urea ( $27.53 \pm 8.03$ ), serum creatinine is ( $1.28 \pm$ ) and BMI is ( $23.33 \pm 2.45$ ) with a p value  $< 0.001$ .

**Conclusion:** Serum Leptin levels were high in patients with chronic kidney disease when compared to healthy individuals even the BMI was in normal range in both patients and healthy individuals. The study concludes that serum Leptin is associated with chronic kidney disease and can be used as a marker for diagnosis and prognosis in chronic kidney disease.

**Keywords:** Chronic kidney disease, serum leptin, blood urea, serum creatinine

### Introduction

Chronic kidney disease is a major public health problem and characterized by a progressive loss in renal function over a period of months or years as defined by structural or functional abnormalities of the kidney. Identification and staging of chronic kidney disease are evaluated by glomerular filtration rate (GFR) and proteinuria<sup>[1]</sup>.

Chronic kidney disease (CKD) is emerging to be an important chronic disease globally<sup>[2]</sup>. One reason is the rapidly increasing worldwide incidence of diabetes and hypertension. On the other hand, because of scarce resources, only 10% of the Indian ESRD patients receive any renal replacement therapy (RRT)<sup>[3]</sup>.

In India, given its population  $>1$  billion, the rising incidence of CKD is likely to pose major problems for both healthcare and the economy in future years<sup>[2]</sup>. Indeed, it has been recently estimated that the age-adjusted incidence rate of ESRD in India to be 229 per million population (pmp), and  $>100,000$  new patients enter renal replacement programs annually in India<sup>[3]</sup>.

Leptin is a adipocytokine belonging to class 1 cytokine super family and is abundantly produced by the adipose tissue<sup>4</sup>. Leptin is synthesized mainly, but not exclusively, by white adipose tissue and circulates as a 16-kDa protein<sup>[5]</sup>. Structural analysis indicates that leptin is similar to cytokines; it contains an intrachain disulfide bond that appears to be necessary for its biological activity<sup>[6]</sup>.

Leptin has been shown to be related to several metabolic, inflammatory, and hemostatic factors involved in the development of hypertension and cardiovascular disease<sup>1</sup>. leptin has been shown to induce natriuresis which may in turn result in an increase in arterial pressure so as to maintain sodium and water balance<sup>1</sup>. Higher leptin levels may cause hyperglycemia, elevations in blood pressure (mediated through increased sympathetic activity), and renal dysfunction<sup>[1]</sup>.

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Leptin has been shown to induce the growth of glomerular endothelial cells and increase the production of transforming-growth-factor-(TGF-) beta 1 [7]. Leptin induces the synthesis of type 1 collagen in mesangial cells, as well as type 4 collagen in glomerular endothelial cells contributing to extracellular matrix deposition, glomerulosclerosis and proteinuria [7].

Leptin is distributed widely in various tissues and is cleared mainly by the Kidney [5]. Leptin is filtered by the glomeruli and is thought to be degraded by renal epithelial cells [5]. High levels of short form leptin receptors are present in the kidney, and leptin binds to the corticomedullary junction and renal papilla [8]. Leptin clearance is consistent with elevation of leptin levels in patients with renal impairment and end-stage renal disease [5].

Hence the present study was conducted to know the levels of Leptin in patients with chronic kidney disease and to understand the Leptin clearance in CKD patients.

### Materials and Methods

**Setting:** A case control study was conducted in the Department of Biochemistry, Osmania General Hospital, Hyderabad

**Sources of samples and Data:** Samples are collected from department of nephrology, Osmania general hospital. Investigations were performed in department of biochemistry, Osmania general hospital.

#### A case-control study of 80 subjects divided into 2 groups.

- Group 1 - Healthy controls
- Group 2 – Patients with known chronic kidney disease.

All the subjects were in the age group of 25 to 60 years and of either sex. Informed oral consent was taken from all individuals who took part in the study.

#### Inclusion criteria

- Group 1 included healthy controls that were matched for age and sex.
- Group 2 included diagnosed CKD patients of 25 to 60 years of age.

#### Exclusion criteria

- Cardiovascular diseases.
- Pregnant females.
- Chronic liver diseases.

**Specimen collection:** 3ml of blood samples were collected in serum tubes (red cap) from cases and controls. Serum urea and serum creatinine was estimated on the same day and the remaining serum was stored at -20<sup>o</sup> c in an aliquot for serum leptin estimation.

#### Parameters estimated.

Body mass index (BMI) was calculated according to the established World Health Organization (WHO) criteria

#### Serum

1. Urea.
2. Creatinine.
3. Leptin.

Estimation of blood urea: Method: Kinetic enzymatic method [9]. Creatinine estimated by Modified Jaffe's reaction [10].

**Leptin was estimated by:** Enzyme-Linked Immunosorbent Assay (ELISA) [11]. The Principle of this enzyme immunoassay is based on a typical two step capture or "sandwich" type assay.

- The assay makes use of two highly specific monoclonal antibodies: A monoclonal antibody specific for leptin is immobilized onto the micro well plate and another monoclonal antibody specific for a different epitope of leptin is conjugated to biotin.
- During the first step, leptin present in the samples and standards is bound to the immobilized antibody and to the biotinylated antibody, thus forming a sandwich complex. Excess and unbound biotinylated antibody is removed by a washing step.
- In the second step, streptavidin -HRP is added, which binds specifically to any bound biotinylated antibody. Again unbound streptavidin-HRP is removed by washing step.
- Next, the enzyme substrate is added (TMB), forming a blue coloured product that is directly proportional to the amount of leptin present. The enzymatic reaction is terminated by the addition of the stopping solution, converting the blue colour to a yellow colour.
- The absorbance is measured on a microtiter plate reader at 450nm.
- A set of standards is used to plot a standard curve from which the amount of leptin in patient samples and controls can be directly read.

### Observations and Results

The present study was undertaken in the Department of Biochemistry, Osmania Medical College and Osmania General Hospital, Hyderabad. A total of 87 patients were recruited for the study which included 43 healthy individuals as controls, 44 cases diagnosed with CKD.

The following parameters were analyzed

- Blood urea.
- Serum Creatinine.
- BMI.
- Serum Leptin.

The data was analyzed using GraphPad Prism software version 7.0. Descriptive results are expressed as mean and SD of various parameters in different groups.

The Mean  $\pm$  SD of all the parameters studied in the total cases were significantly different from those of controls.

**Table 1:** Shows Biochemical parameters in all groups

Parameter	Control			Cases		
	Mean	$\pm$ S. D	SEM	Mean	$\pm$ S.D	SEM
BMI	23.33	2.452	0.3877	24.66	2.667	0.4021
Leptin	7.916	8.24	1.257	55.88	31.59	40763
Urea	27.53	8.031	1.225	75.23	30.91	4.66
Creatinine	1.288	0.4043	0.061	4.093	1.906	0.287

The Mean  $\pm$  S.D of serum leptin levels was increased and statistically significant in CKD cases when compared with controls. The Mean  $\pm$  S.D of blood urea was increased and statistically significant in CKD cases when compared with controls. The Mean  $\pm$  S.D of serum creatinine was increased and statistically significant in CKD cases when compared with controls.

In order to assess the significance of the differences observed in the mean values of different parameters

observed in different groups studied, the data is subjected to unpaired t - test.

The significance of difference of mean values of different groups and within the groups is represented by p values and p value <0.05 is considered as significant.

**Table 2:** Unpaired t- test between the two groups

Parameter	t-value	p value	R2.
BMI	2.384	< 0.019	0.0628
Leptin	9.738	<0.001	0.6595
Urea	9.899	< 0.001	0.6671
Creatinine	9.542	< 0.001	0.6598

P value was significant in all the parameters (BMI, Leptin, Urea, Creatinine). Mean ± SD of BMI in controls is 23.33 ± 2.45 and Mean ± SD of BMI in cases is 24.66 ± 2.66.

The p value for BMI is 0.019.

Serum urea levels are significantly raised in cases when compared to controls. Mean ± SD of urea in cases is 75.23 ± 30.91 where as in controls it is 27.53±8.03. P value is 0.001.

Mean ± SD of creatinine levels in controls is 1.28±0.40 where as in cases it is 4.09 ±1.90. It is significantly high in cases when compared to controls. P value is significant to 0.001.

Serum leptin levels are significantly increased in chronic kidney disease patients when compared to the normal individuals. Mean ± SD of leptin in cases is 55.88±31.59 where as in controls it is 7.91±8.24. P value is 0.001.

**Table 3:** Pearson’s Correlation between different parameters in cases

		BMI	Urea	Creatinine	Leptin
BMI	Pearsons correlation		-0.039	-0.007	0.053
	Sig. (2tailed) N		0.802	0.965	0.732
Urea	Pearsons correlation	-0.039		0.798	-0.115
	Sig. (2tailed) N	0.802		8.660	0.456
Creatinine	Pearson's correlation	-0.007	0.798		-0.003
	Sig. (2tailed) N	0.965	8.660		0.984
Leptin	Pearson's correlation	0.053	-0.115	-0.003	
	Sig. (2tailed) N	0.732	0.456	0.984	

BMI is positively correlated with leptin which was not statistically significant. BMI is negatively correlated with urea and creatinine which was not statistically significant. Urea is positively correlated with creatinine and negatively correlated with leptin and BMI.

**Table 4:** Pearson’s Correlation between different parameters in controls

		BMI	Urea	Creatinine	Leptin
BMI	Pearsons correlation		0.183	0.143	0.183
	Sig. (2tailed) N		0.241	0.361	0.241
Urea	Pearsons correlation	0.183		0.773	0.100
	Sig. (2tailed) N	0.241		1.199	0.522
Creatinine	Pearson's correlation	0.143	0.773		0.137
	Sig. (2tailed) N	0.361	1.199		0.382
Leptin	Pearson's correlation	0.183	0.100	0.137	
	Sig. (2tailed) N	0.241	0.522	0.382	

BMI is positively correlated with urea, creatinine and leptin which were not statistically significant. Urea is positively correlated with BMI, creatinine and leptin which was not statistically significant.

Leptin is positively correlated with BMI, urea and creatinine which was not statistically significant.

**Discussion**

Chronic kidney disease (CKD) is defined as persistent kidney damage accompanied by a reduction in the glomerular filtration rate (GFR) and the presence of albuminuria [12]. The term end-stage renal disease (ESRD) represents a stage of CKD where the accumulation of toxins, fluid, and electrolytes normally excreted by the kidneys results in the uremic syndrome. This syndrome leads to death unless the toxins are removed by renal replacement therapy, using dialysis or kidney transplantation [13].

The progression of metabolic and cellular dysfunction both systemically and locally within kidney tissue is linked to many diverse and complex pathways, which in particular include heightened production of proinflammatory cytokines [14].

CKD is a global threat to health in general and for developing countries in particular, because therapy is expensive and life-long [15].

Individuals with CKD should be included in the highest-risk group for cardiovascular disease and therefore receive aggressive preventive measures to reduce the prevalence and severity of cardiovascular disease [16].

Death from cardiovascular disease is a substantially more common end point of CKD than progression to dialysis [17]. Identifying and treating risk factors for early CKD may be the best approach to prevent and delay adverse outcome [18]. The adverse outcomes of CKD are universal, as are the underlying science and evidence-based strategies for prevention, detection, evaluation, and treatment [18].

Adipose tissue has been identified to secrete more than 50 adipokines, including cytokines, chemokines, hormone-like factors, and other signaling mediators [19]. These adipokines can activate many signal transduction pathways that are essential for the maintenance of energy homeostasis and metabolism [19].

Adipokines have various roles in vivo, including lipid metabolism, inflammation, atherosclerosis, insulin resistance, the immune-stress response, vascular homeostasis, and cell adhesion and migration [19].

Leptin is a small polypeptide (16 kDa), belongs to the category of adipocytokines considered as a pre-inflammatory cytokine that indicates common structural and functional properties, belonging to the IL-6 family of cytokines [20].

Leptin is secreted by adipose tissue and regulates energy homeostasis, neuroendocrine function, metabolism, immune function and other systems through its effects on the central nervous system and peripheral tissues [21].

In the present study the Mean ± S.D of serum Leptin levels in normal individuals is 7.91 ± 8.24 and in chronic kidney disease patients it is 55.88 ± 31.59. It was found that increased serum Leptin concentration in CKD was significant when compared with normal individuals with p value < 0.0001 after adjusting the confounder BMI. Leptin levels were increased due to decreased Leptin clearance.

There is a positive correlation between Leptin and BMI which was not statistically significant and there is negative correlation between urea and creatinine in cases. The results were in concordance with study conducted by Anoop Shankar et. Al. study included 5,820 participants from the United States Third National Health and Nutrition

Examination Survey (NHANES III). According to the study Plasma leptin levels were categorized into quartiles ( $\leq 4.3$  Fg/L, 4.4–8.7 Fg/L, 8.8–16.9 Fg/L,  $>16.9$  Fg/L) [1].

Higher plasma Leptin levels were associated with CKD after adjusting for age, sex, race/ethnicity, education, smoking, alcohol intake, body mass index (BMI), diabetes, hypertension, and serum cholesterol. Compared to quartile 1 of Leptin, the odds ratio (95% confidence interval) of CKD associated with quartile 4 was 3.31 (1.41 to 7.78);

P-trend = 0.0135. Subgroup analyses examining the relation between leptin and CKD by gender, BMI categories, diabetes, and hypertension status also showed a consistent positive association. They concluded higher plasma leptin levels are associated with CKD in a representative sample of US adults [1].

Ponnudhali et.al conducted a study in 90 subjects. Group 1 comprised of 45 non diabetic patients with Chronic Kidney Disease (CKD), diagnosed and staged, based on NKF K/DOQI guidelines [22]. Group 2 comprising of 45 healthy adults with normal renal function (GFR  $> 90$  ml/min), formed the control group. Serum Leptin levels ( $24.15 \pm 17.44$  ng/ml) were increased significantly ( $p=.000$ ) in group 1 patients compared to those in group 2 ( $7.50 \pm 1.28$  ng/ml). Serum insulin levels ( $p=.000$ ) were increased in CKD patients ( $15.49 \pm 9.39$   $\mu$ U/ml) compared to the healthy controls ( $7.50 \pm 1.28$   $\mu$ U/ml). They have also found a negative significant correlation between serum leptin levels and GFR, which confirms that serum leptin is increased due to declining renal function in CKD. In CKD, inflammation is another important factor which contributes to hyperleptinemia. They concluded that Hyperleptinemia and insulin resistance may be responsible for protein energy wasting/cachexia associated with CKD [74].

In comparison to our study, Pecoits-Filho et al..... studied 149 non-obese end-stage renal disease patients in order to analyze the association between serum leptin they identified [23].

A study was conducted by Beberashvili et al..... to determine whether changes of serum leptin levels are correlated with nutritional status over time in a cohort of prevalent hemodialysis patients [24]. They conducted study on 101 hemodialysed patients. No significant associations were noted between leptin levels and changes in dietary protein or energy intake, or laboratory nutritional markers. They concluded leptin levels reflect fat mass depots, rather than independently contributing to uremic anorexia or modifying nutritional status and/or survival in chronic hemodialysis patients [24].

Similar Study conducted by Amal A. El-Mahdy et al..... concluded a highly significant increase in serum leptin level was found in patients with CRF [25]. Mean  $\pm$  SD of leptin in CRF patients before dialysis is  $24.63 \pm 7.42$  and after dialysis is  $10.95 \pm 4.80$  and Mean  $\pm$  SD of leptin in controls is  $6.50 \pm 1.90$ . There is significant increase with a p value of 0.001. Significant elevations in the mean serum insulin and leptin levels is noted in the patients compared to their levels in the controls were found. There were positive correlations in CRF patients group between serum insulin and leptin levels. They concluded that, the significant increase in leptin levels in CRF patients is associated with deteriorated kidney function and decreased renal clearance of leptin.

Study conducted by Dziedzic et al..... [26]. revealed a direct correlation between BMI and leptin levels in hemodialysed

and healthy patients. Additionally, our results found out that the average serum leptin concentration is twice higher in women than in men on dialysis. In hemodialysed patients the serum leptin level is increased as an effect of renal failure.

Similar Study conducted by krizova et al....., show significantly high leptin levels in renal failure patients Mean  $\pm$  SD of leptin in controls is  $9.4 \pm 7.6$  and in patients is  $25.1 \pm 23.5$ . they concluded that soluble leptin receptor levels in patients with chronic renal failure do not differ from those of healthy subjects despite higher serum leptin levels in CRF patients [27].

A study conducted by Kastarinen et. al on 73 CKD patients and 68 controls. The mean leptin levels were increased in the CKD patients ( $24.0$  (SD  $37.1$ ) ng/mL) compared to those in controls ( $9.0$  (SD  $8.5$ ) ng/mL) ( $p = 0.008$ ). Ratio between leptin levels and Body Mass Index (leptin/BMI) was increased in CKD patients (mean  $0.80$  (SD  $1.03$ ) compared to that in control is ( $0.31$  (SD  $0.24$ )) ( $p = 0.001$ ) [28].

Body Mass Index (BMI) is a simple, accurate and reproducible calculation, based on height and weight. It is considered as one of the markers for the estimation of malnutrition [29]. Increase in body mass index are tightly associated with an increased risk in the development of obesity-related CKD [30].

In the present study mean  $\pm$  SD of BMI of cases is  $24.66$  and mean  $\pm$  SD of BMI in controls is  $23.33$  indicating that there is not much difference in BMI of cases when compared to control. As a result BMI is not effecting the increased leptin levels in chronic kidney disease. There is a positive correlation between BMI and leptin.

According to study conducted by Nehus et al..... Leptin levels were significantly increased in obese children with median leptin values of  $33.2$  ng/ml compared with  $3.0$  ng/ml in non-obese children ( $p < 0.001$ ) [31].

Urea is the major nitrogen containing metabolic product of protein catabolism in humans. More than 90% of urea is excreted through the kidneys, with losses through the gastrointestinal tract and skin accounting for most of the remaining minor fraction [32].

In our present study Mean  $\pm$  SD of urea in chronic kidney patients is  $75.23$  where as Mean  $\pm$  SD of urea in normal volunteers is  $27.53$ . there is appositive correlation between urea and creatinine.

This is in concordance with the study conducted by Dileep Singh Nirwan et.al in which mean serum urea level was  $102.34 \pm 24.92$  mg/dl in cases and  $32.78 \pm 4.52$  mg/dl in control group. Mean serum urea level was significantly high in cases compare to control group ( $P=0.0001$ ) [33].

Creatinine is an endogenous substance with a molecular weight of  $113$  Da. It is produced by the muscle from creatine and creatine phosphate through a nonenzymatic dehydration process [34].

In our study serum creatinine was significantly higher in cases when compared to controls. Mean  $\pm$  SD of creatinine in chronic kidney patients is  $4.90 \pm 1.90$  where as Mean  $\pm$  SD of controls is  $1.2 \pm 0.4$ . There is a positive correlation between urea and creatinine.

Serum creatinine level was higher than normal range (up to  $1.4$  mg/dl) in CKD patients. Most of the patients have serum creatinine level between  $7.6$ - $12$  mg/dl (57%) and  $12$ - $15$  mg/dl (27 %) [35].

In a study conducted by Simran kaur et al..... on 80 patients, reported there is significant high levels of

creatinine in chronic kidney disease patients compared to normal individuals. Mean  $\pm$  SD of creatinine in cases is  $11.17 \pm 5.62$  where as mean  $\pm$  SD of creatinine in controls is  $0.63 \pm 0.29$  [36].

### Conclusion

Chronic kidney disease is a major public health problem and characterized by a progressive loss in renal function over a period of months or years as defined by structural or functional abnormalities of the kidney. Identification and staging of chronic kidney disease are evaluated by glomerular filtration rate (GFR) and proteinuria. 80 individuals were included in the study. Cases were 40 diagnosed chronic kidney disease patients. Controls were 40 normal healthy individuals. Patients with CKD have abnormal serum leptin levels which may be considered in the pathogenesis of the disease and should be taken into account in the treatment of such patients. Levels in ESRD are 4 to 7 fold higher than the healthy controls. Therefore evaluation of serum leptin levels in early stages helps in diagnosis and prognosis of cases before they lay into irreversible chronic renal failure.

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