

Original Research Article

SERUM PROLACTIN LEVEL AND INFLAMMATION IN CHRONIC KIDNEY DISEASE

Abstract

Back ground and objectives: Chronic Kidney Disease (CKD) is a major prevalent disease worldwide associated with low grade systemic inflammation, which predisposes to higher incidence of atherosclerotic complications. The pituitary hormone Prolactin (PRL) has recently been regarded as a local regulator of Macrophage responses. Both Prolactin clearance and production are altered in CKD. Emerging evidences suggests that prolactin participates in atherosclerotic processes in cases of peri /post partum cardiomyopathy and hypertension. Given the elevated cardiovascular risk of CKD, this study is intended to estimate the levels of Prolactin and Tumour Necrosis Factor α , an inflammatory marker in CKD and to decipher if there is any association amongst each other.

Methods: The study population included 45 CKD and 45 healthy controls of either gender. Fasting Blood Sugar (FBS) and Serum Creatinine were measured in the fasting sample collected from both the groups. Estimated Glomerular Filtration Rate (eGFR) was calculated using MDRD formula. Serum prolactin levels and Tumour necrosis factor α was estimated by ELISA method.

Results: The study found highly significant increase in serum TNF α levels (8.64 ± 4.97 pg/ml, $p < 0.001$) and serum prolactin levels (61.65 ± 28.9 ng/mL, $p < 0.001$) in cases when compared to controls. There was no correlation between eGFR and Prolactin of cases in different stages of CKD. No correlation was found between prolactin and TNF in all the stages of CKD.

Interpretation & conclusion: The increased prolactin levels acts independently in increasing the risk of cardiovascular diseases in cases of CKD superimposing on low grade inflammation.

Keywords: Atherosclerosis, Chronic kidney disease, inflammation, prolactin,

Introduction and need for the study:

Chronic kidney disease is one of the global health problems with a prevalence of 17.2% as per a cohort study done in India¹. Chronic kidney disease (CKD) has gained epidemic proportions worldwide due to increase in the predominance of systemic arterial hypertension, diabetes mellitus and dyslipidemia, which are found to be associated with elevated risk for cardiovascular disease². Cardiovascular disease is one of the leading causes of morbidity and mortality in all stages of chronic kidney disease.

Subclinical inflammation has been considered to play an important role in the progression of chronic diseases². Activation of the immune system has been reported in various stages of CKD³. Inflammation, associated with the effects of oxidative stress and endothelial dysfunction are considered as cardiovascular risk factors for CKD population⁴.

Prolactin is a multifunctional pituitary hormone and has other metabolic actions that are not confined to the lactating mammary gland. Prolactin has biologic actions that contribute to the atherosclerotic process subsequently predisposing to essential hypertension, acute phase of coronary syndromes, ischemic strokes. Increased expression of prolactin receptors were also found in advanced human atherosclerotic plaque⁵. High levels of serum prolactin in the body can modulate immune function including its involvement in autoimmune disease. At present, little is known about the implications of hyperprolactinemia in CKD.

In CKD, the combination of impaired immune response coupled with persistent immune stimulation and disturbed cytokine network plays a predominant role in low grade systemic inflammation⁶. However the relation of prolactin with inflammation attributing to risk of cardiovascular disease in CKD has not been proved yet. This study is intended to know the

levels of prolactin and Tumor Necrosis Factor α , an inflammatory marker in CKD and to find out if there is any association amid each other.

Materials and methods

The study was conducted at M.S. Ramaiah Medical College, Bangalore after ethical clearance was obtained from the ethical review board of the institution and informed consent was taken from the study population before the collection of the sample. The study population included 45 clinically diagnosed cases of chronic kidney disease in the age group 20-50 years, who were attending the outpatient clinic of the Department of Nephrology and 45 healthy individuals as controls, in the age group 20-50 years who visited hospital for routine health check up and were willing to be a part of this study. The exclusion criteria for the recruitment of cases included CKD patients with history of diabetes mellitus, who were on medications like anti inflammatory drugs, ACE inhibitors, Angiotensin receptor blockers or on diuretics, known cases of cardiovascular disease, with acute illness or infection, chronic kidney disease patients on dialysis. The control subjects also had same exclusion criteria as CKD cases. A detailed history, including drug history and anthropometric measurement was taken from the both the groups. Systemic examination was done and appropriate laboratory investigations were included. 5ml of venous blood sample was collected after overnight fasting of about 10-12 hours from control subjects and CKD patients in vacutainers. The sample collected in lavender vacutainer was used for estimation of hemoglobin as early as possible. The sample collected in yellow vacutainer was allowed to clot and then centrifuged at 5000 rpm for eight minutes. The serum was separated at the earliest and used for the estimation of fasting blood sugar (FBS), blood urea nitrogen (BUN), serum creatinine, uric acid, prolactin and TNF $-\alpha$ level. Blood glucose by hexokinase, Serum creatinine by alkaline picrate, kinetic rate blanked, traceable to IFCC IDMS standardized, BUN by urease-GLDH method and uric acid by uricase method on Roche Cobas 6000. Hemoglobin concentration is estimated in whole blood by the cyanide free- sodium lauryl sulphate method. The Calbiotech, Inc. (CBI) Prolactin ELISA kit is used for the quantitative measurement of prolactin in human serum. This a solid phase sandwich ELISA assay method, based on a streptavidin-biotin principle. The quantitative determination of serum TNF- α was carried out by the Diaclone TNF- α ELISA kit ⁷. This is based on Sandwich enzyme-linked immunosorbent

assay (ELISA) with monoclonal antibody sets, Streptavidin-horseradish peroxidase (HRP) conjugate and recombinant cytokines as the standard were used. The plate was read at 450 nm in an ELISA reader (CPC diagnostics, Stat Fax 4700, microstrip reader).

Estimated GFR (eGFR) was calculated by using the Modification of Diet in Renal Disease (MDRD) Formula⁸.

$$\text{eGFR (mL/min/1.73 m}^2\text{)} = 175 \times (\text{S.cr})^{-1.154} \times (\text{Age})^{-0.203} \times (0.742 \text{ if female}) \times (1.212 \text{ if African American}).$$

CKD patients were grouped based on eGFR calculated by MDRD formula:

Stage I CKD - eGFR: ≥ 90 mL/min with demonstrable kidney damage

Stage II CKD - eGFR: 60-89 mL/min

Stage III CKD - eGFR: 30-59 mL/min

Stage IV CKD - eGFR: 15-29 mL/min

Stage V CKD - eGFR : < 15 mL/min

Statistical Methods

The results were expressed as mean $\pm$ SD. Significance was assessed at 5% level of significance. Categorical data was represented in the form of number (n) and percentage (%). Student “t” test (two tailed, independent) was used to find the significance of study parameters. Pearson correlation was used to study the relation between the various parameters. Statistical analysis was performed using SPSS 12.0 software.

Results:

The data of controls and chronic kidney disease cases are compared with respect to serum creatinine, BUN, uric acid, FBS, hemoglobin, serum prolactin, Tumor necrosis factor- α and calculated parameters like eGFR and BMI. Out of 45 cases included in the study, 65% were males and 35% females (Table 1). There is no evident variation in age, height, weight, BMI, waist and hip circumference between controls and CKD cases as shown in Table 1.

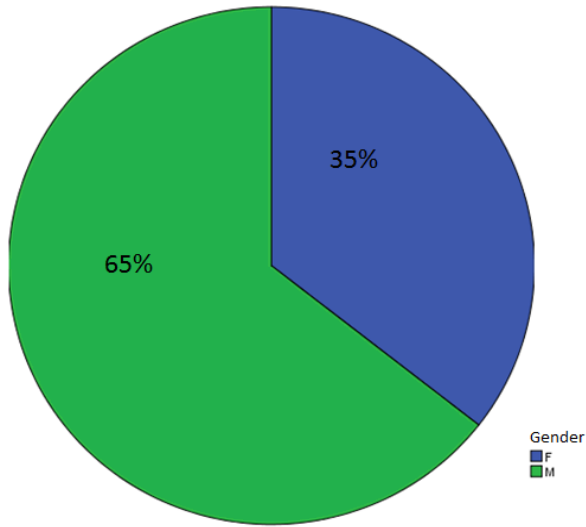


Figure 1: Percentage of male and female gender in CKD cases.

Table 1: Demographic and anthropometric measurements of CKD cases and controls (mean±SD).

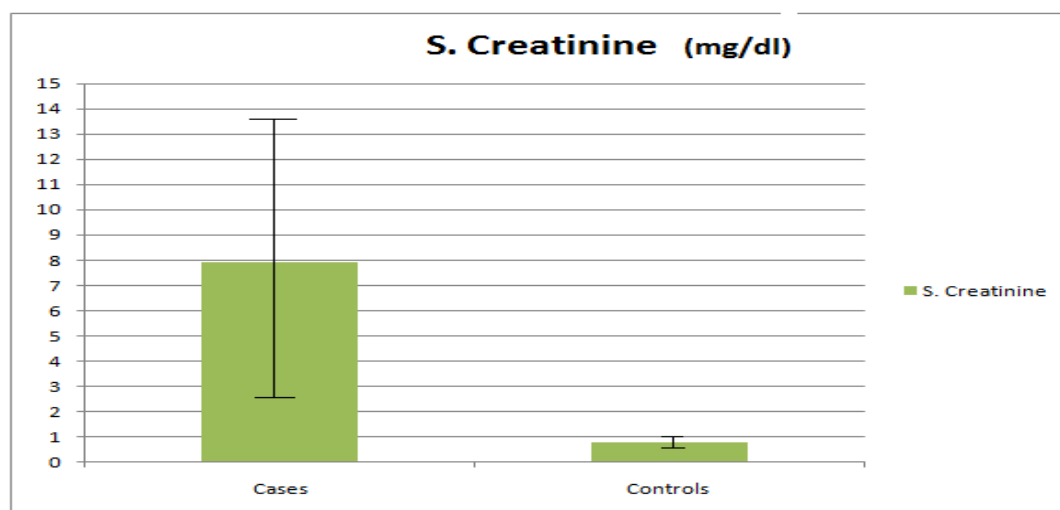
Profiles	CKD cases	Controls	P value
Age (years)	36.1±9.5	35.1±8.7	0.605
Height (cms)	163.5±7.4	164.5±6.8	0.509
Weight (kg)	64.3±10.6	65.2±10.3	0.692
BMI (Kg/m ²)	24±3.6	24±2.8	0.977
Waist circumference (cm)	73.6±11.6	78±13.2	0.096
Hip circumference (cm)	82.4±12.2	86.2±13.8	0.178
Waist Hip Ratio	0.9±0.1	0.9±0.0	0.207

129 **Table 2: Comparison of Biochemical parameters among CKD cases and controls**
 130 **(mean±SD).**

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Biochemical Parameters	CKD cases (mean±SD)	Controls (mean±SD)	P value
Fasting Blood Glucose (mg/dl)	93.9±17.9	90.5±10.5	0.276
Serum Creatinine (mg/dl)	7.9±5.7	0.8±0.2	<0.001**
Serum Uric Acid (mg/dl)	7.7±2.5	4.5±1.5	<0.001**
Blood Urea Nitrogen (mg/dl)	62.1±35.5	8.6±4	<0.001**
Hemoglobin (gm%)	9.2±2.3	13.6±1.9	<0.001**

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135 **Figure 2: Serum Creatinine level (mg/dl) in CKD cases and controls (mean±SD).**

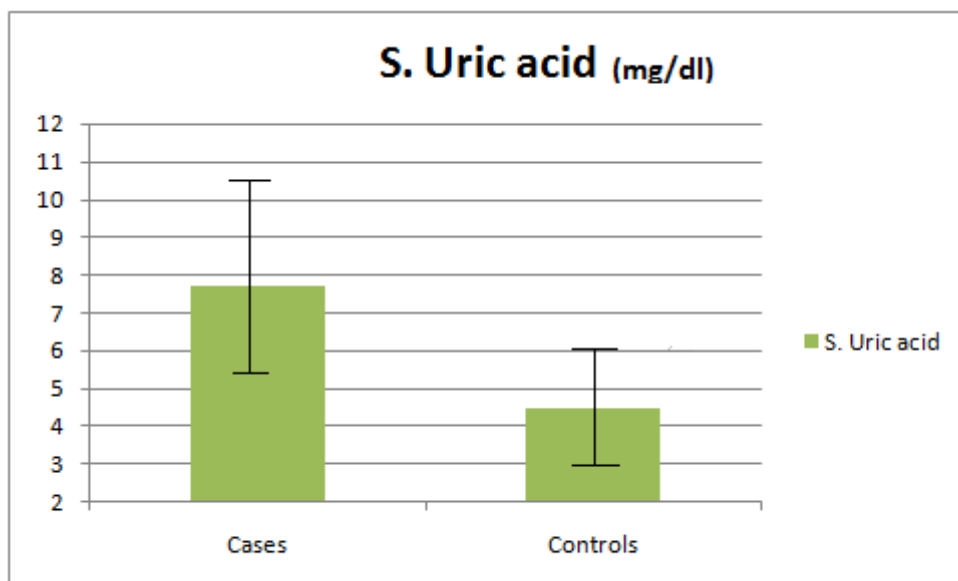


Fig 3: Serum uric acid level (mg/dl) in CKD cases and controls (mean±SD).

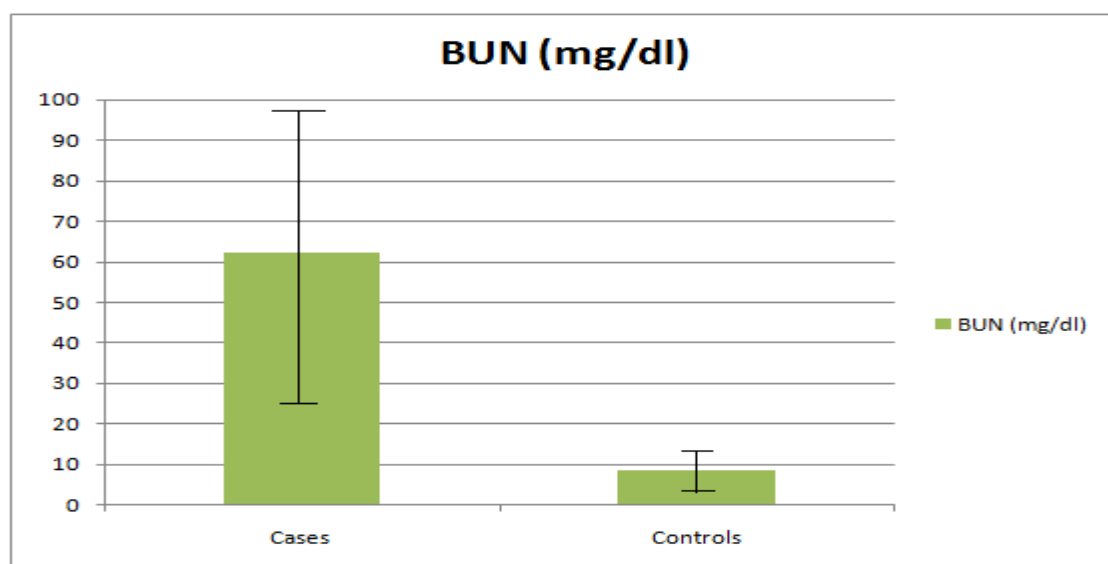


Fig 4: Blood urea nitrogen level (mg/dl) in CKD cases and controls (mean±SD).

Blood urea, S. creatinine, uric acid, eGFR was found to be increased in CKD cases as compared to controls with a p value of <0.001 (Table 2). The blood urea nitrogen levels of both the groups

are shown in fig 4. In CKD, due to compromise in renal functions there is retention of nitrogenous wastes including increase in serum concentration of blood urea nitrogen. As evident from the above table 2, there is significant increase in mean values of serum creatinine, uric acid and BUN ($p < 0.001^{**}$). The study populations recruited were based on the deranged S. creatinine levels for the CKD cases.

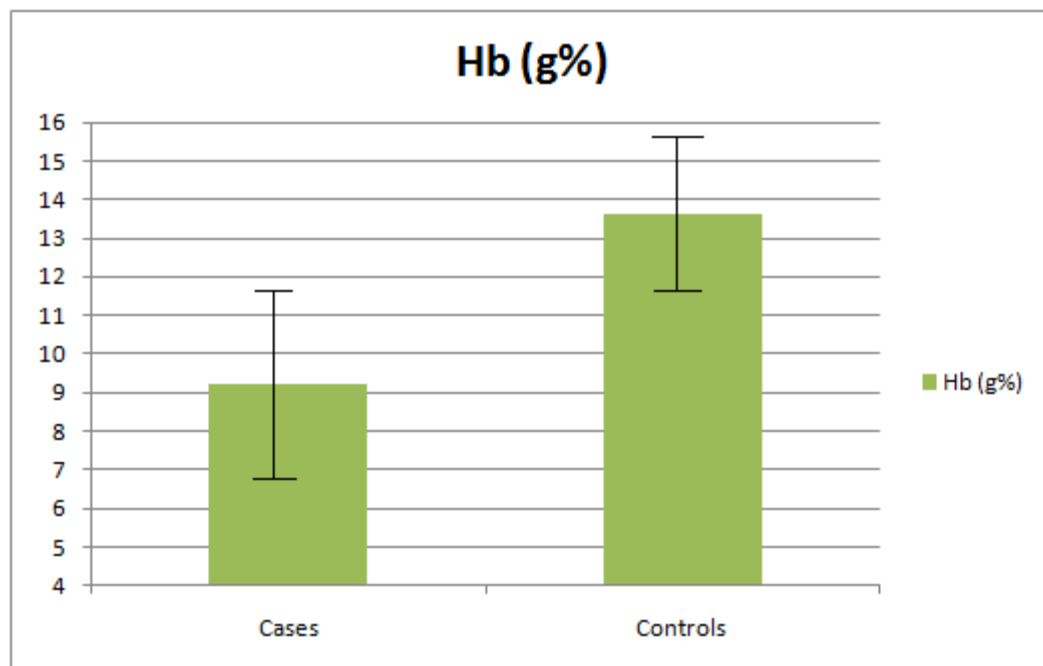
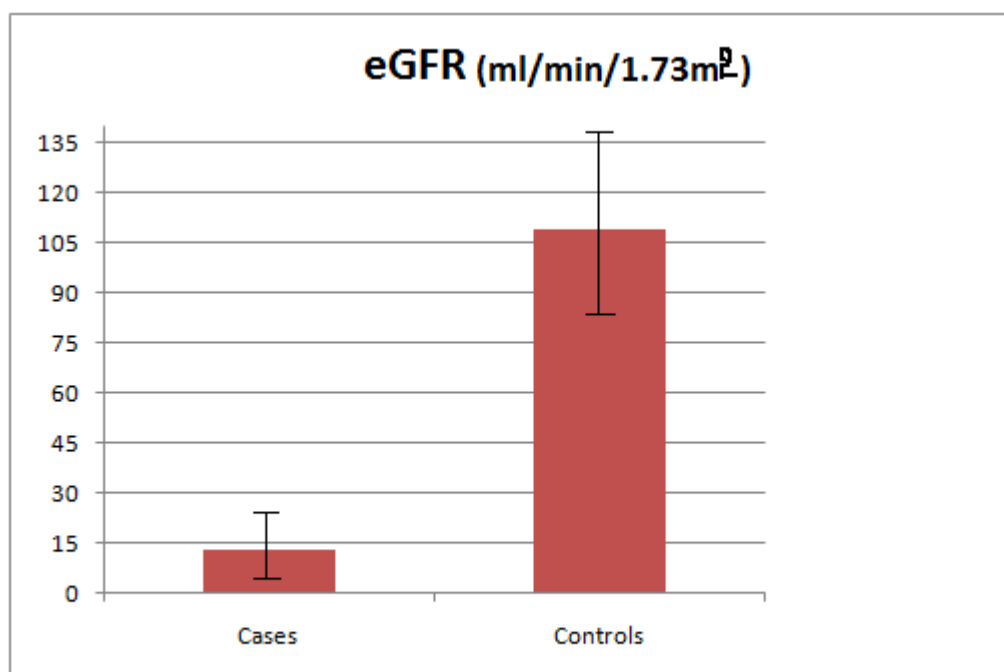


Fig 5: Hemoglobin level (gm %) in CKD cases and controls (mean $\pm$ SD).

There was significant decrease in Hemoglobin concentration (gm %) in CKD cases when compared to controls ($p < 0.001$) (Fig 4). In renal disease there is decrease in the production of hemoglobin resulting in anemia as Erythropoietin is secreted by peri-tubular fibroblasts of kidney.

165 **Table 3: eGFR in CKD cases and controls (mean±SD).**

Parameter	CKD cases	Controls	P value
eGFR(ml/min/1.73m ²)	13.24±8.84	109±27.96	<0.001**



166
167 **Figure 6: Diagram showing eGFR in CKD cases and controls (mean±SD).**

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169 In the study mean eGFR was 13.24±8.84 ml/min/1.73m² in CKD cases and 109±27.96
170 ml/min/1.73m² in controls (Table 4). There is highly significant decrease in eGFR in CKD cases
171 as compared to controls. As CKD progresses, there is fall in Glomerular filtration rate with
172 decrease in clearance of various substances.

Table 4: Serum TNF- α (pg/ml) and Prolactin (ng/ml) level in CKD cases and controls (mean $\pm$ SD).

Parameter	CKD cases	Controls	P value
S. TNF- α (pg/mL)	8.64 $\pm$ 4.97	2.06 $\pm$ 1.34	<0.001**
S. Prolactin (ng/ml)	61.65 $\pm$ 28.9	11.76 $\pm$ 8.55	<0.001**

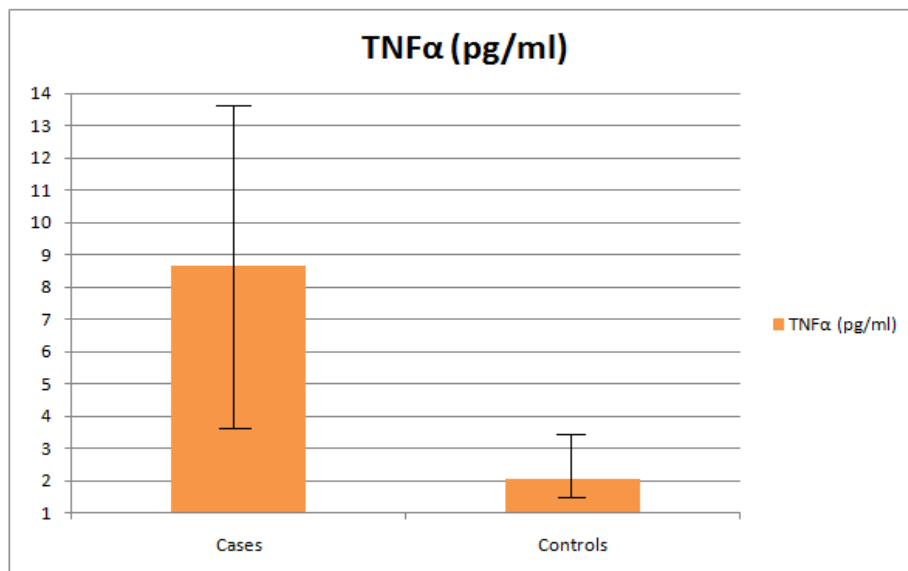


Fig 7: Serum TNF- α in CKD cases and controls (mean $\pm$ SD).

Serum TNF- α level (pg/ml) in CKD cases and controls are shown in fig 7. It is observed that there is a fourfold increase in serum TNF- α in CKD patients when compared to controls. Increased production from activated monocytes and decreased excretion from the diseased kidneys subsequently leads to increased levels of serum TNF- α .

Table 5: eGFR level of cases in different stages of CKD (mean±SD).

CKD stages	N	eGFR(ml/min/1.73m ²) (mean±SD)	P value
Stage 1	3	81.93±27.68	<0.001**
Stage 2	1	60.00±0.00	
Stage 3	5	42.96±3.31	
Stage 4	14	22.71±4.46	
Stage 5	22	9.55±3.56	

Based on eGFR value staging of CKD is done. Table 5 shows mean eGFR in CKD cases and are grouped under different stages.

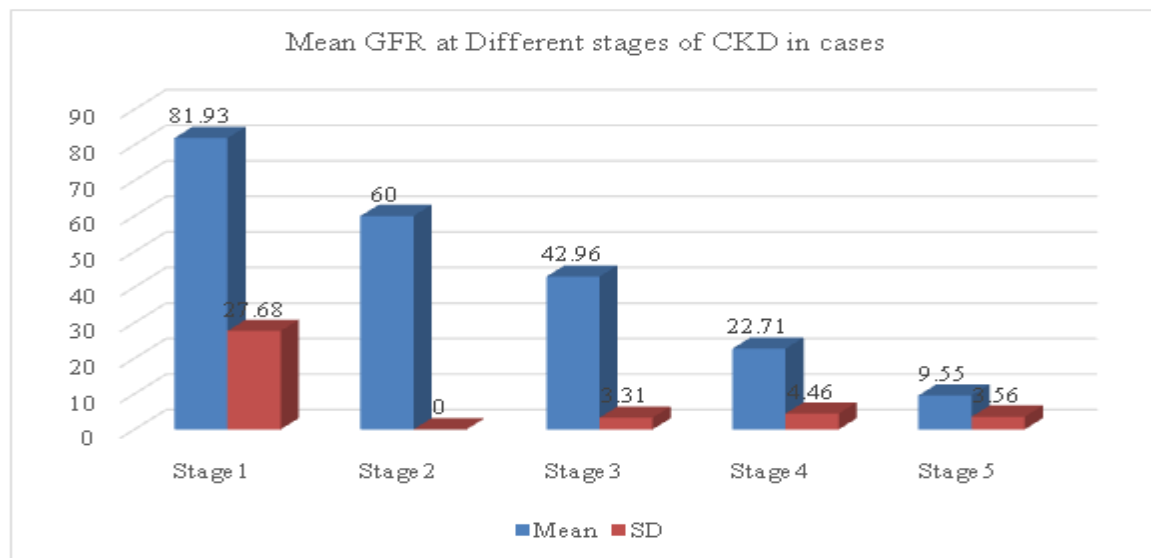


Figure 8: Diagram showing eGFR of cases in different stages of CKD (mean±SD).

Table 6: Correlation of Serum Prolactin with TNF- α and eGFR in CKD cases.

Biochemical parameters	R value	P value
Prolactin and TNF alpha	-0.117	0.467
Prolactin and eGFR	-0.169	0.26

Table 7: Correlation between eGFR and Prolactin of cases in different stages of CKD

STAGES	eGFR(ml/min/1.73m ²) (mean $\pm$ SD)	Prolactin (ng/ml) (mean $\pm$ SD)	No of cases(n)	Correlation coefficient (r)	P value
Stage 1	81.93 $\pm$ 27.68	4.04 $\pm$ 1.8	3	---	---
Stage 2	60.00 $\pm$ 0.00	4.86	1	---	---
Stage 3	42.96 $\pm$ 3.31	6.3 $\pm$ 1.89	5	0.52	0.36
Stage 4	22.71 $\pm$ 4.46	11.11 $\pm$ 8.4	14	-0.002	0.99
Stage 5	9.55 $\pm$ 3.56	63.15 $\pm$ 31	22	-0.04	0.85

Table 6 and 7 show decrease in GFR and elevation in S. Prolactin level in CKD cases, but there is no significant correlation in the present study. The mechanism influencing the rise in S. Prolactin and TNF- α are independent of each other.

219 **Table 8: Correlation between TNF- α and Prolactin in different stages of CKD**

STAGES	TNF- α (pg/ml) (mean $\pm$ SD)	Prolactin (ng/ml) (mean $\pm$ SD)	No of cases (n)	Correlation coefficient (r)	P value
Stage 1	4.35 $\pm$ 2.6	4.04 $\pm$ 1.8	3	---	---
Stage 2	4.2	4.86	1	---	---
Stage 3	4.68 $\pm$ 3.02	6.3 $\pm$ 1.89	5	-0.86	0.06
Stage 4	5.19 $\pm$ 2.57	11.11 $\pm$ 8.4	14	-0.22	0.45
Stage 5	10.36 $\pm$ 5.27	63.15 $\pm$ 31	22	0.14	0.53

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 221 There was no significant correlation between Tumor necrosis factor- α and prolactin in
 222 different stages of CKD (Table 8). Though there has been increase in serum levels of Tumor
 223 necrosis factor- α and prolactin in chronic kidney disease cases but no significant correlation
 224 is evident in the present study indicating the rise in their levels are independent of each other.
 225 The mechanism underlying for their elevation is not interdependent of each other.

226 Discussion

227 Chronic kidney disease is found to be associated with low grade inflammation and
 228 resulting in increased production of atherogenic and proinflammatory compounds ⁹. In the
 229 present study there is increase in serum prolactin and tumor necrosis factor α in chronic
 230 kidney disease. There is gradual increase in inflammation with the progression of CKD. There
 231 are reports which indicate prolactin was able to modulate the inflammatory response, stimulate
 232 adhesion of mononuclear cells to endothelium and enhance vascular smooth muscle cell
 233 proliferation thereby modulate the inflammatory response ¹⁰. Vasoconstrictive effects of
 234 prolactin via β 2-adrenergic and nitric oxide mechanisms further predispose towards increased
 235 cardiovascular risk. There are studies which indicate that endothelial dysfunction and arterial
 236 stiffness are not the sole mechanisms of action mediating this effect ¹¹. Corroero et al ⁵ have
 237 reported selective deteriorating effect of prolactin toward arteriosclerosis.

238 Prolactin retention leads to inhibition of gonadotropic hormone production including
 239 testosterone deficiency in male CKD patients and has been linked to increased atherosclerotic

plaque occurrence, systemic inflammation, cardiovascular risk and mortality¹². In the present study there is fivefold increase in serum prolactin level in CKD cases as compared to controls. Increased prolactinemia can be consequent to decreased dopaminergic activity, which can lead to increased norepinephrine release and can have adverse effects on endothelial function and on other organs consequently predisposing to cardiovascular comorbidities⁵.

The secretion of prolactin is influenced by various substances including monoamines, endogenous opiates and can be altered in uremia¹⁴. Hyperprolactinemia in CKD can be either due to reduced renal clearance or increased production of prolactin¹⁵. Serum prolactin level rise correlates with decline in glomerular filtration rate. Increased serum prolactin levels are also reported in several systemic as well as organ-specific autoimmune diseases. Its elevation can be due to increased release of prolactin from the anterior pituitary due to inflammatory cytokines, reduced production of suppressive dopamine or due to increased production of prolactin in immune system cells¹⁶. Prolactin-receptors are present in T and B lymphocytes, natural killer cells and intestinal epithelial cells. The rise in serum prolactin increases the ability of the immune cells to proliferate and produce cytokines such as TNF-alpha, IL-6, IL-1 beta^{16,17}. Prolactin due to its immune modulatory action can interfere with lymphocyte activation and cytokine production¹⁸. Oxidative stress can bring about proteolytic cleavage of prolactin into a potent antiangiogenic, proapoptotic and proinflammatory 16-kD subform that may initiate the atherosclerotic-related complications¹⁹. In the present study increase in serum prolactin level is very evident in later stages of CKD, as in stage IV and stage V.

CKD is associated with deleterious effect on physiological and metabolic functions including worsening of kidney function, uremia, acidosis, malnutrition and others. The progression of metabolic and cellular dysfunction, both systematically and locally within kidney tissue in CKD eventually can result in increased production of proinflammatory cytokines. These proinflammatory cytokines can increase the production of reactive oxygen species (ROS) which sequentially can further activate cytokine production resulting in detrimental effects on renal function²⁰. Prolactin is metabolized in the kidneys and liver and in case of renal failure due to decreased renal clearance results in accumulation of prolactin in the blood leading to hyperprolactinemia. Morgan et al²¹ have mentioned that proinflammatory cytokines increase the production of reactive oxygen species (ROS) and are themselves regulated in a positive feedback

loop via the nuclear factor kappa beta (NF- κ B) pathway. Nian et al ²² have reported the cellular damage caused by the pro inflammatory response stimulates the ROS production cycle and in turn activating further cytokine production. In CKD, the combination of impaired immune response coupled with persistent immune stimulation and disturbed cytokine network plays a predominant role in low grade systemic inflammation which mediates the process of monocyte influx, proliferation of macrophages and matrix expansion resulting in glomerular sclerosis and tubulointerstitial injuries ²³. These tissue resident macrophages promote the production of pro inflammatory cytokines like C reactive protein, interleukin 6, tumor necrosis factor α and others.

TNF α binds to TNF cell surface receptors on target cells and induces expression of adhesion molecules, chemokines for leucocytes and apoptosis in susceptible cells ²⁴. TNF α can mediate progressive renal injury and can be used as marker of inflammation. In the present study there is nearly four times increase in TNF- α levels in CKD cases as compared to controls. Tonelli et al ²⁵ have reported C reactive protein and TNF α are elevated in the initial stage of renal dysfunction and are independently associated with accelerated rate of loss of kidney function. TNF- α is a proinflammatory cytokine whose production is stimulated by angiotensin II, and which is associated with interstitial fibrosis through myofibroblast differentiation and NF- κ B activation.

Cytokines induce local proliferation of tubular and interstitial cells, synthesis of extracellular matrix, pro-coagulant endothelial activity, formation of oxygen reactive species and increased expression of adhesion molecules and biologically active lipids in renal tissues ²⁶. Cytokine release is also related to the hemodynamic and local effects of the activation of the renin-angiotensin system (RAS). The RAS plays a central role in intracellular signaling processes, possibly by modulating the inflammatory response associated with renal disease progression and susceptibility to cardiovascular dysfunction. RAS and cytokine may lead to modified expression of inflammatory cytokines and, consequently, to renal disease progression and the development of cardiovascular dysfunction in CKD patients ^{27,28}. Though there is significant rise in prolactin and TNF- α level in later stages of CKD, that is in stage IV, and stage V but there is no significant correlation between serum prolactin and TNF- α . In the present study though there is increase in serum prolactin and TNF- α , but the elevation of their levels are independent of each other.

300

301 However, studies need to be carried out to confirm, if therapeutic intervention of
 302 hyperprolactinemia can improve cardiovascular-related outcomes including reduction in arterial
 303 pressure. The limitations of the present study include single measurement of the
 304 proinflammatory cytokines and prolactin which may not represent average levels of these
 305 biomarkers overtime. In conclusion inflammation and alteration in immune response can
 306 aggravate the pathological consequence in chronic kidney disease. Modulation of the immune-
 307 inflammatory response can be targeted towards management of CKD treatment to reduce
 308 cardiovascular risk.
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310 **List of References:**

- 311 1. Singh AK, Farag YMK, Mittal BV, Subramanian KK, Reddy SRK, Acharya VN, et al.
 312 Epidemiology and risk factors of chronic kidney disease in India – results from the SEEK
 313 (Screening and Early Evaluation of Kidney Disease) study. BMC Nephrology. 2013; 14:
 314 114-123.
- 315 2. Vianna HR, Soares CMBM, Tavares MS, Teixeira MM, Silva ACS. Inflammation in
 316 chronic kidney disease: the role of cytokines. Brazilian Journal of Nephrology. 2011; 33(3):
 317 351-364.
- 318 3. Imig JD, Ryan MJ. Immune and Inflammatory Role in Renal Disease. Comprehensive
 319 Physiology. 2013; 3(2): 957-976.
- 320 4. Meenakshi Sundaram SP, Nagarajan S, Manjula Devi AJ. Chronic kidney disease—effect
 321 of oxidative stress. Chinese Journal of Biology. 2014,1-6.
- 322 5. Carrero JJ, Kyriazis J, Sonmez A, Tzanakis I, Qureshi AR, Stenvinkel P, Saglam M,
 323 Stylianou K, Yaman H, Taslipinar A, Vural A. Prolactin levels, endothelial dysfunction,
 324 and the risk of cardiovascular events and mortality in patients with CKD. Clinical Journal
 325 of the American Society of Nephrology. 2012; 7(2): 207-215.
- 326 6. Abdullah AM, Hardan AR, Latif II, Abd-Al-Hassani LJ. Role of hemodialysis and hepatitis
 327 C virus infection in circulating Th1 and Th2 cytokines in patients with chronic renal
 328 disease. Journal of Genetic and Environmental Resources Conservation. 2013; 1(1): 6-11.

- 329 7. Chen G, Shi J, Jin W, Wang L, Xie W, Sun J, Hang C. Progesterone administration
330 modulates TLRS/NF- κ B signaling pathway in rat brain after cortical contusion. *Annals of*
331 *Clinical & Laboratory Science*. 2008; 38(1): 65-74.
- 332 8. Level AS, Eckardt KU, Tsukamoto Y, Levin A, Coresh J, Rossert J, et al. Definition and
333 Classification of chronic kidney disease: A position statement from Kidney Disease
334 Improving global outcomes. *Kidney International*. 2005; 67: 2089-2100.
- 335 9. Suliman ME, Stenvinkel P. Contribution of inflammation to vascular disease in chronic
336 kidney disease patients. *Saudi Journal of Kidney Diseases and Transplantation*. 2008; 19(3):
337 329- 345.
- 338 10. Reuwer AQ, Twickler MT, Hutten BA, Molema FW, Wareham NJ, Dallinga-Thie GM,
339 Bogorad RL, Goffin V, SminkBol M, Kastelein JJ, Boekholdt SM. Prolactin levels and the
340 risk of future coronary artery disease in apparently healthy men and women. *Circulation:*
341 *Cardiovascular Genetics*. 2009; 2(4): 389-395.
- 342 11. Molinari C, Grossini E, Mary DA, Uberti F, Ghigo E, Ribichini F, Surico N, Vacca G.
343 Prolactin induces regional vasoconstriction through the β 2-adrenergic and nitric oxide
344 mechanisms. *Endocrinology*. 2007; 148(8): 4080-4090.
- 345 12. Ros S, Carrero JJ. Endocrine alterations and cardiovascular risk in CKD: is there a link.
346 *Nefrologia*. 2013; 33(2): 181-187.
- 347 13. La Torre D, Falorni A. Pharmacological causes of hyperprolactinemia. *Therapeutics and*
348 *Clinical Risk Management*. 2007; 3(5): 929- 951.
- 349 14. Biasioli S, d'Andrea G, Micieli G, Feriani M, Borin D, Chiaramonte S, Cananzi A, La
350 Greca G. Hyperprolactinemia as a marker of neurotransmitter imbalance in uremic
351 population. *The International journal of artificial organs*. 1987; 10(4): 245-257.
- 352 15. Majumdar A, Mangal NS. Hyperprolactinemia. *J Hum Reprod Sci*. 2013; 6(3): 168–175.
- 353 16. Rathi M, Ramachandran R. Sexual and gonadal dysfunction in chronic kidney disease:
354 Pathophysiology. *Indian J Endocrinol Metab*. 2012; 16(2): 214–219.
- 355 17. Zheng SX, Vrindts Y, Lopez M, De Groote D, Zangerlé PF, Collette J, Franchimont N,
356 Geenen V, Albert A, Reginster JY. Increase in cytokine production (IL-1 β , IL-6, TNF- α
357 but not IFN- γ , GM-CSF or LIF) by stimulated whole blood cells in postmenopausal
358 osteoporosis. *Maturitas*. 1997; 26(1): 63-71.

18. Petrovsky N. Towards a unified model of neuroendocrine – immune interaction. Immunology and cell biology. 2001; 79(4): 350-357.
19. Ferrag F, Lebrun JJ, Touraine P, Nagano M, Dardenne M, Kelly PA. Prolactin and the immune system. Immunomethods. 1994; 5(1): 21-30.
20. Carrero JJ, Kyriazis J, Sonmez A, Tzanakis I, Qureshi AR, Stenvinkel P, Saglam M, Stylianou K, Yaman H, Taslipinar A, Vural A. Prolactin levels, endothelial dysfunction, and the risk of cardiovascular events and mortality in patients with CKD. Clinical Journal of the American Society of Nephrology. 2012; 7(2): 207-215.
21. Yang D, Elner SG, Bian ZM, Till GO, Petty HR, Elner VM. Pro-inflammatory cytokines increase reactive oxygen species through mitochondria and NADPH oxidase in cultured RPE cells. Experimental eye research. 2007; 85(4): 462-472.
22. Morgan MJ, Liu ZG. Crosstalk of reactive oxygen species and NF- κ B signaling. Cell research. 2011; 21(1): 103-115.
23. Nian M, Lee P, Khaper N, Liu P. Inflammatory cytokines and postmyocardial infarction remodeling. Circulation research. 2004; 94(12): 1543-1553.
24. Hildebrandt GC, Olkiewicz KM, Corrión L, Clouthier SG, Pierce EM, Liu C, Cooke KR. A role for TNF receptor type II in leukocyte infiltration into the lung during experimental idiopathic pneumonia syndrome. Biology of Blood and Marrow Transplantation. 2008; 14(4): 385-396.
25. Tonelli M, Sacks F, Pfeffer M, Jhangri GS, Curhan G. Biomarkers of inflammation and progression of chronic kidney disease. Kidney international. 2005; 68(1): 237-245.
26. Dal Canton A. Adhesion molecules in renal disease. Kidney international. 1995; 48(6): 1687-1696.
27. Morgado E, Neves PL. Hypertension and Chronic Kidney Disease: Cause and Consequence—Therapeutic Considerations. Antihypertensive Drugs, Prof. Hossein Babaei (Ed). InTech, Portugal. 2012:45-66.
28. Wong C, Kanetsky P, Raj D. Genetic polymorphisms of the RAS-cytokine pathway and chronic kidney disease. Pediatric Nephrology. 2008; 23(7): 1037-1051.