Plasma pentraxin-3 level as a biomarker in patients with Chronic Kidney Disease and its Association with Cardiovascular complications

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Abstract: Chronic kidney disease (CKD) is a major public health problem world-wide. Recent professional guidelines classify the severity of CKD into five stages starting with stage 1, being the mildest and usually causing few symptoms, to stage 5, being a severe illness with poor life-expectancy if untreated. CKD is recognized as a common condition that is associated with an increased risk of cardiovascular disease and chronic renal failure (CRF). A number of promising markers that help in assessing the progression of CKD are now available as they help to implement potentially effective therapies in a timely manner. Pentraxin 3 (PTX3) is a 40.6 Kd protein that belongs to the pentraxin super family of multifunctional conserved proteins. It is expressed at a low level in some tissues including the kidney. PTX3 is synthesized systemically in response to kidney damage, followed by glomerular filtration and tubular uptake, and it could be produced locally by injured tubules. PTX3 is also identified in vascular endothelial cells (ECs) and monocytes. Human peripheral blood monocytes express significant levels of PTX3 in response to the pro-inflammatory cytokines. PTX3 is rapidly produced from the cells involved in atherosclerotic lesions, namely vascular endothelial cells, vascular smooth muscle cells, macrophages, and neutrophils in response to inflammatory stimuli. In order to evaluate the clinical utility of PTX3 in chronic kidney disease as well as its association with cardiovascular diseases (CVD). Plasma PTX3 levels were measured in 50 adult patients with CKD (stages 3-5 based on estimated glomerular filtration rate (eGFR) by modification of diet in renal disease (MDRD) study equation) divided into two groups: Group 1(Stages 3-4) and Group 2: (Stage 5). Each group was subdivided into two groups according to presence or absence of cardiovascular diseases (CVD). Twenty apparently healthy age- and sex-matched subjects served as a control group. Assay was carried out using an ELISA technique, and results were expressed as ng/mL. Furthermore, kidney function tests (BUN, creatinine, eGFR & calculated GFR), lipid profile (total cholesterol &triglycerides) as well as CRP were also measured. Plasma PTX3 showed a highly significant increase in CKD patients collectively as compared to healthy controls. There was also a highly significant stepwise progressive increase in PTX3 levels from stage 3 through stage 5 indicating that PTX3 is a marker of disease progression. PTX3 levels were also significantly higher in CKD patients with CVD when compared to those without CVD indicating that PTX3 increases in CVD independent of CKD. **Conclusion:** Plasma PTX3 is a promising independent diagnostic marker for identifying patients with CKD as well as it is proposed as a useful indicator of disease progression. Increased plasma level of PTX3 may accelerate the vascular complications in CKD, in addition plasma PTX3 in conjunction with serum CRP can help in differentiation between CKD patients with CVD from those without CVD. **Aim of the Work:** The aim of the present study is to evaluate the plasma levels of pentraxin 3 (PTX3) in patients with chronic kidney disease as a diagnostic and prognostic biomarker and compare its levels with other inflammatory markers as CRP and the possible association of PTX3 with cardiovascular disease in these patients.

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1.Introduction:

Chronic kidney disease (CKD) is a worldwide public health problem. It is recognized as a common condition that is associated with an increased risk of cardiovascular disease and chronic renal failure (CRF) ***(Bash et al., 2009).*** Chronic kidney disease (CKD) is defined as the presence of objective kidney damage and/or the presence of glomerular filtration rate (GFR) of 60 mL/min/1.73 m² body surface area or less for at least three months irrespective of the underlying etiology of the kidney damage ***(Shavit et al., 2012).***

Chronic kidney disease promotes hypertension and dyslipidemia, which in turn can contribute to the

progression of renal failure**.** Furthermore, hypertension and dyslipidemia are major risk factors for the development of endothelial dysfunction and progression of atherosclerosis ***(Ernesto et al., 2007).*** Accelerated atherosclerosis will lead to an increase in the prevalence of coronary and peripheral artery diseases, heart failure and stroke. Consequently, subjects with CKD are exposed to increased morbidity and mortality as a result of cardiovascular events ***(Mantovani et al., 2006 and Inoue et al., 2007).*** Most people with CKD will die as a result of heart disease rather than kidney failure. In fact, heart disease causes 40-50% of all deaths in patients with CKD ***(Schoolwerth et al., 2006).*** The mechanisms for the elevated CVD risk in CKD are complex and may involve changes in both the heart and vasculature already at early stages. Of these, endothelial dysfunction increases in prevalence as renal function declines and is considered a prodromal phase in the atherosclerosis that precedes cardiovascular complications **(*Carrero and Stenvinkel , 2010).*** The etiology of endothelial dysfunction in CKD is likely multifaceted, involving the dysregulation of various pathways.

One of these pathways could be mediated by the TNF-like weak inducer of apoptosis (TWEAK, TNFSF12) **(*Winkles,2008*).** Another potential dysregulated pathway in CKD-associated endothelial dysfunction may involve long pentraxin 3 (PTX3), a multimeric mediator that shares structural homology with hepatic short pentraxins such as C-reactive protein (CRP) and serum amyloid P component, but that it is expressed by many cell types, especially in the vasculature, in response to injury and stress ***(Bottazzi et al., 2009)***.

As a rule, kidney failure due to CKD is preceded by a stage of variable length during which GFR is decreased. GFR is affected by a number of factors in addition to kidney disease, and not all individuals with decreased GFR have CKD. Mild reduction in GFR is defined as chronic kidney disease only in the presence of kidney damage (Stage 2). However, because of the risk of complications, moderate (Stage 3) to severe (Stage 4) reduction in GFR and kidney failure (Stage 5) are defined as CKD, irrespective of the presence of kidney damage. Other than kidney disease, the most important factor affecting GFR is age. Mild reduction in GFR may be "normal" at the extremes of age and, in the absence of kidney damage, is not considered to be CKD ***(National Kidney Foundation K/DOQI, 2002).***

Pentraxin 3 (PTX3) is known as tumor necrosis factor-stimulated gene 14 (TSG-14). It belongs to the pentraxin super family of multifunctional conserved proteins that are characterized by a cyclic multimeric structure and by the presence in their carboxy-terminus of an approximately 200 amino acid long conserved domain, called the 'pentraxin domain'. In addition, all the members of this family share an eight amino acid long domain, called 'pentraxin signature' ***(Fabrizia et al., 2013).*** Proteins of the pentraxin family are involved in acute immunological responses ***(Sébastien et al., 2007).*** They are a class of pattern recognition receptors (PRRs) targeted to various microbial and self determinants including polysaccharides, phosphocholine, and phosphoethanolamine on the surface of microorganisms, apoptotic or necrotic cells, and nuclear autoantigens ***(Jinghua et al., 2011).*** On the basis of the primary structure of the subunit, the pentraxins are divided into two groups: Short pentraxins (*e.g.*, CRP, serum amyloid P) and long pentraxins. The prototype protein of the long pentraxin group is pentraxin 3 (PTX3) ***(Mengli et al., 2007).*** PTX3 is a long pentraxin produced in response to inflammatory signals by immune cells in contrast to CRP that is produced by hepatic cells ***(Robert et al., 2011).***

PTX3 is produced by a variety of cells and tissues, most notably dendritic cells and macrophages, in response to Toll-like receptor (TLR) engagement and inflammatory cytokines. Through interaction with several ligands, including selected pathogens and apoptotic cells, PTX3 plays a role in complement activation, pathogen recognition and apoptotic cell clearance. It is also involved in the deposition of extracellular matrix ***(Deban et al., 2009)***.

PTX3 may amplify the inflammatory response after being produced both in peripheral tissues and in the kidney. The production of PTX3 in human renal epithelial cells was reported, suggesting a role in the innate immune response and inflammatory reactions in the kidney; it may also play an important role in the atherogenic process present in CKD ***(Mengli et al., 2007).***

In cardiovascular system, the role of PTX-3 appears to be primarily protective. Activated platelets cause much of tissue damage in myocardial infarction. Hence, they are the predominant target cells of PTX-3. Upon extrusion into the extracellular space, neutrophil-originating PTX-3 binds to adjacent platelets. PTX-3-stained platelets are thereafter resistant to the formation of platelet-platelet homoaggregates or to platelet-neutrophil or platelet-monocyte heteroaggregates. Furthermore, PTX-3 broadly impacts the adhesion molecule P-selectin, which is known to play distinct roles in atherogenesis. PTX-3 brings about downregulation of P-selectin-dependent neutrophil recruitment to inflammatory sites and of P-selectin-induced cellular heteroaggregate formation. Hence, decreased numbers of microaggregates are present that would impair

blood flow in the microcirculation, not to speak of their additional noxious potential. In acute coronary syndromes, the overall procoagulant state is increased, including excess tissue factor formation by different cell types which makes the blood more sticky ***(Salio et al., 2008).*** Atherosclerosis is considered as an inflammatory process. PTX3 is rapidly produced from the cells involved in atherosclerotic lesions, namely vascular endothelial cells, vascular smooth muscle cells, macrophages and neutrophils, in response to inflammatory stimuli ***(Klouche et al., 2004).***

PTX3 may amplify the inflammatory response after being produced both in peripheral tissues and in the kidney**.** PTX3 is predominantly present in the inflamed tubulointerstitium in close proximity of tubular epithelial cells**.** The production of PTX3 in human renal epithelial cells was reported by ***(Nauta et al., 2005)***, together with an increased PTX3 expression in mesangial cells of renal biopsies obtained from patients with IgA glomerulonephritis. ***Bussolati et al. (2003)*** suggesting a role in the innate immune response and inflammatory reactions in the kidney.

C-reactive protein (CRP) is an acute phase reactant protein that is present in plasma of healthy humans and whose plasma concentration increases significantly during acute and chronic inflammation ***(Pepys et al., 2003).*** CRP is synthesized in a soluble form by hepatocytes from where it is secreted into the circulation. IL-1, IL-6, and TNF are considered important mediators for the modulation of CRP synthesis in the liver ***(Voleti and grawal, 2006)***.

CRP antigens were found in human early atherosclerotic lesions with the location in deep fibroelastic layer and fibromuscular layer of the intima adjacent to the media. Arterial tissue itself is able to produce CRP which is substantially up-regulated in atherosclerotic plaques. The first evidence for the presence of CRP in atherosclerotic lesion was revealed by immuno-histochemical studies ***(Yasojima et al., 2001)*.** CRP binds to modified LDL. CRP readily gets complexed in a calcium dependent manner to modified (oxidized and enzymatically-treated) LDL but not to native LDL. Binding of CRP to LDL is mediated by the PCh-binding site in CRP that interacts with the PCh and cholesterol moieties present on LDL. CRP has been found deposited and localized with LDL in human atherosclerotic lesions ***(Zacho et al., 2008).***

CRP concentration is associated with the incidence of atherothrombotic events in humans, most notably myocardial infarction ***(Ridker, 2004).*** CRP level increases in individuals with cardiovascular complications as unstable angina and myocardial infarction. In addition, an elevated CRP concentration in blood serum predicts the risk of sudden death and restenosis after percutaneous coronary intervention ***(Albert et al., 2002)***. CRP increases the risk of ischemic vascular events, such as myocardial infarction, not by promoting atherosclerotic plaque size, but rather by activating the blood coagulation system and increasing the risk of thrombosis ***(Kovacs et al., 2007).*** Measurement of serum CRP is recommended for use as an indicator of arterial inflammation and predictor of future cardiovascular events ***(Zacho et al., 2008).***

CRP is filtered in the glomerulus and reabsorbed by the distal renal tubules. CRP concentration increases with CKD due to diminished filtration of CRP ***(Stuveling et al., 2003).*** CKD causes renal inflammation and scarring of renal cortex, this inflammation leads to increase CRP level in patients of CKD. The manifested link between CRP as a marker of inflammation and CKD is the significant negative correlation of CRP with lower GFR ***(Tarver-Carr et al., 2002).***

2.Subjects and methods: This study included Fifty (50) adult patients with chronic kidney disease stages 3-5 from Alzahraa hospital in Cairo from the outpatient clinic of nephrology Their ages ranged between 25-65 years. In accordance with National Kidney Foundation K/DOQI (2002), they were classified based on the estimated GFR (eGFR), using the MDRD equation, into the following 2 CKD stage groups:

Stage 3-4 group: This stage included CKD patients with eGFR (15-59 mL/min).This group was further subdivided into 2 subgroups:

a) Stage 3-4 with CVD (n=12).

b) Stage 3-4 without CVD (n=13)

Stage 5 group: This stage included CKD patients with kidney failure (eGFR < 15 mL/min) or dialysis. This group was further subdivided into 2 subgroups:

a) Stage 5 with CVD (n=13).

b) Stage 5 without CVD (n=12) without CVD

Twenty age and sex matched healthy subjects served as control group. All groups were subjected to full history, complete clinical examination, radiological investigations of CVD as: echocardiography, CT scan, angiography and ECG and laboratory investigations (Kidney function tests including serum creatinine and blood urea nitrogen (BUN), Creatinine clearance using the estimated (eGFR) by MDRD equation, and the calculated (GFR) method ,Lipid profile including (serum cholesterol and triglycerides).Estimation of serum high sensitive C-reactive protein (hs-CRP) by ELISA: assays were carried out in serum by a sandwich enzyme-linked immunosorbent assay (ELISA) technique using reagents provided by DRG

International Inc., hs (C-reactive protein) ELISA - 3954 KIT.USA (Macy et al., 1997). Measuring of plasma Pentraxin 3 by Enzyme Linked Immunosorbant Assay (ELISA) using reagents provided by Quantikine (R&D International, Inc., 614 McKinly Place N.E., Minnespolis, and MN55413 USA.

Statistical Analysis:

Data was analyzed by Microsoft Office 2003 (excel) and Statistical Package for Social Science (SPSS) version 16.

3. Results:

The results were presented in the following figures and tables:

Table (1):-Comparative statistical study of measured plasma PTX-3 (ng/ml) and serum CRP (mg/l) among control and patients with CKD.

Parameters	Control	Patients with CKD	
	Mean ± SD (min-max)	Mean ± SD (min-max)	*P* value
PTX-3 (ng/ml)	0.41 ± 0.39 (0.1-1.2)	8.19 ± 9.28 (0.6-29.1)	<0.0001
CRP (mg/l)	0.40 ± 0.28 (0.1-1)	7.60 ± 5.01 (1-18.25)	<0.0001

❖ Statistical analysis showed a high significant increase in PTX-3 and CRP levels in all cases with CKD more than control (P<0.0001).

Table (2):-Comparative statistical study of different laboratory parameter among control and patients with CKD.

Parameters	Control	patients with CKD	*P* value
	Mean ± SD (min-max)	**Mean ± SD (min-max)**	
BUN (mg/dL)	22.50 ± 5.46 (15-31)	72.06 ± 18.52 (40-110)	<0.0001
Creatinine (mg/dL)	0.67 ± 0.20 (0.4-1.1)	5.40 ± 2.80 (2-11)	<0.0001
eGFR (mL/min)	99.92 ± 3.61 (92-105)	21.31 ± 13.20 (7-45)	<0.0001
GFR (ccc) (mL/min)	101.70 ± 4.53 (93-107)	21.39 ± 13.17 (7.5-46)	<0.0001
T-CHOL (mg/dL)	130.75 ±6.14 (120-142)	183.74 ± 26.71 (140-250)	<0.0001
TAG (mg/dl)	132.30 ±7.11 (120-145)	164.92 ± 29.62 (98-210)	<0.0001

Descriptive and comparative statistics of the various studied parameters among CKD patients and the healthy control group revealed a highly significant decrease in the eGFR and calculated GFR of CKD patients (both p<0.001). Meanwhile, CKD patients showed a highly significant increase in serum creatinine, BUN, total cholesterol and triglycerides more than control (p<0.001).

Table (3):- Mean ± SD (min-max) of plasma PTX-3 and serum CRP for the 4 studied groups compared to the control group.

	Control	Stage (3-4) without (CVD)	Stage (3-4) with (CVD)	Stage (5) without (CVD)	Stage (5) with (CVD)
	Mean ± SD (min-max)	Mean ± SD (min-max)	Mean ± SD (min-max)	Mean ± SD (min-max)	Mean ± SD (min-max)
PTX-3 (ng/ml)	0.41 ± 0.39 (0.1-1.2)	2.98±2.19 (0.6-8.7) p<0.001	3.04±2.47 (0.9-10.3) p<0.003	9.44± 6.97 (2.5-21.6) p<0.001	17.28±12.51(2.9-29.1) p<0.0001
CRP (mg/l)	0.40 ± 0.28 (0.1-1)	2.92±1.52 (1-6.5) p<0.0001	4.76±1.83 (2-8) p<0.0001	8.31±1.87 (6-11.5) p<0.0001	14.27±3.67 (9-18.25) p<0.0001

Concerning plasma PTX-3 levels, statistical analysis showed a high significant increase in each group when compared to control group.

Concerning serum CRP levels, statistical analysis showed a high significant increase in each group when compared to control group.

Table (4):- Mean ± SD (min-max) of different laboratory parameters for the 4 studied groups compared to the control group.

Parameters	Control	Stage (3-4) without (CVD)	Stage (3-4) with (CVD)	Stage (5) without (CVD)	Stage (5) with (CVD)
	Mean ± SD (min-max)	Mean ± SD (min-max)	Mean ± SD (min-max)	Mean ± SD (min-max)	Mean ± SD (min-max)
BUN (mg/dL)	22.50±5.46 (1 5-31)	54±9.03 (40-70) *p*<0.001	60.92± 10.17 (45-80) *p*<0.001	81.67± 9.72 (70-100) *p*<0.001	91.54±12.53 (75-110) p<0.001
Creatinine (mg/dL)	0.67±0.20 (0.4-1.1)	3.19±0.77 (2-4.75) *p* <0.001	3.24± 0.82 (2.5-5) *p*<0.001	6.88 ± 1.92 (4.5-10) p<0.001	8.23± 2.57 (5-11) p<0.001
eGFR (mL/min)	99.92±3.61 (92-105)	36.04±5.79 (25-45) *p*<0.001	30.56±7.11 (17-40) *p*<0.001	9.03 ±1.49 (7.5-12) p<0.001	9.40± 1.99 (7-12) p<0.001
GFR (ccc) (mL/min)	101.70±4.53 (93-107)	36.77± 5.71 (28-46) *p*<0.001	29.74± 7.06 (16-39) *p*<0.001	9.73 ± 1.14 (8-11) p<0.001	9.08± 1.45 (7.5-11) p<0.001

- Descriptive and statistical comparison between the various studied parameters in CKD patients at various stages of the disease as compared to control show a highly significant increase regarding serum creatinine, BUN (p<0.001) and a highly significant decrease in eGFR and calculated GFR (p<0.001).

Table (5): Comparative statistical study of measured plasma PTX-3 and serum CRP among patients without and with CVD.

Parameters	Patients without CVD	Patients with CVD	*P* value
	Mean± SD (min-max)	Mean± SD (min-max)	
PTX-3 (ng/ml)	5.94 ± 5.61 (0.6-18.1)	10.45 ±11.57 (0.9-29.1)	<0.05
CRP (mg/l)	5.50 ± 3.21 (1-11.5)	9.70 ±5.64 (2-18.25)	<0.01

Statistical analysis showed a significant increase in plasma PTX-3 and serum CRP levels in patients with CVD more than patients without CVD.

Table (6): Correlation between plasma PTX-3 and serum CRP in all patients with CKD.

	Patients with CKD
CRP	r= 0.895 *P*<0.01 H.S

Statistical analysis showed a highly significant positive correlation between plasma PTX-3 and serum CRP in all patients with CKD (P<0.01).

Table (7): Correlation between plasma PTX-3 and serum CRP among the studied group.

	Stage(3-4) without CVD	Stage(3-4) with CVD	Stage(5) without CVD	Stage(5) with CVD
CRP	r=0.987 *P*<0.01 H.S	r= 0.821 *P*<0.01 H.S	r=0.986 *P*<0.01 H.S	r= 0.980 *P*<0.01 H.S

Statistical analysis showed a highly significant positive correlation between plasma PTX-3(ng\ml) and serum CRP (mg/L) among studied groups (P<0.01).

Table (8): Sensitivity and specificity of plasma PTX-3 and serum CRP in prediction of CKD.

	Cut off point	sensitivity	Specificity	PPV	NPV
PTX-3	0.95ng/ml	100%	87%	95%	100%
CRP	0.65mg/l	100%	80%	92.59%	100%

N.B. PPV: Positive predictive value; NPV: negative predictive value

Plasma PTX-3 had a diagnostic sensitivity, specificity, negative predictive value and positive predictive value of 100%, 87%, 100% and 95% respectively.

In regard to serum CRP, it had a diagnostic sensitivity, specificity, negative predictive value and positive predictive value of 100%, 80% , 100% and 92.59% respectively .

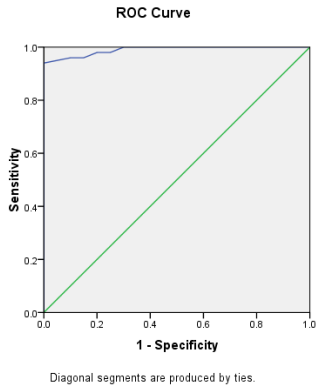

Fig. (2) :- Diagram illustrates Receiver- operating characteristics (ROC) curve for PTX-3.

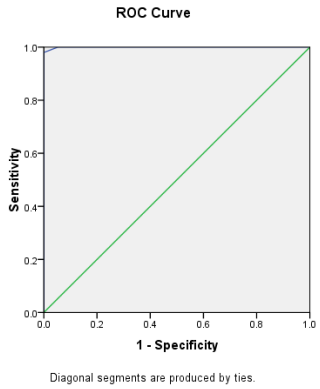

Fig. (3):- Diagram illustrates Receiver- operating characteristics (ROC) curve for CRP.

4. Discussion :

The Kidney Diseases Outcomes Quality Initiative (K/DOQI) of the National Kidney Foundation (NKF) defines chronic kidney disease (CKD) as either kidney damage or a decreased kidney glomerular filtration rate (GFR) of < 60 mL/min/1.73 m^2 for 3 months or more, irrespective of diagnosis ***(Shavit et al., 2012)***. The attention being paid globally to chronic kidney disease is attributable to five factors: the rapid increase in its prevalence, the enormous cost of treatment, data indicating that overt disease is the tip of an iceberg of covert disease, an appreciation of its major role in increasing the risk of CVD, and the discovery of effective measures to prevent its progression ***(Barsom ,2002).*** In current practice, serum creatinine is an imperfect measurement to assess GFR, because the total amount of creatinine in plasma depends on variables of age, gender, muscle mass, metabolism, medications and hydration status. Efforts to estimate GFR from a single measurement of creatinine have used various formulae, to account for dependent variables, such as in the MDRD ***(Malyszko et al., 2010)***.

Long pentraxin 3 (PTX3) is a recently discovered multimeric inflammatory mediator that is structurally linked to short pentraxins, such as C-reactive protein (CRP) and serum amyloid P component ***(Fabrizia et al., 2013)***. PTX3 is an inflammatory marker produced by mesangial cells, human renal epithelial cells as well as monocytes, macrophages, neutrophils, and fibroblasts, in response to inflammatory stimuli including IL-1, TNFα, and LPS. PTX3 is thought to be related to vascular inflammation ***(Dubin et al., 2011).*** PTX-3 levels may directly reflect the inflammatory status ***(Inoue et al., 2007)***. Because of its extrahepatic synthesis (in contrast to CRP), the PTX3 level is believed to be a true independent indicator of disease activity because PTX3 is produced at sites of inflammation and is intimately linked to endothelial dysfunction***(Fabrizia et al., 2013)***. PTX3 release is likely a specific response to vascular damage, PTX3 levels may provide more explicit information on development and progression of atherosclerosis than nonspecific markers like CRP and interleukin-6 ***(Parlak et al., 2012).*** The production of PTX3 in human renal epithelial cells, together with an increased PTX3 expression in mesangial cells of renal biopsies obtained from patients with IgA glomerulonephritis , suggesting a role in the innate immune response and inflammatory reactions in the kidney. ***(Nauta et al., 2005)***.

Indeed PTX3 is associated with impaired kidney function which may be due to endovascular inflammation, a mechanism for initiation and progression of GFR loss ***(Souza et al., 2009).*** PTX3 levels are increased in individuals undergoing hemodialysis, contributing to increase both the cardiovascular and mortality risk by pathways independent of its homologous CRP ***(Suliman et al., 2008)***.

Therefore, this study aimed at examining the levels of PTX3 in patients with CKD and correlating its levels with other inflammatory markers as CRP. The possible association of PTX3 with CVD in these patients was also investigated.

As regards routine kidney function tests, data of the present study revealed that BUN and serum creatinine were significantly higher in CKD patients when compared to the healthy control group. This is in agreement with the findings of ***Mitsnefes et al. (2007)*** who reported that higher levels of BUN and creatinine indicate a falling of GFR as a result of decreased capability of the kidney to excrete waste products.

Concerning serum creatinine, the current National Kidney Foundation K/DOQI guidelines (2002) highlighted that it is not very sensitive to changes in GFR. Hence, these guidelines advocated creatinine-based MDRD equations for estimating GFR

to identify patients with potential kidney disease. In this context, our results revealed that eGFR based on the MDRD study equation, was significantly lower in CKD patients than that of the healthy control group. Similar findings were previously confirmed by ***Devarajan (2008)***.

In the present study, plasma PTX3 showed a highly significant increase in CKD patients collectively when compared with control group levels ($P<0.01$). This finding was in agreement with ***(Pradeep et al., 2012)***. They found that patient groups with CKD had higher plasma PTX3 concentrations than control subjects**.**

These results also were in agreement with ***(Nauta et al., 2005)*** as they found that PTX3 is an important factor in the regulation of inflammatory reactions of innate immunity. The gradual increase of PTX3 concomitant to the decline in GFR could be explained by an inadequate clearance because PTX3 is a large molecular weight substance (molecular weight 40.6 KD) characterized by a multimeric, usually pentameric, structure, but it could also be explained by an enhanced synthesis/release upon stimulation in peripheral tissues and also perhaps in the remaining functioning kidney ***(Mantovani et al., 2006)***.

It was previously shown that proinflammatory cytokines can upregulate the expression of PTX3 from endothelial cells and macrophages. The chronically elevated cytokine levels in patients with CKD are likely to be at least partially responsible for PTX3 induction, and the retention of these and other toxins might altogether contribute to the overall inflammatory effect reflected by CRP values ***(Yano et al., 2005).*** Therefore, this study suggests that PTX3 may provide additional prognostic information to that obtained from CRP (short pentraxin) and that PTX3 may play an important active role in the inflammatory and atherogenic processes in patients with CKD.

Data of the present study not only demonstrate the presence of a statistically significant increase in plasma PTX3 levels in each CKD patients, but also a highly significant stepwise progressive increase in the marker level from stage 3 through stage 5. These findings are in accordance with the findings of ***(Tong et al., 2007) and*** (***Nishi et al., 2011)*** who concluded that PTX3 is a useful marker for CKD progression**.** (***Gursu et al., 2011)*** speculated that the progression of chronic inflammation in renal tissues of CKD is associated with increased levels of cytokines.

As regards the association of plasma PTX3 levels with CVD in our CKD patients. This study showed that plasma PTX3 levels were significantly higher in CKD patients with CVD than those without CVD. It revealed also a significant rise in levels of PTX3 in each stage of patients of CKD associated with CVD than those without CVD within the same stage. These data demonstrate that PTX3 levels increase in CVD independently of CKD. These findings were in agreement with ***Nishi et al., (2011)***. They found that PTX3, hsCRP, and TNFα, but not MCP-1 could predict the presence of CVD as a complication associated with CKD. Additionally, PTX3 might be a more sensitive marker for the association of CVD than hsCRP and TNFα in patients with advanced CKD.

Jenny et al. (2009) reported a strong expression of PTX3 and atherosclerosis, suggesting that PTX3 may be involved in the pathogenesis of atherosclerosis.

The main autoantigen involved in the development of atherosclerosis is the LDL which, on reaching the oxidative stage, is recognized and internalized by macrophages through scavenger receptors. Oxidized LDL increases PTX3 mRNA expression by vascular smooth muscle cells more than 70-fold. In contrast, native LDL does not demonstrate any effect on its expression. Vascular smooth muscle cells actively participate in the atherogenic process by promoting local inflammatory reactions, which lead to in situ vascular damage by the activation of complement via the classical pathway ***(Klouche et al., 2004).*** In addition, PTX3 is rapidly produced from vascular endothelial cells, macrophages and neutrophils in response to inflammatory stimuli ***(Latini et al., 2004).*** Moreover, amplification of endothelial cell procoagulant activity by PTX3 has been demonstrated ***(Nauta et al., 2003).***

Inflammation is a common feature of CKD ***(Stenvinkel et al., 2005)*** and an important prognostic and diagnostic tool for ischemic heart disorders as well . Strong expression of PTX3 in advanced human atherosclerotic lesions by endothelial cells and macrophages was described, suggesting that PTX3 may be involved in the pathogenesis of atherosclerosis.

In this study, it was found that plasma PTX3 was significantly positively correlated with triglycerides and total cholesterol. These findings were in agreement with study done by ***Nishi et al. (2011)***. ***Napoleone et al. (2002)*** explained that by showing to PTX3 which induced in endothelial cells by oxidized LDL might promote thrombogenesis and vascular ischemia leading to CVD.

These results also were in agreement with ***Iwata et al. (2012)***. They found that, the level of PTX3 was positively correlated with the percentage of lipid volume.

Furthermore, this correlation study in CKD patients revealed a significant positive correlation between plasma PTX3 and serum creatinine, BUN, in addition to a significant negative correlation with eGFR. These findings are in accordance with the findings of ***(Nauta et al. 2005 and Tong et al. 2007).***

In the present study, it was found that plasma PTX3 was significantly positively correlated with

CRP. These findings are in agreement with study done by ***Hollan et al. (2010).***

Receiver-operating characteristic (ROC) curve analysis was applied to assess the diagnostic performance of PTX3 in CKD patients. The optimum cut-off level was 0.95ng/mL. This had a diagnostic sensitivity, specificity, negative predictive value and positive predictive value of 100%, 87%, 100% and 95% respectively. Receiver-operating characteristic (ROC) curve analysis for CRP to assess its diagnostic performance in CKD patients was also done. The optimum cut-off level was 0.65 mg/L. This had a diagnostic sensitivity, specificity, negative predictive value and positive predictive value of 100%, 80%, 100% and 92,59% respectively. These findings highlight the possible role of these markers in discriminating CKD patient and also show that PTX3 is more specific than CRP in diagnosing the patients with CKD.

It was hypothesized that PTX3, unlike CRP, may represent a rapid marker for primary local activation of innate immunity and inflammation and an indicator of disease activity ***(Muller et al., 2001)***. Because of this extrahepatic synthesis and in contrast to CRP, PTX3 levels are believed to be a true independent indicator of disease activity produced at sites of inflammation ***(Fazzini et al., 2001).***

Conclusion

- Plasma PTX-3 is a promising independent diagnostic marker for identifying patients with CKD as well as it is proposed as a useful indicator of disease progression.
- Increased plasma level of PTX-3 may accelerate the vascular complications in CKD, In addition plasma PTX-3 in conjunction with serum CRP can help in differentiation between CKD patients with CVD from those without CVD.

Recommendation

- The value of PTX-3 in monitoring response to therapy is worth exploration.
- Further studies are needed in order to establish PTX-3 as a prognostic & diagnostic biomarker in the acute setting of renal failure.
- PTX-3 could be an independent indicator of myocyte. Besides common cardiac markers used in patients with ACS as (cardiac troponin I) (cTnI), PTX-3 can be used by clinicians for diagnosing of ACS, particularly in the first few hours.

References

1. **Albert CM, Rifai N, Stampfer MJ and Ridker PM (2002):** Prospective study of C-reactive protein, homocysteine, and plasma lipid levels as predictors of sudden cardiac death; Circulation .105: 2595–2599.
2. **Barsom R (2002):** Subjective and objective physical limitations in high functioning renal dialysis patients. New Eng. J. Med, 354: 997-999.
3. **Bash LD, Erlinger TP, Coresh J, Marsh-Manzi J, Folsom AR and Astor BC (2009):** Inflammation, hemostasis, and the risk of kidney function decline in the Atherosclerosis Risk in Communities (ARIC) Study. *Am J Kidney Dis.* Apr; 53(4):596-605.
4. **Bottazzi B, Garlanda C, Cotena A, Moalli F, Jaillon S, Deban L and Mantovani A (2009):** The long pentraxin PTX3 as a prototypic humoral pattern recognition receptor: Interplay with cellular innate immunity. Immunol Rev; 227: 9–18.
5. **Bussolati B, Peri G, Salvidio G, Verzola D, Mantovani A and Camussi G (2003):** The PTX3 is synthesized in IgA glomerulonephritis and activates mesangial cells. J. Immunol.; 170:1466–1472.
6. **Carrero JJ and Stenvinkel P (2010):** Inflammation in end-stage renal disease: What have we learned in 10 years? Semin Dial; 23: 498–509.
7. **Deban L and Mantovani A (2009):** The long pentraxin PTX3 as a prototypic humoral pattern recognition receptor: Interplay with cellular innate immunity. Immunol Rev; 227: 9–18.
8. **Devarajan P (2008):** Neutrophil gelatinase-associated lipocalin:a new marker of kidney disease. Scandinavian Journal of Clinical and Laboratory Investigations; 68 (S241): 89- 94.
9. **Dubin R, Shlipak M, Li Y, Ix J, de Boer IH, Jenny N and Peralta CA (2011):** Racial differences in the association of pentraxin-3 with kidney dysfunction: the Multi-Ethnic Study of Atherosclerosis. Nephrol Dial Transplant; 26(6):1903-8.
10. **Ernesto L, Mark L and Johannes F (2007):** Cardiovascular Involvement in General Medical Condition. Circulation; 116:85-97.
11. **Fabrizia Bonacina, Andrea Baragetti, Alberico Luigi Catapano and Giuseppe Danilo Norata (2013):** Long Pentraxin 3: Experimental and Clinical Relevance in Cardiovascular Diseases; 1-10.
12. **Fazzini F, Peri G, Doni A, Dell'Antonio G, Dal Cin E, Bozzolo E, D'Auria F, Praderio L, Ciboddo G, Sabbadini MG, Manfredi AA, Mantovani A and Querini PR (2001):** PTX3 in small-vessel vasculitides: An independent indicator of disease activity produced at sites of inflammation. Arthritis Rheum.; 44:2841– 2850.
13. **Gursu M, Aydin Z, Karadag S, Uzun S, Ogul S, Kiris A, Doventas Y, Koldas M, Ozturk S and Kazancioglu R (2011):** PTX3 a better marker of inflammation in uremic patients. Oxford Journals, medicine, Vol. 2 (supl 4).
14. **Hollan I, Bottazzi B, Cuccovillo I, Førre ØT, Mikkelsen K, Saatvedt K, Almdahl SM, Mantovani A, Meroni PL; Feiring Heart Biopsy**

Study Group (2010): Increased levels of serum pentraxin 3, a novel cardiovascular biomarker, in patients with inflammatory rheumatic disease. Arthritis Care Res (Hoboken); 62(3):378-85.

15. **Inoue K, Sugiyama A and Reid P.C (2007):** Establishment of a high sensitivity plasma assay for human pentraxin-3 as a marker for unstable angina pectoris. Arterioscler Thromb Vasc Biol.; 27:161-7.
16. **Iwata A, Miura S, Tanaka T, Ike A, Sugihara M, Nishikawa H, Kawamura A and Saku K(2012):** Plasma pentraxin-3 levels are associated with coronary plaque vulnerability and are decreased by statin; 23(5):315-21.
17. **Jenny NS, Arnold AM, Kuller LH, Tracy RP and Psaty BM (2009):** Associations of pentraxin 3 with cardiovascular disease and all-cause death: the Cardiovascular Health Study. Arterioscler Thromb Vasc Biol.; 29(4):594-9.
18. **Jinghua Lu, Kristopher D and Lorraine L (2011):** Recognition and functional activation of the human IgA receptor (FcαRI) by C-reactive protein. March 22; 108(12): 4974–4979.
19. **Klouche M, Peri G, Knabbe C, Eckstein HH, Schmid FX and Schmitz G (2004):** Modified atherogenic lipoproteins induce expression of pentraxin-3 by human vascular smooth muscle cells. Atherosclerosis; 175:221-8.
20. **Kovacs A, Tornvall P, Nilsson R, Tegnér J, Hamsten A and Björkegren J (2007):** Human C-reactive protein slows atherosclerosis development in a mouse model with human-like hypercholesterolemia; Proc. Natl. Acad. Sci. USA; 104:13768–13773.
21. **Latini R, Maggioni AP, Peri G, Gonzini L, Lucci D, Mocarelli P, Vago L, Pasqualini F, Signorini S, Soldateschi D, Tarli L, Schweiger C, Fresco C, Cecere R, Tognoni G, Mantovani A; Lipid Assessment Trial Italian Network (LATIN) Investigators (2004):** Prognostic significance of the long pentraxin PTX3 in acute myocardial infarction. Circulation. 19; 110(16):2349-54.
22. **Macy E, Hays T and Tracy R (1997):** Variability in the measurement of C-reactive protein in healthy subjects: implication for references interval and epidemiological application. Clin Chem; 43(1):52-58.
23. **Malyszko, J.; Przybylowski, P. and Malyszko J.S (2010):** Lipocalin Correlates With Kidney Function in Heart Allograft Recipients. Trans. Proce.; 41: 158–161.
24. **Mantovani A**, **Garlanda C**, **Bottazzi B**, **Peri G**, **Doni A**, **Martinez de la Torre Y and Latini R (2006):** The long pentraxin PTX3 in vascular pathology. Vascul Pharmacol. Nov.; 45(5):326-30.
25. **Mantovani A**, **Garlanda C**, **Bottazzi B**, **Peri G**, **Doni A**, **Martinez de la Torre Y and Latini R (2006):** The long pentraxin PTX3 in vascular pathology. Vascul Pharmacol. Nov.; 45(5):326-30.
26. **Mengli Tong, Juan Jesús, A. Rashid and Björn Anderstam (2007):** plasma Pentraxin 3 in Patients with Chronic Kidney Disease: Associations with Renal Function, Protein-Energy wasting, Cardiovascular Disease, and Mortality**.CJASN;** 2(5):889 -897.
27. **Mitsnefes M.M, Kathman T.S and Mishra J (2007):** Serum neutrophil gelatinase-associated lipocalin as a marker of renal function in children with chronic kidney disease. Pediatr. Nephrol.; 22:101–108.
28. **Muller B, Peri G, Doni A, Torri V, Landmann R, Bottazzi B and Mantovani A (2001):** Circulating levels of the long pentraxin PTX3 correlate with severity of infection in critically ill patients. Crit Care Med.**;** 29:1404– 1407.
29. **Napoleone E, Di Santo A and Bastone A (2002):** Long pentraxin PTX3 up regulates tissue factor expression in human endothelial Cells: A novel link between vascular inflammation and clotting activation. Arterioscler Thromb Vasc Biol.; 22: 782–787.
30. **National Kidney Foundation K/DOQI (2002):** Clinical practice guidelines for chronic kidney disease: evaluation, classification and stratification. Am. J. Kidney Dis.; 39: S1 – S266.
31. **Nauta AJ, Bottazzi B, Mantovani A, Salvatori G, Kishore U and Schwaeble WJ (2003):** Biochemical and functional characterization of the interaction between pentraxin 3 and C1q. Eur J. Immunol.; 33:465-73.
32. **Nauta AJ, De Haij S, Bottazzi B, Mantovani A, Borrias MC, Aten J, Rastaldi MP, Daha MR, Van Kooten C and Roos A (2005):** Human renal epithelial cells produce the long pentraxin PTX3. Kidney Int.; 67:543 –553.
33. **Nauta AJ, De Haij S, Bottazzi B, Mantovani A, Borrias MC, Aten J, Rastaldi MP, Daha MR, Van Kooten C and Roos A (2005):** Human renal epithelial cells produce the long pentraxin PTX3. Kidney Int.; 67:543 –553.
34. **Nishi K, Imamura T, Kitamura K, Ogawa T, Fujimoto S, Kakitsubata Y, Ishikawa T, Asada Y and Kodama T (2011):** Associations of plasma pentraxin 3 and monocyte chemoattractant protein-1 concentrations with cardiovascular disease in patients with chronic kidney disease. Ren Fail. 2011; 33(4):398-404.
35. **Nishi K, Imamura T, Kitamura K, Ogawa T, Fujimoto S, Kakitsubata Y, Ishikawa T, Asada Y and Kodama T (2011):** Associations of plasma pentraxin 3 and monocyte chemoattractant protein-1 concentrations with cardiovascular disease in patients with chronic kidney disease. Ren Fail. 2011; 33(4):398-404.
36. **Parlak A, Iyisoy A, Aydogan U, Cakir E and Saglam K (2012)**The effect of valsartan and nebivolol treatment on ADMA and pentraxin-3 levels inhypertensive patients ;79(3):294-8.

37. **Pepys MB and Hirschfield GM (2003):** C-reactive protein a critical update. J. Clin Invest.;111:1805–1812.
38. **Pradeep AR, Kathariya R, Arjun Raju P, Sushma Rani R, Sharma A and Raghavendra NM (2012):** Risk factors for chronic kidney diseases may include periodontal diseases, as estimated by the correlations of plasma pentraxin-3 levels: a case-control study; 44(3):829-39.
39. **Ridker PM (2004):** High-sensitivity C-reactive protein, inflammation, and cardiovascular risk from concept to clinical practice to clinical benefit. Am. Heart J.; 148:S19–S26.
40. **Robert G, Sree K, Glenda C, Jeff S and Coombes (2011):** Biomarkers in chronic kidney disease .Kidney International**;** 80: 806–821.
41. **Salio, Chimenti and Angelis (2008):** Cardioprotective function of the long pentraxin PTX3 in acute myocardial infarction. Circulation; 117 (8): 1055-1064.
42. **Schoolwerth A, Engelgau MM, Hostetter TH, Rufo KH, Chianchiano D. and McClellan WM (2006):** Chronic kidney disease: a public health problem that needs a public health action plan. Am. J. Kidney Dis.; 51(4 Suppl 2):S30-37.
43. **Sébastien Jaillon, Giuseppe Peri and Yves Delneste (2007):** The humoral pattern recognition receptor PTX3 is stored in neutrophil granules and localizes in extracellular traps. April 16; 204(4): 793–804.
44. **Shavit L, Lifschitz MD and Epstein M (2012) :** Aldosterone blockade and the mineralocorticoid receptor in the management of chronic kidney disease: current concepts and emerging treatment paradigms**;** 81(10):955-68.
45. **Souza DG, Amaral FA and Fagundes CT (2009):** The long pentraxin PTX3 is crucial for tissue inflammation after intestinal ischemia and reperfusion in mice. Am .J. Pathol.; 174: 1309–1318.
46. **Stenvinkel P, Ketteler M, Johnson RJ, Lindholm B, Pecoits-Filho R, Riella M, Heimburger O, CederholmT and Girndt M (2005):** IL-10, IL-6, and TNF-alpha, central factors in the altered cytokine network of uremia–the good, the bad, and the ugly. Kidney Int.; 67: 1216– 1233.
47. **Stuveling E, Hillege H, Bakker S, Gans R, De Jong P and De Zeeuw D (2003):** C-reactive protein is associated with renal function abnormalities in a non-diabetic population. Kidney Int.; 63(2):654-61.
48. **Suliman ME, Qureshi AR, Carrero JJ, Barany P, Yilmaz MI, Snaedal-Jonsdottir S, Alvestrand A, Heimburger O, Lindholm B and Stenvinkel P (2008):** The long pentraxin PTX-3 in prevalent hemodialysis patients: Associations with comorbidities and mortality. QJM 101: 397–405.
49. **Tarver-Carr M, Powe N and Eberhardt M (2002):** Excess risk of chronic kidney disease among African-American versus white subjects in the United States: a population-based study of potential explanatory factors. J. Am. Soc. Nephrol.; 13(9):2363-70.
50. **Tong M, Carrero JJ, Qureshi AR, Anderstam B, Heimbürger O, Bárány P, Axelsson J, Alvestrand A, Stenvinkel P, Lindholm B and Suliman ME (2007):** Plasma pentraxin 3 in patients with chronic kidney disease: associations with renal function, protein-energy wasting, cardiovascular disease, and mortality. Clin J Am Soc Nephrol.; 2(5):889-97.
51. **Tong M, Carrero JJ, Qureshi AR, Anderstam B, Heimbürger O, Bárány P, Axelsson J, Alvestrand A, Stenvinkel P, Lindholm B and Suliman ME (2007):** Plasma pentraxin 3 in patients with chronic kidney disease: associations with renal function, protein-energy wasting, cardiovascular disease, and mortality. Clin J Am Soc Nephrol.; 2(5):889-97.
52. **Voleti B and Agrawal A (2006):** Statins and nitric oxide reduce C-reactive protein production while inflammatory conditions persist. Mol. Immunol.; 43: 891–896.
53. **Winkles JA (2008):** The TWEAK-Fn14 cytokine-receptor axis: Discovery, biology and therapeutic targeting. Nat Rev 7: 411–425.
54. **Yano A, Nakao K, Sarai A, Akagi S, Kihara T, Morimoto H, Nakamura A, Hiramatsu M, Nagake Y and Makino H (2005)**: Elevated serum interleukin-18 levels might reflect the high risk of hospitalization in patients on peritoneal dialysis. Nephrology (Carlton); 10:576– 582**.**
55. **Yasojima K, Schwab C, Mcgeer E and Mcgeer P (2001):** Generation of C-reactive protein and complement components in atherosclerotic plaques. Am. J. Pathol.; 158:1039–1051.
56. **Zacho J, Tybjaerg-Hansen A, Jensen J, Grande P, Sillesen H and Nordestgaard B (2008):** Genetically elevated C-reactive protein and ischemic vascular disease. N. Engl. J Med.; 359:1897–1908.

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