IS PENTRAXIN-3 STRONGER THAN C-REACTIVE PROTEIN TO DETERMINE INFLAMMATION IN PERITONEAL DIALYSIS PATIENTS?

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Pentraxin–3 (PTX-3) is the prototype of long pentraxins and is produced by many tissues and organs including vascular endothelial cells in response to pro-inflammatory signals. It is thought to be an independent indicator of disease activity. We analyzed the correlation of PTX-3 with other markers of inflammation in peritoneal dialysis (PD) patients. Non-diabetic patients on chronic PD program who meet the dialysis adequacy criteria and who had no active infectious/inflammatory disease were included. Demographic and clinical parameters were recorded as well as hsCRP, fibrinogen, interleukin-6 (IL-6) and PTX-3 levels; and the correlation between them were studied. Twenty-five patients (mean age: 45.7±12.5 years; female/male ratio: 16/9) were included. Mean PTX-3 level was 2.16±2.76ng/ml. PTX-3 was found to be correlated positively with only IL-6 among inflammatory markers (r=0.827; p<0.001) but not with hsCRP. With linear regression model, IL-6 was the only independent determinant of PTX-3 levels. PTX-3 may be a more valuable marker of inflammation than CRP in patients on PD.

There is chronic inflammation manifested by increased inflammatory markers like C-reactive protein (CRP), interleukin-6 (IL-6), fibrinogen together with decreased albumin and fetuin-A levels in patients with uremia (1). The relationship between chronic inflammation and cardiovascular disease risk has been demonstrated by many studies, but there is no standard approach for these patients since there is insufficient proof of the causes and the ideal treatment of chronic inflammation in uremic patients (2). Moreover; the diagnostic role of these inflammatory markers is debatable. Some studies have found a correlation between clinical, laboratory and radiological findings while some others have

not (3), therefore the method of choice seems to be the morphological and functional evaluation of the vascular system.

Pentraxins form a pattern recognition family. Short pentraxins, namely CRP and amyloid-p protein, are produced in the liver in response to inflammatory signals, mainly IL-6 (4). Pentraxin–3 (PTX-3) is the prototype of long pentraxins and has a molecular weight of 40.6 kDa. PTX-3 is produced by many tissues and organs including vascular endothelial cells, smooth muscle cells, macrophages and adipose tissue in response to pro-inflammatory signals (lipopolysaccharides, tumor necrosis factor, IL-1, IL–6) and stimulation of Toll-like receptors (4).

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PTX-3 production and expression has been shown in renal proximal tubular cells, renal fibroblasts and mesangial cells (5). PTX-3 stimulates opsonization of apoptotic cells and microorganisms as well as activation of the complement system through binding to C1q (6). It is thought to be an independent indicator of disease activity because of its production at the center of inflammation; and to be related with endothelial dysfunction.

Thinking of the epidemic nature of chronic kidney disease, its coexistence with chronic inflammation and the associated increased cardiovascular risk; it is imperative to detect early the chronic inflammation by non-invasive laboratory methods and to treat the underlying reason in this population of patients. The role of the commonly-used inflammatory marker CRP is controversial in the dialysis population. In our study we analyzed the correlation of PTX-3 with other inflammatory markers in peritoneal dialysis (PD) patients.

MATERIALS AND METHODS

Patients on PD program for at least three months who met the dialysis adequacy criteria advised by international guidelines and who gave informed consent were included in the study, and are referred to as the PD group. The modality of PD treatment was not a criterion for inclusion to or exclusion from the study. Patients younger than 18 or older than 80 years of age, those with diabetic nephropathy as the primary kidney disease, those with peritonitis or any other infectious or inflammatory disease within the last three months or history of acute ischemic vascular disease, and patients with chronic liver disease, hepatitis or any other disease that may lead to inflammation were excluded from the study.

Age, gender, primary kidney disease, comorbidities, dialysis duration, the current medications, body mass index (BMI), systolic and diastolic blood pressures (SBP and DBP) of all patients were recorded. Coronary or peripheral artery diseases were recorded only if they were shown definitely by angiography or other radiological methods. Weekly creatinine clearance and Kt/V values were also recorded.

The patient group was compared with the control group consisting of healthy volunteers. For the control group, age, gender, systolic and diastolic blood pressures were recorded as well as laboratory parameters.

The blood sample for hematological and biochemical measurements were obtained after 12-hour fasting.

Glucose, urea, creatinine, uric acid, calcium (Ca), phosphorus (P), parathyroid hormone (PTH), albumin, total cholesterol, LDL cholesterol, HDL cholesterol, triglyceride, transferrin saturation, ferritin, vitamin B12, folic acid, hemoglobin (Hb), hematocrit (Hct), high sensitive CRP (hsCRP), fibrinogen, IL-6 and PTX-3 levels were measured. PTX–3 levels were measured by ELISA method using Cusabio KIT (Cosmo Bio, California, USA) and IL-6 levels by enzyme sensitive immunoassay method using EASIA Biosource kit (BioSource Europe, Belgium).

Data analysis was conducted using SPSS (Statistical Package for Social Sciences) 15 for Windows standard version. Numerical parameters were expressed as mean±standard deviation (SD). Intergroup comparisons were made by paired Student t-test or Mann Whitney U test when necessary. Correlation analyses of numerical parameters with normal and abnormal distribution were performed using Pearson and Spearman's rho correlation tests, respectively. The parameters found to be related with IL-6 on univariate analysis were studied by multivariate analysis (enter method). P values less than 0.05 were regarded as statistically significant.

RESULTS

Among the 69 patients followed-up in our unit, 25 patients fulfilling the inclusion and exclusion criteria and who gave informed consent were included in the study. Eighteen healthy volunteers were selected as the control group. The mean age and female/male ratio of the groups were 45.7±12.5 years vs 54.8±8.34 years (p=0.01); and 16/9 vs 10/8 (p=0.57), respectively. Among the patients, primary kidney disease was autosomal dominant polycystic kidney disease in five, chronic glomerulonephritis in five, nephrosclerosis in four, chronic pyelonephritis in one and amyloidosis in one, while it was unknown in eight patients. The mean PD duration was 32.12±19.67 months. Recorded comorbidities were hypertension in 21 and hyperlipidemia in seven patients. The drugs used by the patients alone or in combination were angiotensin converting enzyme inhibitors/angiotensin receptor blockers (n=9), calcium channel blockers (n=10), beta blockers (n=10), diuretics (n=17), alpha blockers (n=8), statins (n=6), fibrate derivatives (n=1), acetyl salicylic acid (n=3), folic acid preparations (n=6), vitamin B complexes (n=11) and active vitamin D (n=17).

Table I. *Laboratory results of the study gr*

Parameter	PD group	Control group	P
Glucose (mg/dl)	104±17	100±9	0.285
Urea (mg/dl)	99±19	31±10	<0.001
Creatinine (mg/dl)	7.9±2.6	0.7±0.1	<0.001
Uric acid (mg/dl)	5.9±1.4	5.0±1.3	0.047
Ca (mg/dl)	9.06±0.60	9.8±0.5	<0.001
P (mg/dl)	5.1±1.4	3.5±0.5	<0.001
PTH (pg/ml)	548±445	67±44	<0.001
Albumin (gr/dl)	3.6±0.3	4.2±0.3	<0.001
Total cholesterol (mg/dl)	193±44	209±36	0.14
HDL cholesterol (mg/dl)	44±15	49±13	0.275
LDL cholesterol (mg/dl)	112±35	137±30	0.010
Triglyceride (mg/dl)	202±222	114±57	0.030
Hemoglobin (gr/dl)	10.54±1.68	13.5±1.1	<0.001
Hematocrit (%)	31.7±5.2	40.4±3.2	<0.001
Ferritin (ng/ml)	259±125	81±55	<0.001
Vitamin B12 (pg/ml)	575±394	282±76	0.002
Folic acid (ng/ml)	9.9±7.9	7.7±2.0	0.202
PTX-3 (ng/ml)	2.16±2.76	0.73±0.96	0.019
hsCRP (mg/l)	1.02±1.29	0.47±0.59	0.046
IL-6 (pg/ml)	46.6±101.2	16.0±28.3	0.035
Fibrinogen (mg/dl)	568±110	347±104	<0.001

Table II. *Results of multivariate analysis showing the parameter related to IL-6 levels in both groups.*

GROUP		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
PD group	(Constant)	-79.214	67.148		-1.180	0.253
	Age	0.361	1.007	0.044	0.359	0.724
	hsCRP	17.240	9.953	0.212	1.732	0.100
	Gender	19.853	28.248	0.092	0.703	0.491
	PTX-3	31.115	4.787	0.844	6.499	<0.001
Control group	(Constant)	62.040	43.467		1.427	0.177
	Age	-0.811	0.793	-0.239	-1.023	0.325
	hsCRP	-10.812	10.668	-0.227	-1.013	0.329
	Gender	-6.264	12.679	-0.113	-0.494	0.630
	PTX-3	17.346	7.128	0.591	2.433	0.030

On physical examination, mean BMI were similar in the PD and the control group (28.1±7.2 kg/m² vs 27.9±4.5 kg/m²; p=0.806); while mean systolic (136±29 mmHg vs 115±10 mmHg; p=0.011) and diastolic (81±13 mmHg vs 71±8 mmHg; p=0.027) blood pressures were significantly higher in the PD group. All patients and members of the control group were clinically euvolemic.

All PD patients had Kt/V values above the limit described by ISPD guidelines (14). Mean Kt/V and creatinine clearance values were 2.63±0.65 and 4.06±4.24 ml/min, respectively. The mean urine output was 866±781 ml/day while eight patients were anuric. Thirteen patients had proteinuria less than 1 gr/day and four had proteinuria of 1-3 gr/day. There was no correlation of PTX-3 levels with residual creatinine clearance and proteinuria levels. The results of the laboratory analysis are presented in Table I.

Between the inflammatory markers the significant correlations were between hsCRP and fibrinogen (r=0.462; p=0.040); and between PTX-3 and IL-6 (r=0.574; p=0.003). Additionally, hsCRP was positively correlated with BMI (r=0.580; p=0.004), iron (r=-0.458; p=0.028), folic acid (r=-0.475; p=0.022) and age (r=0.480; p=0.020). In the control group the correlation between PTX-3 and IL-6 was not significant (r=0.066; p=0.795). PTX-3 was positively correlated with TIBC (r=-0.545; p=0.019) and age (r=0.492; p=0.038). hsCRP was correlated positively with fibrinogen (r=0.530; p=0.029) and

BMI (r=0.0538; p=0.021) while it was negative with iron level (r=-0.6567; p=0.03). Fibrinogen and age were positively correlated also (r=0.646; p=0.005). The correlations in both groups are presented in Figs. 1-3. With linear regression analyses; the major determinant of IL-6 level was found to be PTX-3 in both groups (Table II).

DISCUSSION

Although kidney transplantation is the superior treatment modality by means of physical capacity and cardiovascular diseases in end stage renal disease patients, because of the shortage of kidney donors, many of these patients can not have a kidney transplantation (7). Many studies have shown that there is chronic inflammation in chronic kidney disease and this process is coupled with increased cardiovascular risk. Many biochemical markers have been described for detection of patients under risk; but the usefulness of these markers for clinical diagnosis and follow-up is still debatable. In fact, some authors have claimed that CRP and homocystein known as inflammatory markers may be anti-atherogenic (8-10). Kalantar-Zadeh named the studies regarding this subject as *'inflammatory marker mania'* in his paper published in 2007 (11). In our cross-sectional study we aimed to determine the status of PD patients in the inflammatory process.

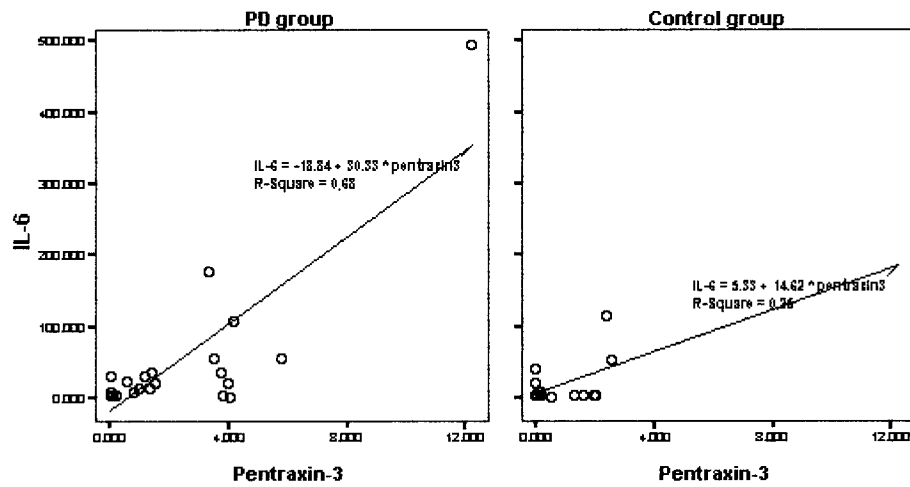PTX-3 is thought to have a different role in inflammation from other markers. Some studies have

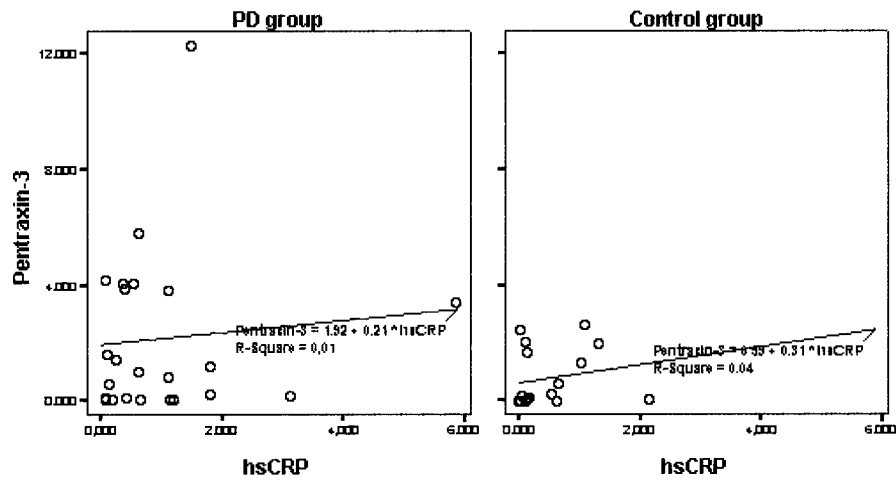**Fig. 1.** *The graphical presentation of the relation between PTX-3 and IL-6.*

Fig. 2. *The graphical presentation of the relation between PTX-3 and hsCRP.*
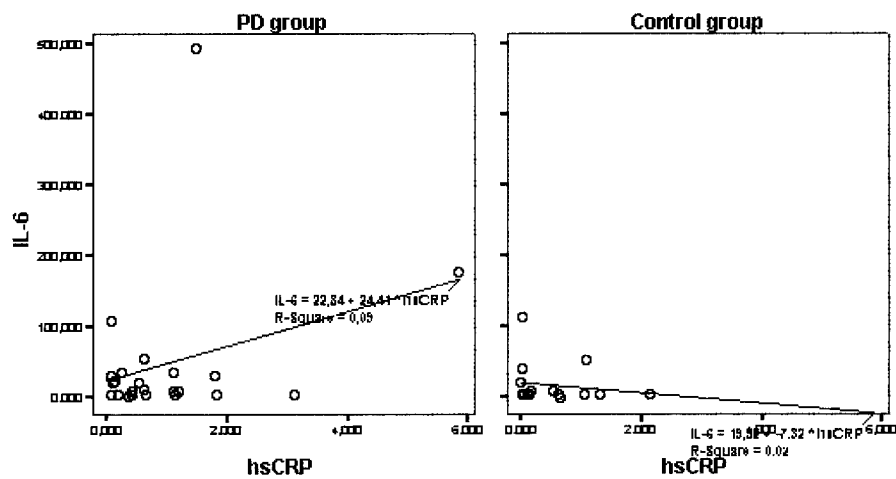

Fig. 3. *The graphical presentation of the relation between IL-6 and hsCRP.*

found the relationship between CRP and PTX-3 as weak, even insignificant as opposed to the previous thoughts (8, 12). The most striking result of our study is the strong correlation between IL-6 and PTX-3 while there was no relation of CRP with either IL-6 or PTX-3. In multivariate analysis, PTX-3 was found to be the only determinant of IL-6 which is known to have a central role in the start of inflammation (pro-inflammatory effect, stimulation in the hepatocytes of production of precursor proteins of inflammatory response, activation of peripheral mononuclear cells, and stimulation of B cell differentiation) and to be a strong stimulus for PTX-3 production (13). The strong correlation between PTX-3 and IL-6 is a clue for the value of PTX-3 as a marker of inflammation. Some other studies are consistent with our findings. PTX-3 levels have been reported to be high independently from CRP levels in dialysis population; and to be related with increased mortality rates (14, 15). It has been claimed to have a more stable course than CRP (16). Tong et al. (14) studied PTX-3 levels in chronic kidney disease stage 3-5, and found the highest levels in stage-5. They reported that PTX-3 was related with increased mortality rate in a manner similar to IL-6 and stronger than CRP. PTX-3 levels were higher in patients with protein-energy malnutrition, inflammation and cardiovascular disease, were correlated negatively with GFR and positively with other inflammatory markers. The same group reported that the predictive value of CRP for mortality was lower when corrected for PTX-3. This finding leads to the idea that PTX-3 is more valuable than CRP as a marker of cardiovascular risk.

Another study showed elevated PTX-3 levels to be related with increased mortality in patients followed up in intensive care unit but to be not related with CRP (12). A study conducted in our country has reported that PTX-3 levels were elevated in patients with chronic kidney disease stage 4 and 5 compared with the control group; and were related with endothelial dysfunction (17).

In our study, hsCRP was found to be related only with fibrinogen levels. Other studies have shown that PTX-3 has a more stable course (16), relation between CRP and PTX-3 is weak (8, 12), PTX-3 is related with IL-6 more strongly than CRP (15) and the relationship between IL-6 levels and cardiovascular disease risk is stronger (2). From

our findings, together with these literature data, it may be said that PTX-3 is superior to CRP as an inflammation marker.

No comment can be made regarding relationship of the mentioned parameters with mortality due to the cross-sectional nature of the study. Long-term follow-up of both radiological and biochemical measurements may give more detailed information about the process.

PTX-3 may be a more valuable marker of inflammation than CRP in patients on PD.

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