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C-terminal Propeptide of Type-I Procollagen as a Possible Biochemical Marker for Preclinical Detection of Cardiac Disease in Chronic Renal Failure

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The aim of present research was to investigate the possible role of C-terminal propeptide of type-I procollagen as a biochemical marker for early preclinical detection of Left Ventricular (LV) dysfunction in children with CRF. The study included 25 children with CRF undergoing hemodialysis for at least six months. Their age ranged from 8-16 years. Patients were receiving erythropoietin and anti-hypertensive medications. Serum was withdrawn from patients during the time interval between two dialytic sessions for estimation of CICP. The systolic and diastolic functions of the LV were investigated by echocardiography. Mean level of CICP was significantly higher in patients than in controls. Echocardiographic findings revealed significantly higher mean LV mass index, interventricular septal thickness, LV posterior wall thickness and E/A ratio in patients versus controls. Left atrial and ventricular diameters were significantly higher, whereas EF and FS% were lower in patients than controls. There were positive correlations between CICP levels and LVMI, IVST, LVPWT and E/A ratio. CICP correlated with the duration of illness, blood pressure, BUN and creatinine. We conclude that CICP levels are strongly correlated with the early changes that occur in the heart of CRF children, namely LV hypertrophy and diastolic filling abnormalities. CICP could be used as a reliable preclinical serological marker for detection of these changes.

Key words: C-terminal propeptide of type-I procollagen, echocardiography, left ventricular abnormalities, chronic renal failure

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INTRODUCTION

Cardiovascular disease is the leading cause of morbidity and mortality in children with chronic renal failure (CRF) on hemodialysis (Byung-Soo *et al.*, 2005; Lima, 2006). Left ventricular (LV) abnormalities, so called uremic cardiomyopathy, are associated with poorer outcome (Foley *et al.*, 1998; Fathi *et al.*, 2003). Three forms of uremic cardiomyopathy are described in literature, namely, LV hypertrophy (LVH), dilation and systolic dysfunction. However, it was shown that LVH is the predominant cardiomyopathy specific to uremia (Mark *et al.*, 2006).

CRF is also accompanied by biochemical and hormonal disturbances which lead to the loss of bone mass and the destruction of bone microarchitecture (Polak- Jonkisz *et al.*, 2003). One of the serological markers of bone turnover used in assessment of bone formation in children with CRF is procollagen type I carboxy -terminal propeptide (a peptide that is cleaved from procollagen type I during the synthesis of fibril-forming collagen type I) (CICP) (Sawamura *et al.*, 1998).

Interstitial collagen is essential for LV integrity and function (Schwartzkopff *et al.*, 2002). The myocardial fibrillar extracellular matrix is mainly composed of type I and III fibrillar collagens forming a three -dimensional framework, which is important for normal LV function and allows for an effective transmission of force in systole as well as in diastole (Spinale *et al.*, 1998).

Mitchell *et al.* (2005) mentioned that measurement of CICP in cardiac diseases could be useful as a non-invasive serological marker to detect myocardial fibrosis.

The aim of the present research is to investigate the value of serum CICP (a marker of collagen and bone turnover) as a biochemical marker for preclinical detection of LV dysfunction in children with CRF.

MATERIALS AND METHODS

The study was conducted on twenty five children (12 males and 13 females) with chronic renal failure during the period February to June, 2006. Patients were attending the Pediatric Dialysis Unit, Ain Shams University Hospital and were undergoing regular hemodialysis for at least 6 months (mean 3.8 ± 3.27 , range 0.5 to 12 years), three times weekly - three hours each session. Their age ranged from 8-16 years (mean 12.88 ± 2.55). The patients were divided into two groups according to their total body bone mineral density (BMD) z-score (Stein *et al.*, 1996); Group I : BMD z-score $> - 2$ (mean $- 0.52\pm 1.16$), duration on dialysis 0.5-1.5 year (mean 1.00 ± 0.47) and considered to have normal bone mass. In addition, calcium, phosphorus,

parathormone levels were within normal limits and they had no X-ray evidence of bone affection; Group II: osteopenic/osteoporotic group with BMD z-score < -2 ($- 2.94\pm 0.7$) and duration on dialysis 2-12 year (mean 5.27 ± 3.2) All patients were receiving erythropoietin (150 IU/kg/wk subcutaneously). They also received anti-hypertensive medications in the form of angiotensin-converting enzyme inhibitors (40%), calcium channel blockers (24%), α - and β -blockers (24 and 12%, respectively). Non of our patients had intercurrent illness, infection, liver failure, autoimmune disorders or clinical manifestations of cardiac disease. Twenty age- and sex-matched apparently healthy children recruited from the Pediatric Clinic, National Research Center served as a control group. Their BMD z-score was matched with Group I patients ($- 0.18\pm 0.82$).

Blood sample collection: For each patient, 3 mL whole blood was withdrawn via venipuncture during the time interval between two dialytic sessions. Whole blood withdrawn from each patient and control was immediately centrifuged and serum was separated and stored at -20°C until assayed.

All subjects had the following completed:

- Full history taking with emphasis on duration of illness, duration on dialysis, medications, other medical problems or complications.
- Thorough clinical examination with emphasis on cardiac examination and measurement of systolic and diastolic blood pressures at the time of echocardiography, after 15 min of rest.
- Complete blood picture using Coulter Counter T660 (Coulter Corporation, Miami, Florida, USA).
- Routine renal investigations using Synchron CX9 auto-analyzer (Beckman Instruments, Brea, California, USA), which included blood urea nitrogen (BUN), serum creatinine and albumin.
- Dual-energy X-ray absorptiometry (DEXA) (Norland-XR- 46, USA) performed at the Medical Services' Unit, National Research Center for estimation of bone mass by assessment of total body Bone Mineral Density (BMD). Absolute values were converted to z-scores (standard deviations from the mean of a healthy age- and sex-matched reference population) (Stein *et al.*, 1996; Barnas *et al.*, 2001).
- Two- dimensional Echocardiography (performed at Medical Services' Unit, National Research Center) using Vivid 3 Pro (General Electric Medical Systems, Norway) with a 3-MHz multiphase array probe was performed immediately after blood sample collection. Left ventricular mass (LVM) was corrected for body

surface area and expressed as LVM index (LVMI) and left ventricular end diastolic volume (LVED), interventricular septal thickness (IVST), left ventricular posterior wall thickness (LVPWT), early-to-atrial mitral peak flow velocity (E/A), ejection fraction (EF%) and fractional shortening (FS%) were calculated.

- Estimation of C-terminal Propeptide of Type I Procollagen (CICP) in serum: using Metra CICP ELISA kit (Quidel Corporation, San Diego, USA). It is a sandwich enzyme immunoassay in a microtiter plate format utilizing a monoclonal anti-CICP antibody coated on the plate, a rabbit anti-CICP antiserum, a goat anti-rabbit alkaline phosphatase conjugate and a pNPP substrate to quantify CICP in human serum.

Statistical analysis: Standard computer program SPSS for Windows, release 10.0 (SPSS Inc, USA) was used for data entry and analysis. All numeric variables were expressed as Mean±Standard Deviation (SD). Comparison of different variables in various groups was done using student t-test and Mann Whitney test for normal and nonparametric variables, respectively. Comparisons of multiple subgroups were done using ANOVA and Kruskal Wallis tests for normal and nonparametric variables, respectively. Pearson's and Spearman's correlation test (r = correlation coefficient) were used for correlating normal and non-parametric variables, respectively. For all tests a probability (p) less than 0.05 was considered significant.

RESULTS AND DISCUSSION

Children with CRF often have multiple cardiovascular risk factors and a high incidence of cardiovascular morbidity and mortality (Fathi *et al.*, 2003). LV hypertrophy which is the most frequent cardiac alteration in CRF patients develops early and occurs at a rate that is inverse to the level of renal function (McMahon *et al.*, 2004).

In the current study, the hypothesis of impaired collagen turnover in children with CRF was tested by measuring serum levels of CICP and correlating them with echocardiographic parameters of LV function. There was a significant increase in mean serum levels of CICP in patients compared to healthy children (Table 1). These results came in agreement with Fatema *et al.* (2002) and Fathi *et al.* (2003). In order to exclude the possible influence of bone mass loss on CICP (a marker of bone turnover), this study patients were divided into two groups according to BMD z-score. The group of patients with osteopenia/osteoporosis showed significantly higher

levels of CICP than in healthy controls indicating increased bone formation in these patients (Table 1). Hampson *et al.* (2002) presented similar results and concluded that measurement of CICP in serum is useful for monitoring metabolic bone diseases in hemodialysis patients. However, significantly higher mean CICP levels still existed when group I patients (with normal bone mass and mineral content) were compared to controls. We deduced that, after excluding the effect of osteopenia/osteoporosis, high levels of CICP probably would be attributable to the ongoing process of myocardial fibrosis as suggested by Lopez *et al.* (2001).

In a study by McMahon *et al.* (2004) LV hypertrophy was detected by elevations in echocardiography parameters: LVMI, IVST and LVPWT. In confirmation, this study detected significantly higher mean levels of LVMI, IVST and LVPWT in patients with CRF compared to controls (Table 2). When the mean LVMI in group I patients was compared to group II, a highly significant difference was obtained (Table 1). This result suggests that cardiac affection (LVH) might start earlier than bone changes in CRF children.

Factors leading to LV hypertrophy in CRF children are pressure and volume overloads. Pressure overload is induced by hypertension which leads to LV hypertrophy, diastolic dysfunction and eventually clinical heart failure (Mitchell *et al.*, 2005). A significant positive correlation existed between CICP and both systolic and diastolic blood pressures in the present study (Table 3). Although our patients were receiving anti-hypertensive treatment,

Table 1: Comparison of CICP and Left Ventricular Mass Index(LVMI) Between Group I (with normal bone mass), Group II (osteopenic/osteoporotic) and Healthy Children

Variable (mean±SD)	Group I n = 10	Group II n = 15	Healthy children n = 12	p-value
CICP (ng mL ⁻¹)*	274.6±56.8	432.8±77.9	191.00±32.9	p ₁ <0.001 p ₂ <0.001 p ₃ <0.001
LVMI (g m ⁻²)	84.39±22.5	146.1±35.6	48.6±14.9	p ₁ <0.001 p ₂ <0.001 p ₃ <0.001

p₁ = comparison between Group I and control children, p₂ = comparison between Group II and control children, p₃ = comparison between Group I and Group II,*Mean level of CICP was significantly higher in patients compared to control group (369.5±104.95 vs 202.15±31.73 ng mL⁻¹), p-value = <0.001

Table 2: Echocardiographic findings in the studied population

Variable (mean±SD)	Patients with CRF (n = 25)	Healthy children (n = 20)	p-value
EF%	71.56±7.9	76.1±5.8	< 0.05
FS%	35.35±6.26	38.1±5.0	> 0.05
IVST (cm)	0.75±0.28	0.55±0.1	< 0.05
LVPWT (cm)	0.82±0.24	0.53±0.12	< 0.001
LVMI (g m ⁻²)	121.43±43.37	61.95±24.5	< 0.001
LAD (cm)	2.58±0.57	2.1±0.5	< 0.05
LVED (cm)	4.24±0.57	3.57±0.64	< 0.05
E : A ratio:	1.431±0.24	1.17±0.09	< 0.001

Table 3: Correlations between CICP and clinical and laboratory findings of studied patients

	Age	Duration of disease	SBP	DBP	Hb	BUN	Creatinine	Albumin
Variable	r	r	r	r	r	r	r	r
CICP (ng mL ⁻¹)	-0.18	0.89**	0.41*	0.42*	0.05	0.9**	0.81**	-0.17

SBP = Systolic Blood Pressure, DBP = Diastolic Blood Pressure, *p<0.05, **p<0.001

Table 4: Correlations between CICP and the variable echocardiographic findings

	EF (%)	FS (%)	IVST	LVPWT	LVMI	LAD	LVED	E : A
Variable	r	r	r	r	r	r	r	r
CICP (ng mL ⁻¹)	-0.28	-0.26	0.9**	0.9**	0.9**	0.03	0.31	0.98**

**p<0.001

the serum levels of CICP were high denoting ongoing process of cardiac fibrosis. In agreement, Lopez *et al.* (2001) showed that the ability of anti-hypertensive therapy to reduce blood pressure did not predict its capacity to either regress myocardial fibrosis or normalize CICP synthesis in patients with CRF.

Anemia is an important risk factor in CRF patients which leads to volume overload. Numerous studies have demonstrated a close relationship between LV hypertrophy and anemia (Foley *et al.*, 1996; Levin *et al.*, 1999). However, in our study, no correlation existed between hemoglobin concentrations and CICP levels (Table 3). In accordance with the recommendations of the American Dialysis Outcomes Quality Initiative (Parfrey and Foley, 1999), patients in the present study received recombinant human erythropoietin for correction of anemia and yet these children displayed different degrees of LV hypertrophy. Previous studies explained that correction of anemia led to partial rather than complete regression of LV hypertrophy (Yang *et al.*, 1996; Foley *et al.*, 1998).

LV hypertrophy begins early in an attempt to accommodate pressure or volume overload. Later on, it becomes a maladaptive phenomenon leading to diastolic dysfunction (London, 2003). In the present work, the early- to - atrial mitral peak flow velocity (E/A ratio) was used for assessment of diastolic function. Our CRF children showed significantly higher E/A ratio in comparison to healthy children denoting enhanced diastolic function (Table 2). Escudero *et al.* (2004) mentioned that the increase in LV mass and wall thickness found in renal failure hypertensive patients was associated with diastolic filling abnormalities and if neglected, would lead to overt diastolic dysfunction later on. On the other hand, Ibrahim *et al.* (1995) detected impaired left ventricular diastolic function in their studied children, evidenced by reversal of E/A ratio.

Children in the current study showed left-chamber dilatation as well as LV hypertrophy (Table 2). LAD and

LVED were found to be significantly higher than in healthy children, in accordance with the results reported by Ibrahim *et al.* (1995).

In the present work, significantly lower mean EF% was obtained, whereas FS% showed non-significant lower mean value in patients compared to healthy children (Table 2). This result denotes impending systolic dysfunction, similar to that reported by Russu *et al.* (2003). Another study in adults revealed both LV systolic and diastolic dysfunctions in patients with CRF on hemodialysis (Wanic-Kossowska *et al.*, 2003).

As mentioned before, CICP is involved in the process of ongoing myocardial fibrosis. A question was set forth by the authors. Could measurement of CICP levels in serum predict changes that occur in the heart in CRF patients preclinically? Results of the present study revealed the existence of a positive correlation between levels of CICP and echocardiographic parameters of LV hypertrophy, namely: LVMI, IVST and LVPWT (Table 4). These results came in agreement with Fort (2005). Sundstrom and Vasan (2006) confirmed that the marker for type I collagen synthesis, the C-terminal propeptide could predict adverse outcomes following cardiac affection. Moreover, our study revealed a positive correlation between serum levels of CICP and diastolic E/A ratio in accordance with a previous study by Fassbach and Schwartzkopff (2005). In the present study, we found no significant correlations between CICP levels and either LAD or LVED (Table 4). This could be explained by the fact that CICP is mainly involved in the process of myocardial fibrosis and hypertrophy (Mitchell *et al.*, 2005). Furthermore, we found a non-significant inverse correlation between CICP and both EF and FS% (Table 4), which is in agreement with a previous study by Querejeta *et al.* (2004).

Significant positive correlations were present between the duration of illness, levels of BUN and serum creatinine on one hand and CICP levels on the other hand (Table 3). In previous studies by Foley *et al.* (1998),

Lopez *et al.* (2001) and Fort (2005) it was found that cardiac affection in renal failure patients appeared to progress over time as renal function declined. They stressed upon the importance of early intervention and appropriate management of children with CRF to delay progression of cardiac disease and its various complications.

In conclusion results of the present study revealed that CICP serum levels were strongly correlated with the early changes that occur in the heart of CRF children, namely LVH and diastolic filling abnormalities. Although our results seem promising, yet they have been performed on a small sample size. Further studies are needed on larger scale to validate our results before the use of CICP, as a reliable serological marker for preclinical detection of these changes, could be justified.

RECOMMENDATIONS

Findings of this study imply the importance of active attention to risk factor control by early detection and more aggressive treatment of those with preclinical dysfunction. Recently, treatment options have focused heavily on the antifibrotic effects of inhibitors of the Renin Angiotensin Aldosterone System (RAAS), perhaps supplanting β - blockers as first line agents to regress LV hypertrophy. The ultimate goal is to preserve normal cardiovascular function in patients with CRF.

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