Diagnostic utility of Amino-terminal pro-B type natriuretic peptide (NT pro BNP) in adult patients with chronic kidney diseases.


ABSTRACT:
In recent studies, suggestions were made that the diagnostic utility of Amino-terminal pro-B type natriuretic peptide (NT-proBNP), a known biomarkers of myocardial dysfunction, be extended to chronic kidney disease (CKD) patients that are devoid of any cardiovascular abnormalities or disease, however may develop same in future. The goal of present study was to further evaluate the relationship between CKD and NT-proBNP concentration in patients with varying levels of renal dysfunction including End Stage Renal Disease (ESRD). Stable adult patients of both gender (n = 76) with CKD were included in the study during January 2007 to September 2008, who had been on hemodialysis (HD). The patients were divided into two groups depending on Left ventricular ejection fraction (EF)<50%. Other parameters such as mean arterial pressure (MAP), NT proBNP and biochemical parameters were measured using standard procedures. The result strongly presents a correlation between NT-proBNP levels in CKD patients, who were without a definite onset of LVD. Furthermore, NT pro-BNP significantly correlated with an elevated protein to creatinine ratio and blood urea nitrogen (P < 0.01) suggesting that onset and presence of CKD influenced the concentration of natriuretic peptides. The present study, regarding assessment of NT proBNP in CKD patients, provide evidence that renal dysfunction and natriuretic peptides, especially NT-proBNP are correlated with each other. In addition, further prospective studies are underway to validate and better define this relationship.

KEY WORDS: Natriuretic peptides, amino-terminal pro-B type natriuretic peptide (NT-proBNP), Left Ventricle Dysfunction (LVD), Ejection Fraction (EF)

INTROCUCTION:
In recent decade or so, studies revealed that patients with chronic kidney disease (CKD) are at increased risk of cardiovascular dysfunctions and adverse onset of this fatal disease. Natriuretic peptides (NPs), especially amino-terminal pro-B type natriuretic peptide (NT-proBNP) and BNP biomarkers of myocardial dysfunction, provides clinicians and cardiologists, the potential for early detection and management of cardiac disease, mostly in emergency department, cardiac and intensive care units. It was researched and ultimately suggested that its screening utility could be extended to CKD patients that are devoid of any symptoms relating to cardiovascular disease. Clearly noted dependence of plasma NT-BNP on glomerular filtration rate (GFR) has been reported among patients with and without heart failure (HF). But it was argued that this relationship may not be independent of cardiac or volume-related factors. It was documented that patients with CKD, including those with stage 5, have one of the highest cardiovascular risk scores. In this type of specific population, the clinical benefit of NT-pro BNP measurements has not been thoroughly researched, evaluated and established yet and the fact remains under continued evaluation that patients with CKD have significantly increased BNP and NT-pro-BNP levels.

Therefore, to contribute and established the hypothesis that CKD have influence on NT-pro-BNP levels and thus advocates in clinical value, NT-BNP measurements need to be explored within the context of renal function only. Thus present study was undertaken to further evaluate the relationship between CKD and NT-BNP concentration in group selected patients with varying levels of renal function. 

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dysfunctions including End Stage Renal Disease (ESRD).

**METHODS AND MATERIALS**

**Patients and Procedures:**
We studied 76 stable adult patients with CKD who had been on hemodialysis (HD) for at least 15 weeks prior to the present prospective study. The study was conducted during the period January 2007 to September 2008. For most of protocols of David et al., 2008 was followed for proper management of the analyses. All patients were clinically stable and those with acute cardiac disease and/or acute cardiac failure were excluded from the study. Major causes of CKD leading to renal failure are shown in Fig 1. Blood pressure was measured and MAP was calculated accordingly. All measurements were performed twice and the means were used for analysis. According to grading described earlier, the presence of oedema was assessed clinically and determined according to the following scheme: grade I—absence of oedema, grade II—mild oedema, grade III—moderate oedema and grade IV—severe oedema (Fig 2). Clinical data were obtained from patients' records. Echocardiography (trans-thoracic) measurement was performed in all patients and left ventricular ejection fraction (LVEF) was measured according to established method. This procedure has been performed twice and the mean EF value was used for analysis. To avoid bias, the investigators who performed the echocardiography were not informed about the volume status and NT-pro-BNP level of patients examined. Left ventricular dysfunction (LVD) defined as LVEF<50%.

**Biochemical measurement**
Booild samples from all patients were collected during the post-dialysis periods to avoid discrimination in volume status. After centrifugation (2000 rpm/min), samples were stored at -20°C until further analysis. NT proBNP was measured using a commercially available electro-chemoluminescence immunoassay (Roche, Basel, Switzerland) that was performed on a Roche Elecsys 2010 analytical system. All other biochemical parameters, including BUN, creatinine, calcium phosphorus, albumin, C-reactive protein (CRP), were measured with routine laboratory methods on Hitachi 912 Chemistry analyzer (Roche) and electrolytes (Na, K, Cl, HCO3) on NOVA Crt 4 (Nova Biomedical, USA). Results were expressed as mean ± SD.

**Statistical Analysis**
SPSS statistic software 13.0 (Lead Technology Inc., Chicago, USA) were used for statistical analysis. Data are expressed as mean ± standard deviation. Subgroups were compared using one-way ANOVA. To compare NT-proBNP levels between groups Pearson's correlation analysis was applied. Correlation analysis between different variables was done by using Pearson's correlation coefficient. Independent variables associated with LVD, i.e. an EF<50% multivariate regression analysis was performed using variables which were correlated with EF in the univariate regression analysis was performed using which were correlated with EF in the univariate analysis e.g. NT proBNP, age, haemoglobin, hematocrit, left ventricular hypertrophy (LVH). P values less than < 0.05 was considered to be significant.

**RESULTS**
Around 120 eligible patients were screened for present study and only 76 met criteria for CKD and negative for CHF. Etiology of CKD is summarized in Fig 1. Four point scale of edema as per description stated earlier is in Fig 2, which shows presence of edema in more than 60% patients. Four patients were related to status of ESRD with increased level of protein to creatinine ratio of 3.0, BUN 64 mg/dl (pre) and 38 (post-dialysis) and NT-proBNP 28,129 ng/l. In overall group of patients, increasing severity of underlying comparatively low ejection fraction or a possible congestive heart failure was found associated with a higher NT pro-BNP (P<0.05), but not as significant as was assumed (Table 1). In addition, there was a significant inverse association between NT pro-BNP and low EF (<50%) (P<0.001), with higher NT pro-BNP levels observed in those with lower <50% EF. As regard the biochemical parameters, NT pro-BNP correlated significantly with a higher protein to creatinine ratio and blood urea nitrogen (P<0.01) suggesting that onset and presence of CKD influenced the levels of natriuretic peptides. MAP, both pre and post-dialysis, Hb, Hct, CRP and albumin shows no significant correlation with patients showing either lower or higher than 50% EF (depicting LVD or normal
cardiac functions respectively) and NT-proBNP. The results of present study clearly indicates the NT-proBNP represented itself as an independent diagnostic factor which may be useful clinically to evaluate onset or severity of patients of CKD and renal failure.

DISCUSSION:
BNP (B type natriuretic peptide) belongs to a family of natriuretic proteins (NP). Their physiological function is to maintain sodium homeostasis and protection of the cardiovascular system from volume overload. Its sister component, NT-proBNP, represent clearance products of the precursor pro-BNP which is synthesized by ventricular myocytes in response to physiological signals such as stretching of the ventricular wall, changes in systemic blood pressure, sodium levels or extracellular volume. It is reported that the action of BNP is mediated through metabolism by specific natriuretic peptide receptors of kidney, lung, liver and along the vascular endothelium, while the NT BNP is mainly cleared by the kidneys. What is more disastrous is the notion and current status that CKD and consequent end-stage renal failure are going to reach epidemic proportions worldwide over the next decade. Thus, it was pointed out that an increasing number of CKD patients will require early initiation of management protocols and screening for co-morbidity, such as cardiac problems. For this purpose considerable attention has been given to the issue of how to differentially interpret BNP in the presence or absence of CHF and within the context of renal function only. However, it remains a considerable important factor but remains incompletely resolved from a clinical perspective. Currently, usual clinical decisions for CHF, which incorporate BNP results, are made without evaluating or assessing renal function status of patients.

In the present study, we hypothesized and then investigated whether the natriuretic peptide NT-proBNP may be of any clinical value in the assessment of CKD in patients with or without underlying LVD or vice versa. The idea came through review of recent and past studies that stated diagnostic potential of NT-proBNP in CHF in patients with normal kidney function. We observed significantly elevated NT-proBNP levels in our CKD patients; but greater in patients with EF < 50% than in patients with EF > 50%. Our results are in agreement with earlier reported studies showing increased levels of natriuretic peptides as a result of reduced renal excretion and chronic volume overload in CKD patients without LVD. However as data and reports suggested earlier that a definite specific reference value of NT-proBNP, that can be used routinely as a diagnostic tool, is not decided nor recommended yet to separate LVD from over-hydration in CKD patients. In addition, the researcher also recommended that with respect to the previously demonstrated impact of NT-proBNP as a predictor of mortality in CKD patients, the need to define an NT-proBNP cut-off value for such use in clinical practice is mandatory. It was extensively argued by few authors that studying the temporary changes of NT-proBNP levels as a result of HD-related ultrafiltration was not the target, since this is not helpful in the diagnosis of LVD in patients requiring HD therapy. The assessment of NT-proBNP levels, therefore, was recommended in post-dialytic period in order to minimise the chance of fluid overload. Moreover, NT-proBNP level was more quickly manifested by candidate variables, most notably GFR, or more commonly the kidney functions and not the pre-dialytic state. A correlation of NT-proBNP levels with age has been reported in both Western and Asian populations. A study reported that age is a dependent variable correlated with plasma NP levels in univariate analysis, however lacked independent predictive value. The researchers argued that this is not unexpected in a CKD cohort study where LV hypertrophy, a variable highly correlated with age is known.

A study of a cohort of 3916 patients with heart failure suggested that BNP and NT-proBNP were independent markers correlating strongly with outcomes of CHF including: mortality, morbidity and hospitalization. Other factors that have correlated with BNP levels included age, NYHA class, ventricular function, body mass index, cardiac arrhythmia, ischemia, diuretics, bilirubin, creatinine, and C-reactive protein. In another study of 213 subjects, it was demonstrated that as renal function declined, BNP levels increased, especially among the subset of patients with ventricular hypertrophy. Similarly, another group of researchers found that among 389 patients, with and without de-compensated heart failure, those with eGFR greater than 60 ml/min/1.73m² had lower BNP levels than patients whose eGFR was less than 60 ml/min/1.73m². BNP levels, when assessed in dialysis patients, can be predictor of presence of left ventricular dysfunction, cardiac events and survival in
the presence of end stage renal disease, thus further concluding that BNP levels may provide information regarding overall status of renal function. A more comprehensive study was conducted, where patients were recruited to cover a variable range of renal function including patients on hemodialysis, with functional renal allograft and patients assessed by creatinine clearance measurements. Patients were further evaluated for heart function by echocardiography for cardiac hypertrophy, dilatation, systolic and/or diastolic dysfunction, pharmacological treatment and blood chemistry. The authors concluded that out of full range of renal function factors represented in the population studied, it was the factor-GFR, which superseded ventricular function as the more important determinant deciding serum BNP levels. It was also further noted that in addition to GFR, hypoalbuminemia, anemia, use of beta blockers and age were significant confounders of serum BNP levels, as has also been reported in earlier studies.

CONCLUSION:
In present study we presented a strong correlation of NT-proBNP levels with CKD patients. Such correlation previously had only been confirmed by patients with LVD but not in patients without LVD. However before availability of any such study or outcome, it was concluded earlier and well supported by literature that increasing BNP levels is related to worsening heart failure, however, lacked clear recommendations regarding renal function. After much deliberation and studies, it was than strongly suggested and recommended by several scientists and researchers that understanding the relationship of eGFR, CKD and BNP in the presence and absence of clinical heart failure is beneficial to understanding of clinicians interpretation of BNP and NT -pro BNP levels when making diagnostic and treatment decisions. As suggested earlier that the goal of studies, such as ours, regarding assessment of NT-pro BNP in CKD patients, was solely to provide compelling evidence of the association of renal function and BNP. Nonetheless, it was suggested that additional prospective studies must be performed to validate and better define this relationship. As concluded earlier, further determinations and assessments are necessary and needed to confirm the outcome, such as presented here, in larger cohorts and to further validate cut-off values of NT pro-BNP in CKD patients with our without CHD/CHF.

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REFERENCES:


16. Lang RM, Bierig M, Devereux RB, Flachskampf FA, Foster E, Pellikka PA, Picard MH, Roman MJ, Seward J, Shewerd J, Shanewise JS, Solomon SD, Spencer KT, Sutton MS, Stewart WJ. Recommendations for chamber quantification: a report from the American Society of Echocardiography's Guidelines and Standards Committee and the Chamber Quantification Writing Group, developed in conjunction with the European Association of Echocardiography, a branch of the European Society of Cardiology. J Am Soc Echocardiogr 18: 1440–1463, 2005


24. Sharma R, Gaze DC, Pellerin D, Mehta RL, Gregson


Fig. 1: Etiology of chronic kidney diseases leading to terminal renal failure

Fig. 2: Four Point Scale of Clinical Presence of Edema (Ref.:16) in CKD patients (n = 76)
Table 1: Clinical characteristics and laboratory data of patients categorized in two groups according to their left ventricular ejection fraction.

<table>
<thead>
<tr>
<th></th>
<th>EF &lt; 50% Left Ventricular Dysfunction (LVD)</th>
<th>EF &gt; 50% Normal Left Ventricular Function</th>
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<tbody>
<tr>
<td></td>
<td>n</td>
<td>P value</td>
</tr>
<tr>
<td>Age [years]</td>
<td>60 ± 5.10</td>
<td>0.8</td>
</tr>
<tr>
<td>Weight [kg]</td>
<td>62.2 ± 15.2</td>
<td>0.809</td>
</tr>
<tr>
<td>ECW / Weight [l/kg]</td>
<td>0.30 ± 0.002</td>
<td>0.004*</td>
</tr>
<tr>
<td>EF [%]</td>
<td>45.20 ± 2.10</td>
<td>0.817</td>
</tr>
<tr>
<td>NT-proBNP [ng/l] post-HD</td>
<td>26,824 ± 5,210</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>P:C ratio</td>
<td>3.9 ± 0.01</td>
<td>&lt;0.01*</td>
</tr>
<tr>
<td>BUN (mg/dl)</td>
<td>60.72 ± 3.41</td>
<td>&lt;0.01*</td>
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<tr>
<td>MAP pre-dialysis</td>
<td>90.1 ± 2.5</td>
<td>0.330</td>
</tr>
<tr>
<td>Post-dialysis</td>
<td>81.2 ± 1.7</td>
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</tr>
<tr>
<td>Hb [g/dl]</td>
<td>13.0 ± 0.5</td>
<td>0.612</td>
</tr>
<tr>
<td>Hct</td>
<td>0.35 ± 0.02</td>
<td>0.529</td>
</tr>
<tr>
<td>CRP [mg/l]</td>
<td>10.2 ± 2.3</td>
<td>0.421</td>
</tr>
<tr>
<td>Albumin [g/l]</td>
<td>40.25 ± 1.4</td>
<td>0.873</td>
</tr>
</tbody>
</table>

* Significantly differ with corresponding group. NTproBNP - N-terminal pro-B-type natriuretic peptide; BP - blood pressure; MAP – Mean arterial pressure; P:C – protein to creatinine ratio; Hb - haemoglobin; Hct - haematocrit; CRP - C-reactive protein; NS - not significant (P < 0.05); EF - ejection fraction.