

NT-pro-BNP IN DIABETES MELLITUSKamakhya Kumar¹, Arunima Datta², Pramod Kumar³, Anant Kumar⁴, Arup Bandyopadhyay⁵**HOW TO CITE THIS ARTICLE:**

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ABSTRACT: BACKGROUND: Brain natriuretic peptide (BNP) is a peptide hormone that is synthesized largely by the heart in the form of pre-pro-BNP which is cleaved to pro-BNP, and then proteolyzed to BNP and NT-pro-BNP. ANP, BNP and NT-pro-BNP are sensitive diagnostic markers for heart failure. NT-pro-BNP being more sensitive is preferable to BNP and ANP for the detection of heart failure. Several studies have shown that NT-pro-BNP level is higher in diabetics. We have here tried to study the same dividing the diabetic population duration-wise. **MATERIALS AND METHODS:** Patients with uncomplicated diabetes mellitus aged below 50 years were selected irrespective of sex. Patients without diabetes mellitus were taken as controls (Group A). Others viz., Group B consists of DM patients suffering from diabetes mellitus for a period <1 year, Group C consists of DM patients suffering for a period >2 year but <6 years and Group D comprises patients of uncomplicated DM for a period >10 years. NT-pro-BNP values in plasma are determined by AQT 90 FLEX NT-pro BNP Assay. Blood samples are collected with EDTA as anti-coagulant and plasma is separated. A Roche Modular Analytics E170 immuno assay analyser was used. **RESULTS:** The results show that the plasma NT-pro-BNP becomes progressively higher in diabetic patients with increased duration of diabetes when compared to the normal healthy controls. The difference in plasma NT-pro-BNP levels of Groups A and D showed a high degree of statistical significance but the difference was not statistically significant in case of Group B and Group C. **CONCLUSION:** It was found that uncomplicated DM patients of short and moderate duration had greater plasma NT-pro-BNP levels than the age matched non-diabetic healthy group though the difference is not significant. However the levels of NT-pro-BNP in patients of Group D were even higher than Group B and C (Gr A vs. Gr D: $p < 0.005$). Diabetic patients can be periodically checked for NT-pro BNP to detect early onset Diabetic cardiomyopathy or other cardiac disorders as suggested by high NT-pro-BNP levels.

KEYWORDS: NT- pro-BNP; Diabetic Cardiomyopathy, Diabetes Mellitus.

INTRODUCTION: Cardiomyopathy as per recent definition of the World Health Organization is ventricular dysfunction due to inability to correct volume or pressure overload in valvular diseases, hypertension, coronary artery disease (CAD), Diabetes Mellitus (DM), alcoholism, viral myocarditis and genetic preponderance etc.¹ DM is a not so uncommon cause of cardiomyopathy when the former is particularly long standing and both are becoming increasingly more common. Since NT-pro-BNP and other natriuretic cardiac markers are enhanced in cardiac ailments as noted above, it will not be unreasonable to study NT-pro-BNP levels in diabetic patients particularly when cardiomyopathy is suspected to be setting in.

NT- pro-BNP belongs to the natriuretic peptide (NP) family of hormones which contribute to the control of body fluid homeostasis and blood pressure regulation through their combined actions on vasculature, kidneys, and adrenal glands.² The best-known members of these families are ANP,³ BNP,⁴ NT-pro-ANP, NT pro-BNP^{5,6} and C-type natriuretic peptide (CNP).⁷ ANP and BNP are produced predominantly by the cardiac atrium and ventricle respectively in response to increased atrial and

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ventricular transmural pressure, although BNP was first isolated from brain. These two natriuretic peptides have pronounced hypotensive, diuretic and natriuretic effects.² BNP was first identified in the brain, in the form of pre-pro-BNP which is cleaved to pro-BNP. Proteolysis of pro-BNP (108 amino acids) results in BNP (32 amino acids) and the N-terminal piece of pro-BNP (NT-pro-BNP: 76 amino acids). Both BNP and NT-pro-BNP are sensitive diagnostic markers for heart failure along with Atrial Natriuretic Peptide (ANP).

BNP and NT-pro-BNP are better markers than ANP. Again, NT-pro-BNP is even preferable to BNP for the detection of heart failure and dilated cardiomyopathy, particularly in asymptomatic patients, as the NT-pro-BNP is more sensitive than BNP.

Plasma levels of ANP and BNP are markedly elevated in heart failure⁸ and after acute myocardial infarction (AMI),⁹ and are powerful predictors of ventricular dysfunction and mortality.¹⁰ Moreover, within heart tissue gene expression of both ANP and BNP is reportedly unregulated in animal models of MI and heart failure and human heart disease.¹¹⁻¹⁶ Although ANP is expressed primarily in the atria in adults, the ventricle is the major site of ANP and BNP expression in embryos.¹⁷

The mechanism of natriuretic peptide expression is very characteristic. The cellular response to cardiac injury is initiated by an invasion of inflammatory cells, followed by infiltration by endothelial cells and fibroblasts to form granulation tissues. A complex interplay of paracrine factors released by macrophages and injured myocytes triggers the phenotypic switch of fibroblasts to myofibroblasts, which deposit collagen to form the fibrotic scar. Changes at the site of injuries are accompanied by on-going hypertrophy and remodelling of the non-infarcted myocardium.¹⁸ ANP and BNP are synthesised by cardiac myofibroblast. Cardiac fibroblasts express all three NP receptors and can generate the second messenger cGMP in response to both ANP and BNP.¹⁹ ANP inhibits the DNA synthesis and proliferation of cardiac fibroblast in culture.^{20,21} It also inhibits the synthesis of collagen by rat and human cardiac fibroblast via cGMP.²² During regional mechanical stress and hemodynamic overload to the left ventricle there is marked increase in mRNA related to both ANP and BNP.²³

NT-pro-BNP is a marker for functional cardiac impairment and is increased in heart disease with or without symptoms. It is a hormonally active natriuretic peptide i.e., mainly released from cardiomyocytes in left ventricular wall in reaction to stretch and tension of the myocardial wall. The prohormone pro-BNP splits into BNP and the hormonally inactive remnant NT-pro-BNP by proteolytic cleavage.^{24,25} Therefore NT-pro-BNP is not hormonally active but a good marker of heart disease. The half-life of NT-pro-BNP in circulation is around 120 minutes. The normal range for circulating NT-pro-BNP is 100pg/ml.²⁵

Diabetic cardiomyopathy is a not so uncommon clinical condition. It is a measurable deterioration of myocardial ability to contract due to any reason usually leading to heart failure. Common symptoms include dyspnoea and peripheral oedema and these patients are often at risks of dangerous forms of arrhythmia and sudden cardiac death.²⁶

The term cardiomyopathy came into use only 50 years ago. In 2008, the European society of cardiology (ESC) defined it as a myocardial disorder in which the heart muscle is structurally and functionally abnormal.²⁷ Since cardiomyopathy is a dangerous and often fatal disease an early diagnosis of cardiomyopathy in the natural history of DM is crucial. Periodic estimation of plasma NT-pro-BNP will be a valuable tool for this.

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MATERIALS AND METHODS: The study was done in four groups of patients: Group A, Group B, Group C and Group D. Group A consists of perfectly healthy volunteers below the age of 50 years belonging to both sexes and free from any cardiac or liver disease or DM. Group B consists of DM patients without any complications particularly cardiac or hepatic, suffering from diabetes mellitus for a period <1 year. Group C consists of DM patients who are proven patients of DM for a period >2 year but <6 years. Group D comprises patients of DM for a period of >10 years. 30 subjects are selected for each of Group A and Group B, but Group C contains 11 subjects and Group D contains 17 subjects as we could not get more number of subjects with established and proven diabetes mellitus suffering from the ailment for such a prolonged period without developing any complications particularly cardiac and hepatic, in our set up. History and clinical profile of all the patients of all the four groups are taken first. All the patients were also subjected to skiagram of chest (PA view), ECG, echocardiography with colour Doppler studies, USG (Whole abdomen) and liver function tests.

Patients aged above 50 years are deliberately excluded because NT-pro-BNP level becomes higher and higher even in otherwise healthy non-diabetic old persons as their age progresses. Diabetic patients with other disorders like cirrhosis of liver congestive cardiac failure are also excluded because they also show high plasma NT-pro BNP levels. The NT-pro-BNP level cut off value is taken as 100pg/ml. Institutional ethical committee approval and patient's written consent were taken.

NT-pro-BNP values in plasma are determined by AQT 90 FLEX NT-pro BNP Assay. Blood samples are collected by venepuncture with EDTA as anti-coagulate and from that plasma is separated. A Roche Modular Analytics E170 immune assay analyser was used. Data for statistical analysis were expressed as (mean±SD) and statistical significance were computed by "Unpaired t-test". The level of significance was fixed at 5% (p<0.05). The statistical analysis was done using software SPSS 16 version.

RESULTS: The results of our studies are given in following Tables 1 & 2:

Group	Group A	Group B	Group C	Group D
Type	Controls	Diabetic Duration<1yr	Diabetic Duration >2yr-<6yrs	Diabetic Duration >10yrs
NT-pro-BNP Level (pg/ml) Mean±SD	49.47±18.86	102.47±21.97	258.71±139.96	342.73±101.33

TABLE 1: Comparison of NT-pro-BNP Level (pg/ml) (mean±SD) in Groups A, B, C & D

Comparison between	Group A & Group B	Group A & Group C	Group A & Group D
t-value	1.413	0.0001469	6.02055
p-value	>0.05	>0.05	<0.005
Level of significance	Not significant	Not significant	Highly significant

TABLE 2: Comparison of level of significance in Group A, B, C & D

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The results show that the plasma NT-pro-BNP becomes progressively higher compared to the normal healthy controls (Group A) in short duration DM (Group B), moderate duration DM (Group C) and long duration DM (Group D) respectively. Although short duration diabetics without complications have higher plasma NT-pro-BNP levels than healthy controls but the difference is not statistically significant. The plasma NT-pro-BNP difference between non-diabetic controls and long duration diabetics however shows a high degree of statistical significance (<0.005).

DISCUSSION: It was found in our experiments that the plasma NT-pro-BNP level is higher in short and moderate duration diabetics than in non-diabetic healthy controls. However this difference is not statistically significant. Again when the plasma NT-pro-BNP level of the controls and compared with patients of long duration diabetics the difference becomes statistically highly significant.

The concept that there may be a direct relation between diabetes and heart failure and cardiomyopathy is not new. In 1954, Lundbaek published an article on clinically important complications in patients with diabetes underlining that heart disease was common in patients with DM and was present in two-thirds of elderly diabetic subjects.²⁸ He was the first to suggest the presence of a diabetes specific cardiomyopathy.²⁹ Currently, IHD is the leading cause of CHF in Industrialized society with diabetes as a rapidly emerging risk factor.^{30,31} Considering the rapidly growing prevalence of diabetes this means that the combination of diabetes and CHF will become increasingly more common in the future.³²

In our study the plasma NT-pro -BNP level was definitely higher in short and moderate duration diabetics than that in normal controls but the difference was not statistically significant. Another study in which NT-pro-BNP levels are shown to be significantly elevated in cohort of patients with diabetes, but the duration of diabetes was not considered.³³ Charlotte et al³⁴ had convincingly demonstrated earlier a similar finding and suggested NT-pro-BNP as a screening marker as increased risk predictor of CVD in diabetes giving a strong base to our study, but in their study also the diabetic population was not divided in groups according to duration of the disease. There are several possible explanations for elevated NT-pro-BNP levels in patients with diabetes as they have a higher prevalence of diastolic dysfunction, more peripheral and distal atherosclerotic changes in the coronary tree due to increased blood lipids. Hearts from patients with diabetes have increased collagen content as have been verified in autopsy studies and it is proposed that natriuretic peptide synthesis increases by the same mechanism that transform cardiac fibroblast into a collagen-secreting cell.³⁵ Another possible mechanism working from the very start of diabetes could be decreased relaxation because of ATP deficiency.

The intracellular glucose deficiency among patients with diabetes leads to a higher use of free fatty acids through beta oxidation in the myocardium. A sufficient amount of carbohydrate break down is of great importance for assuming an adequate function of the ion pumps meaning Na⁺/K⁺-ATPase and Ca⁺⁺ ATPase, which maintains the right cardiomyocyte membrane potential and intracellular Ca⁺⁺ transport, that triggers relaxation. In the diabetes heart, this balance is disturbed, proposing a functional explanation to the impaired relaxation in the myocardium.³⁶⁻³⁸ Thus, NT-pro-BNP might be especially useful for screening CVD risks in diabetes subjects.

CONCLUSION: Our studies have shown that in established diabetes mellitus of moderate to long duration the plasma NT-pro-BNP level is much higher than that in uncomplicated non-diabetic population and the difference is statistically highly significant.

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This suggests that at the beginning in uncomplicated state the plasma NT-pro-BNP level is slightly higher than normal keeps on increasing as cardiomyopathy or any gross cardiac disease for that matter gradually sets in and its severity increases. Therefore, measurement of NT-pro-BNP might be a simple but useful screening tool for early diagnosis of CVD in diabetics. However, more elaborate studies are needed to confirm the findings of this small study.

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