Plasma N-Terminal Pro B-Type Natriuretic Peptide Reference Values in Subjects without Heart Disease

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ABSTRACT

Background: *B-type natriuretic peptide (BNP) and its terminal fragment (NT-proBNP) are released from ventricular cardiomyocytes in response to increase in ventricular wall stress and to myocardial ischaemia. Both BNP and NT-proBNP have been described as reliable diagnostic and prognostic biomarkers in patients with heart failure. There are no previous reports on the reference values for both markers in the Nigerian population ; an essential pre-requisite for effective utilization of the biomarkers in the management of the indigenous patients.*

Objective: *The aim of this study was to determine the upper reference value for NT-proBNP in apparently healthy Nigerian adults with neither cardiovascular nor renal disease.*

Subjects and methods: *A group of 34 subjects were recruited into the study; equally divided between the sexes. They were aged between 19 and 42 years. Subjects' blood pressure, weight and height were measured. Plasma NT-proBNP was analysed using the electrochemiluminescence immunoassay method on elecsys 2010 machine, while creatinine and blood glucose were measured by routine methods.*

Results: *The mean values for BMI, plasma glucose, creatinine clearance, systolic blood pressure, diastolic blood pressure were 21.2 kg/m^2, 4.1 mmol/L, 91.2 ml/min, 119 mmHg, 79 mmHg respectively. The mean concentration of NT-proBNP in males and females were 24.7 pg/ml and 32.5 pg/ml respectively, while the upper reference value for NT-proBNP was found to be 99.5 pg/ml.*

Conclusion: *We therefore suggest that NT-proBNP values below 100 pg/ml be considered as the cut-off point for normal ventricular function or structure in our clinical setting.*

Keywords: B-type natriuretic peptides, NT-proBNP, BNP, Upper reference value

Introduction

B-type natriuretic peptide, which is also called brain-type natriuretic peptide (BNP), belongs to the natriuretic peptide family together with other structurally similar peptides, namely atrial natriuretic peptide (ANP), C-type natriuretic peptide (CNP) and Urodilatin.[1] It was first described in 1981 after isolation from porcine brain. However, it was soon found to originate mainly from the heart, thus representing a cardiac hormone.[2]

The major source of BNP synthesis and secretion is the ventricular myocardium. BNP

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is derived from a preprohormone (preproBNP, 134 amino acids) containing a signal peptide sequence at the amino-terminal end. The prohormone, proBNP (108 amino acids), is formed after cleavage of the signal peptide (26 amino acids). ProBNP is further split into a longer inactive amino-terminal fragment, NT-proBNP (amino acids 1-76), and a shorter biologically active fragment, BNP (amino acids 77-108), both of which are released into the circulation in equal proportion.[2,3]

The main stimulus for increased synthesis and secretion of both molecules i.e BNP and NT-proBNP, is myocardial wall stress. Furthermore, factors such as myocardial ischaemia and endocrine (paracrine) modulation by other neurohormones and cytokines are also of importance.[4]

The physiological effects of BNP are manifold and comprise natriuresis/diuresis, peripheral vasodilation, and inhibition of rennin-angiotensin-aldosterone-system (RAAS) and the sympathetic nervous system (SNS).

BNP is cleared from plasma by binding to the natriuretic peptide receptor type C (NPR-C) and through proteolysis by neutral peptidases.[2,3] In contrast, NT-proBNP which is mainly cleared by renal excretion, lacks a clearance receptor, and thus has a longer half-life in serum than the active hormone. The circulating concentration of NT-proBNP is believed to be less influenced by conditions under which blood sample is taken.The half-life of BNP is 20 minutes whereas NT-proBNP has a half-life of 120 minutes.[4,5,6]

The inactive propeptide, NT-proBNP better fits the definition of a disease marker than the circulating concentrations of BNP.[7] NT-proBNP is relatively stable in EDTA plasma for up to 48 hours at room temperature whereas measurable BNP levels drop significantly after only 4 hours and the level falls by half after 48 hours. When stored refrigerated at 4^{o}C plasma BNP will also decrease significantly while NT-proBNP levels will remain stable for up to 6 days.[8]

Several studies have demonstrated that measurement of BNP and/or NT-proBNP concentration represents a useful addition to chest x-ray, electrocardiogram and Doppler echocardiography in the clinical assessment of patients suspected to have heart failure, both in the out-patient and in the emergency care settings.[9,10,11,12,13] They are useful in screening for left ventricular systolic dysfunction[14,15,16] and for monitoring therapy for heart failure.[17,18,19]

Generally, plasma BNP and NT-proBNP concentrations are raised in patients with heart failure, rising in line with the New York Heart Association(NYHA) classification. Furthermore studies have shown that the greater the cardiac damage, the higher the plasma BNP and NT-proBNP concentration.[20] In acute heart failure, NT-proBNP has been shown to be more sensitive than BNP (90% versus 80% sensitivity overall), for diagnosis.[21]

Most of the reported studies cited above, were carried out on Caucasian populations hence the need for the determination of local reference values to investigate the usefulness or otherwise, of NT-proBNP assay in the management of our indigenous patients with cardiac diseases.

The aim of this study was therefore to determine the upper reference value for plasma NT-proBNP in healthy Nigerian subjects without heart or renal diseases.

Subjects and Methods

Study centre

The study was carried out at the Chemical Pathology and Immunology Laboratory of Aminu Kano Teaching Hospital, Kano, Nigeria.

Subjects

Thirty-four subjects were studied. They were selected from among the undergraduate students on Students Industrial Work Experience Scheme (SIWES), Laboratory staff and Resident Doctors (17 males and 17 females, aged between 19 and 42 years of age).

Detailed history was taken from all subjects and comprehensive physical examination carried out. Subjects with history and clinical evidence of any of the following disease conditions were excluded from the study, namely: cardiovascular diseases, renal diseases, thyroid disorders, diabetes mellitus and pregnancy. In accordance with Helsinki Declaration II, the design and execution of the study were explained to the subjects and informed consent was obtained. The Ethical Committee of the hospital gave approval for the conduct of the study.

Methods

Blood Pressure was measured in the sitting position after a 10 minutes rest. Hypertension - was defined as systolic blood pressure e"140 mmHg or diastolic blood pressure e"90 mmHg. Height and weight were measured without shoes and heavy clothing and the Body Mass Index (BMI) was calculated as weight (kg)/height (meters^2).

Blood was collected under standardized conditions to minimize source of pre-analytic variation. After an overnight fast, five milliliters of blood was collected from each of the subjects by the conventional venipuncture between 08:00 and 09:00 hours with the subjects in the sitting position, into a lithium heparinized tube. All samples were then centrifuged at 3000 g for 10 minutes within 30 minutes of collection. Plasma was separated and an aliquot was stored at -70^{o}c until NT-proBNP analysis while the remaining aliquot was used for the measurement of glucose and creatinine on the same day of blood collection using routine laboratory methods.[22,23] The creatinine clearance was estimated from the plasma creatinine value using the Cockcroft and Gault formular.[24]

At the end of the collection period, all frozen samples were thawed, mixed, and centrifuged for the analysis of NT-proBNP in a single run in duplicates. NT- proBNP concentrations were determined by an electrochemiluminescence sandwich immunoassay (Roche Diagnostics)[25] using two polyclonal antibodies directed at the NT-proBNP molecule. The assay was performed on an Elecsys 2010 system. ((All measurements

Table 1: Baseline characteristics

	MALE	FEMALE	TOTAL
Number of Subjects	17	17	34
Age (years)	25.5 ± 1.5 (19- 42)	23.5 ± 0.8 (20 – 32)	24.5 ± 0.9 (19 – 42)
NT-proBNP (pg/ml)	24.7 ± 6.2 (6.23 – 99.5)	32.5 ± (5.34 – 87.05)	28.7 ± (5.34 – 99.5)
BMI (kg/m^2)	22.0 ± 0.9 (14 – 28)	20.4 ± 0.7 (16 – 25)	21.2 ± 0.58 (14 – 28)
Glucose (mmol/L)	4.0 ± 0.15 (3.0 – 5.3)	4.2 ± 0.16 (2.9 – 5.4)	4.1 ± 0.11 (2.9 – 5.4)
Creatinine Clearance (ml/min)	88.3 ± 2.4 (75 – 92)	95.8± 2.3 (77 – 99)	90.2 ± 1.7 (75 – 99)
Systolic Blood Pressure (mmHg)	118.5 ± 2. (100 – 135)	119.4 ± 2.6 (90 – 130)	119 ± 1.8 (90 – 135)
Diastolic Blood Pressure	76.8 ± 1.7 (60 – 90)	81.2 ± 2.1 (60 – 90)	79 ± 1.4 (60 – 90)

Data is presented as mean ± SEM and Range

were performed at the Department of Chemical Pathology and Immunology, Aminu Kano Teaching Hospital, Kano-Nigeria.))

Statistical Analysis

All data are expressed as Mean with Standard Error of Mean (SEM), Range and Percentiles. Comparison between groups were performed by Students' t-test and test for trend by linear regression. Associations were tested by univariate and multi-variate linear regression analysis. Confidence intervals - for the estimates were calculated using Boostrap estimation with 2.5th and 97.5th percentile. $P < 0.05$ on two-sided tests was considered significant. All Statistical analyses were performed using SPSS version 14.0 and Minitab statistical software systems. The upper reference value for NT-proBNP was defined as the 97.5th percentile of NT-proBNP level.

Results

The baseline characteristics of the subjects is shown in table 1. There was no significant difference ($p < 0.05$) in the characteristics between both sexes except for age and the plasma concentration of NT-proBNP.

Table 2 shows plasma NT-proBNP levels in normal subjects, with upper reference values in bold while table 3 depicts the serum NT-proBNP concentration of the subjects in percentiles.

Discussion

This study is one of the first clinical studies to determine the upper reference value for NT-proBNP in our environment.

The mean systolic blood pressure and diastolic blood pressure of the subjects were 119 mmHg and 79 mmHg respectively. None of the subjects was found to be hypertensive.

Studies have shown that assays for both BNP and NT-proBNP show similar clinical performance in the diagnosis of congestive heart failure.[13,19] Prognostic information is also gained via these assays in acute coronary syndrome [13,2] including myocardial infarction.[21]

Table 2: Serum NT-proBNP levels in subjects by gender

Median NT-proBNP (pg/ml)	Males	Females
Mean NT-proBNP (pg/ml)	16.0	30.0
Mean + 2 SD NT-proBNP (pg/ml)	24.7	32.5
97.5th Percentile for NT-proBNP (pg/ml)	27.2	34.9
	99.5 (6.23 – 99.5)	**87.05** (5.34 – 87.05)
Number of Subjects	17	17

NT-proBNP upper reference values are defined as 97.5th percentile for NT-proBNP serum concentration stratified by Gender(in bold)

This study showed that the upper reference value for NT-proBNP was 99.5 pg/ml for males and 87.05 pg/ml for females and 99.5 pg/ml

Table 3: NT-proBNP of the subjects in percentiles

Percentiles	2.5th	5th	95th	97.5th
NT-proBNP(pg/ml)	5.34	6.01	90.16	99.5

for both sexes combined. These values are lower than the reported cut-off value of 125 pg/ml for patients younger than 75 years of age and 450 pg/ml in patients 75 years or older in the Roche study.[6] Johnston *et al*[26] using the same NT-proBNP assay found 97.5th percentile value of 184 pg/ml for men and 268 pg/ml for women less than 65 years of age , again higher than the values observed in the present study. The higher cut-off values observed in the Roche study and that of Johnston et al may be accounted for by the older age of their studied population compared to the age of the subjects that participated in this study. Both BNP and NT-proBNP levels in blood increase with age regardless of cardiac status, presumably due to natural stiffening of the left ventricle, due to age-related fibrosis which stimulates BNP and NT-proBNP production and subtle diastolic dysfunction.[27,28] Also the reduced renal clearance, with the mean estimated creatinine clearance in normal subjects > 60 years old significantly lower than in normal subjects < 60 years old (74 versus 91 ml/min respectively)[23] could result in increased levels of NT-proBNP in individuals >60 years of age. The mean creatinine clearance obtained for the subjects studied was 90.2 ml/min.

This study also shows that the mean NT-proBNP level was higher in the females (32.5 pg/ml) than in males(24.7 pg/ml). Similar observations have been reported in the literature from other parts of the world.[22,24,25] The mechanism underlying increased natriuretic peptide levels with female gender is unclear, although Redfield *et al* found 21% higher natriuretic levels in women taking hormone replacement therapy suggesting a role of oestrogen status.[29]

A recent study has also documented that obesity significantly lowers the BNP concentration.[30] However, the BMI of the subjects in this study were within the reference limits, hence no case of obesity was recorded. An no correlation was established between BMI and NT-proBNP in the present study.

Thus age, sex and body mass index should be taken into consideration for the appropriate interpretation of BNP or NT-proBNP result, but currently the effects of these factors on the reference interval requires further study and quantification.

Study Limitation

The cohort size of this study was small. The cost of the reagents was exorbitantly high to allow for more subjects to be included in the study.

The small sample size could potentially affect the reliability of the results. However, as similar results have been seen elsewhere, this seems less likely. It was also observed that the mean age of this study population is relatively young compared to the quoted references, which actually explained the slightly lower values for NT-proBNP observed in our study. More elderly subjects probably require higher cut-off values, hence such analysis involving them is still required. However, this study has truly demonstrated the upper reference values for subjects with normal renal and cardiac function in our environment, the first of its kind.

Conclusion

In conclusion, we suggest that NT-proBNP values of 100 pg/ml can be used as a diagnostic cut-off value for normal ventricular structure and function in our clinical setting. Therefore higher values could prove useful in the diagnosis of heart failure.

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