

# The association between brain natriuretic peptide and tissue Doppler parameters in children with hypertrophic cardiomyopathy

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## ABSTRACT

In this study, we investigated the association between brain natriuretic peptide (BNP) levels and tissue Doppler imaging measurements and also screening for deadly mutations in patients with hypertrophic cardiomyopathy (HCM). We enrolled 20 patients diagnosed with HCM (age:10.7±5 years (1-17), 85% male, weight:42.25±23.10 kg, height:141.80±32.45 cm) and 20 age, gender and body weight-matched control subjects. We performed electrocardiography, transthoracic echocardiography, and tissue Doppler echocardiography in each group, as well as genetic tests (for *Arg403Gln*, *Arg453Cys*, *Arg719Trp* and *Arg719Gln* mutations in *MYH7 Exons* 13, 14, 19) and BNP in the patients. The patients were divided into two groups according to the presence (Group 1) or absence (Group 2) of left ventricular (LV) outflow tract obstruction. QTc dispersion and the LV ejection fraction and left atrial (LA) volume index were increased in Group 1. The LA volume index and the mitral and septal E/Ea ratio and septum Z-score were increased while the mitral lateral annulus and septal annulus Ea wave velocities and the mitral and tricuspid E/A ratio were decreased in patients with high levels of BNP compared to those with normal BNP levels. There were no mutations that are associated with increased risk of sudden death found in patients included in this study. In the light of our data, we conclude that such parameters BNP levels above the 98 pg/mL, septal thickness Z-score >6, and higher mitral and septal E/Ea ratios can be used for management of patients with HCM according to life-threatening conditions.

KEY WORDS: Hypertrophic cardiomyopathy; molecular genetics; brain natriuretic peptide; tissue Doppler flow; left ventricular outflow tract obstruction

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## INTRODUCTION

Hypertrophic cardiomyopathy (HCM) is a common hereditary condition with an incidence of 1/500 in the general population. The manifestations of this genetically heterogeneous disease vary from asymptomatic or mildly symptomatic patients to patients with severe heart failure, syncope, or sudden cardiac death due to malignant ventricular arrhythmias. The cardiac pathology of the disease is related to myocyte hypertrophy and sarcomere irregularity [1]. More than 1400 mutations of at least 11 genes coding the cardiac sarcomere proteins are known to be causative factors of this condition [2]. Molecular genetics studies were conducted in

relatives of the patients to detect individuals before the onset of symptoms and hypertrophy to decrease the rate of sudden death by changing the lifestyle of those with a malignant mutation, encouraging them to avoid competitive sports, and implanting defibrillators.

Echocardiography is a sensitive and specific clinical method for the diagnosis of HCM. Tissue Doppler imaging (TDI) echocardiography has been investigated in the pre-clinical diagnosis of HCM. Studies from transgenic animal models revealed some abnormal myocardial function at a time preceding the development of left ventricular (LV) hypertrophy, firstly due to alterations in Ca<sup>++</sup> sensitivity which probably induce low TDI velocities at annular mitral level [3]. Brain natriuretic peptide (BNP) levels were thought to be potentially useful for the determination of early ventricular changes in carriers before the description of conventional echocardiographic abnormalities. TDI E/Ea ratio and BNP were more accurate in identifying patients with increased

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filling pressures and tracked well the changes in mean wedge pressure with therapy [4]. Lateral Ea velocity is usually higher than septal Ea velocity [5]. This difference can be exaggerated or reversed depending on the presence and extent of cardiac pathology. An animal study [6] showed that in the setting of myocardial ischemia, Ea is dependent not only on global LV systolic and diastolic function but also on regional thickening. Accordingly, it is imperative to use the average of septal and lateral Ea velocities when drawing conclusions on LV diastolic function in patients with regional dysfunction.

In this study, we aimed to assess two-dimensional (2D) and tissue Doppler characteristics and BNP levels of patients with HCM and to find, if present, a correlation between BNP and tissue Doppler; echocardiographic measurements in these patients. We investigated the mutations related to a high risk of sudden cardiac death in patients with HCM, as well.

## MATERIALS AND METHODS

20 patients diagnosed with HCM between 2007 and 2011 and 20 age, sex, body weight, and age-matched healthy subjects, diagnosed with an innocent murmur who were admitted to our outpatient clinic were enrolled into this study. Medical history was obtained, and a physical examination was performed for each patient. All of the patients underwent electrocardiographic and echocardiographic examination using both 2D and tissue Doppler echocardiography.

A local ethical committee has not been established in our hospital at the time of the study. Therefore, the study was approved by a scientific committee comprising the hospital administration and the lecturers.

HCM diagnosis was based on an LV wall thickness of  $\geq 15$  mm or  $\geq$  standard deviation above the mean (Z score-2) for age, sex, and body size in 2D echocardiography and on the presence of no other causative factor for hypertrophy [7,8].

Venous blood samples of the patients were collected with ethylenediaminetetraacetic acid tubes. DNAs were obtained from the peripheral blood leukocytes. The following primers were used for the polymerase chain reaction amplification of exons 13, 14, and 19 of the *MYH7* gene associated with the *Arg403Gln*, *Arg453Cys*, *Arg719Trp* and *Arg719Gln* mutations, which are the most common mutations according to the literature [9].

Exon 13 (*Arg403Gln*): Fragment size: 267 base pairs; Forward primer: 5' *TTA CAG GCA TGA ACC ACA CAC C* 3'; Reverse primer: 5' *GTG AAC TTG AAA ACT CTC ATC CC* 3'

Exon 14 (*Arg453Cys*): Fragment size: 287 base pairs; Forward primer: 5' *CAC TCT TCC CAA CCC TG* 3'; Reverse primer: 5' *GGT CCA CAG CTG GCT CTA AG* 3'

Exon 19 (*Arg719Trp*, *Arg719Gln*): Fragment size: 200 base pairs; Forward primer: 5' *CTC ACA GAC TCC TCC TAC*

*TTC CTT C* 3'; Reverse primer: 5' *GCC TGG CTC CCC CTG TTC TAT GAG C* 3'

## Echocardiography

Transthoracic 2D and Doppler echocardiographic evaluations of the patients were performed with the GE Vivid 3 device (GE Healthcare, Milwaukee, WI, USA) using 3 and 7 MHz transducers for coronary assessment. M-mode echocardiography measurements were obtained from the level of the posterior mitral valve, according to the recommendations of the American Society of Echocardiography [10]. Septal curvature and cavity contours were evaluated in the long axis view at the end-diastole. In the apical four-chamber view, the maximum pulse waves Doppler flow rates were recorded with a sample volume situated at the mitral and tricuspid valve endings. LV outflow tract obstruction was defined as a subvalvular gradient  $>30$  mmHg at rest [8].

## Tissue Doppler imaging (TDI)

The sample volume was situated in a 3 mHz probe apical four-chamber view at the intersection of the LV mitral lateral annulus, the mitral septal annulus, and the right ventricle tricuspid lateral annulus. Recording was performed when the patients were calm and in at least five cardiac cycles to prevent the flow from a respiration affect. The Sa, Ea, Aa waves from the mitral lateral annulus, the Sa, Ea, Aa waves from the mitral septal annulus, and the Sa, Ea, Aa waves from the tricuspid lateral annulus were recorded [11]. E/Ea: This shows the filling pressure of the LV. There are limited studies on the E/Ea ratio in pediatric groups. This ratio reflects the pulmonary capillary wedge pressure (88% specificity, 78% sensitivity) and is useful for determining the prognosis in congestive heart failure [12].

## Left atrial (LA) volume

The oval-ellipsoid method was used. LA diameters were determined from the apical four-chamber and parasternal long axis sections (Transverse [D1], longitudinal [D2], parasternal long axis [D3], the formula:  $D1 \times D2 \times D3 \times 0.523$  was used) [13].

## LV mass

The following formula developed by Devereux et al. was used to calculate the following:

LV mass =  $0.8 (1.04 [\text{interventricular septum thickness} + \text{posterior wall thickness} + \text{LV end diastolic diameter}]^3 - [\text{LV end diastolic diameter}]^3) + 0.6$  [14].

## Myocardial performance index (Tei)

This value can be calculated by, dividing the sum of isovolumic contraction time and isovolumic relaxation time by the

ejection time and extracting the LV ejection time from the time interval between the ending and the beginning of mitral flow for the LV and dividing it by the ejection time [15].

### Biochemical myocardial function marker

BNP levels were measured in ethylenediaminetetraacetic acid-blood samples from 18 patients using the Triage Meter Plus device (Biosite, San Diego, CA, USA) to investigate ventricular functions at a cut-off point of 98 pg/mL [16].

### Statistical assessment

Statistical analyzes were performed using the “SPSS for Windows 17.0” (SPSS, Chicago, IL, USA) program. Normal distribution of the variables was assessed by the Kolmogorov–Smirnov test. The *t*-test was used to investigate the significance of differences between the control and the patient groups for the series with regular distribution, and the Mann–Whitney-U-test was used for the series with irregular distribution. *p* values below 0.05 were considered statistically significant.

## RESULTS

In this study, 85% (17/20) of the patients were male, and the mean age was  $10.7 \pm 5$  years (1-17). At first presentation, 45% (9/20) of the patients were asymptomatic while 15% (3/20) had syncope, 15% (3/20) had palpitations, and 25% (5/20) had chest pain. Of the patients, 15% (3/20) had consanguineous parents. There was no family history in 40% (8/20) of the patients, while 35% (7/20) had a family history of sudden death and 25% (5/20) had relatives diagnosed with HCM (Table 1). All of the patients were receiving beta-blocker treatment, and one subject had undergone septal myectomy.

Echocardiography revealed that HCM was sigmoidal in 40% (8/20), reverse-curved in 20% (4/20), and apical in 40% (8/20) of the patients [12]. Septum thickness was 15-20 mm in 60% of the patients and 20-30 mm in 40% (8/20); septum z-score was <4 in 30% (6/20), and 4-6 in 50% (10/20) and >6 in 20% (4/20) of the patients. Five patients (25%) had LV outflow tract obstruction while 5% (1/20) had right ventricular outflow tract obstruction, 60% (12/20) had mitral valve regurgitation, and 5% (1/20) had aortic valve regurgitation (Table 1).

Comparison of the control group and the patient group showed that the mitral Sa-Ea wave and the septal Sa-Ea wave evaluated using TDI were significantly lower, while the LV Tei index-LV E/Ea ratio assessed using pulse wave Doppler and EF – fractional shortening (FS) – LA volume index were significantly increased in the patient group compared to the control group (Table 2).

The QTc dispersion and the LV fraction – FS – LA volume index were increased in the group with LV outflow tract

**TABLE 1.** Clinical and echocardiographic characteristics of patients with HCMP

Demographic characteristics	Percentage
Rate of symptomatic cases	55
Syncope	15
Palpitation	15
Chest pain	25
Rate of asymptomatic cases	45
Rate of consanguineous marriage	15
Rate of patients without family history	40
Rate of patients with family history of sudden death	35
Rate of patients with family history of HCMP	25
HCMP type sigmoid type	40
Reverse curve type	20
Apical type	40
Septum thickness of 15-20 mm	60
Septum thickness of 20-30 mm	40
Rate of LVOTO	25
Rate of mitral regurgitation	60
Rate of aortic regurgitation	5

HCMP: Hypertrophic cardiomyopathy; LVOTO: Left ventricular outflow tract obstruction

**TABLE 2.** Echocardiographic-electrocardiographic characteristics of the HCMP group and the control group

Variable	HCMP group	Control group	<i>p</i>
Number	20	20	
Age range (mean-year)	1-17 (10.7±5)	7-14 (11±1.9)	NS
Gender distribution (boys/girls)	85% male	60% male	
LV - EF (%)	79.8±9.6	69.8±4.8	0.000
LV - FS (%)	49.3±10.4	38.8±4.1	0.000
LA - Volume index (mL/m <sup>2</sup> )	19.3±6.4	14.8±3.8	0.01
LV - Tei index	0.56±0.15	0.35±0.09	0.000
LV - E/Ea	9.9±6.3	5.1±1.1	0.000
ECG - Heart rate	85.2±25.1	98.95±12.9	0.004
ECG - QTc dispersion (s)	0.03±0.016	0.03±0.014	NS
Tissue Doppler mitral Sa (m/s)	0.08±0.02	0.12±0.02	0.000
Mitral Ea (m/s)	0.12±0.05	0.18±0.02	0.000
Mitral Aa (m/s)	0.07±0.02	0.08±0.02	NS
Tissue Doppler septal Sa (m/s)	0.06±0.01	0.07±0.01	0.000
Septal Ea (m/s)	0.07±0.02	0.13±0.01	0.000
Septal Aa (m/s)	0.05±0.01	0.06±0.01	NS

HCM: Hypertrophic cardiomyopathy; LV: Left ventricle; EF: Ejection fraction; FS: Fractional shortening; LA: Left atrial; E: Peak early diastolic flow velocity; Ea: Annular tissue Doppler component during early diastole; ECG: Electrocardiography; Sa: Peak velocity during ventricular systole; Aa: Peak velocity during atrial contraction; NS: Non-significant

obstruction compared to the group without LV outflow tract obstruction (Table 3).

The mitral lateral annulus and septal annulus Ea wave velocities were significantly decreased while the septum Z-score was significantly increased in patients with elevated BNP levels compared to those with normal BNP levels (Table 4).

The screening for mutations associated with the risk of sudden death (*Arg403Gln*, *Arg453Cys*, *Arg719Trp* and *Arg719Gln* mutations in *MYH7* Exons 13, 14, 19) revealed no mutations in any of the patients included in the study.

**TABLE 3.** Comparison of patient groups with and without LVOTO

Parameter	With LVOTO	Without LVOTO	<i>p</i>
QTc dispersion (s)	0.0520±0.01483	0.0273±0.0116	0.007
EF (%)	90±6.272	77±8.549	0.017
FS (%)	61.25±9.430	46±8.199	0.015
LA volume index (mL/m <sup>2</sup> )	23.96±2.73	17.80±6.63	0.016
LV Tei index (PW)	0.60±0.20	0.54±0.14	0.61
Right ventricular Tei index (PW)	0.34±0.11	0.38±0.09	0.45
BNP (pg/mL)	362±290	114±197	0.04
Tricuspid E/A (PW)	1.20±0.11	1.46±0.28	0.01
Mitral E/Ea	16.34±9.77	7.60±2.18	0.04

LVOTO: Left ventricular outflow tract obstruction; EF: Ejection fraction; FS: Fractional shortening; LA: Left atrial; E: Peak early diastolic flow velocity; PW: Pulse wave; Ea: Annular tissue Doppler component during early diastole

**TABLE 4.** Comparison of patient groups with normal and abnormal BNP levels

Parameter	BNP<98 pg/mL (normal)	BNP>98 pg/mL (abnormal)	<i>p</i>
Z-score of septal thickness	4.09±0.79	6.05±0.49	0.000
LA volume index (mL/m <sup>2</sup> )	16.47±3.30	23.64±7.75	0.03
LV Tei index (PW)	0.52±0.14	0.61±0.17	0.30
Right ventricular Tei index (PW)	0.36±0.10	0.38±0.08	0.52
Mitral E/A (PW)	1.79±0.44	1.22±0.17	0.004
Tricuspid E/A (PW)	1.52±0.30	1.23±0.10	0.018
Mitral E/Ea	7.31±2.35	13.45±8.41	0.039
Septal E/Ea	12.03±3.28	17.36±4.94	0.021
Tissue doppler mitral Sa (m/s)	0.086±0.021	0.085±0.026	0.6
Mitral Ea (m/s)	0.15±0.038	0.095±0.05	0.02
Mitral Aa (m/s)	0.072±0.025	0.077±0.017	0.55
Tissue doppler septal Sa (m/s)	0.059±0.013	0.066±0.014	0.36
Septal Ea (m/s)	0.089±0.023	0.063±0.027	0.03
Septal Aa (m/s)	0.054±0.015	0.067±0.018	0.13

BNP: Brain natriuretic peptide; EF: Ejection fraction; FS: Fractional shortening; LA: Left atrial; E: Peak early diastolic flow velocity; PW: Pulse wave; Ea: Annular tissue Doppler component during early diastole; Sa: Peak velocity during ventricular systole; Aa: Peak velocity during atrial contraction

## DISCUSSION

The risk of sudden death associated with HCM is independent of the severity and extent of LV hypertrophy. The most common gene mutations in HCM are observed in the beta-myosin heavy chain (B-MHC) gene with a rate of 35-50%, in the myosin binding protein C gene with 15-25%, in the cardiac troponin T gene with 15-20%, in the tropomyosin gene with <5%, and in other genes. The most commonly defined mutation from B-MHC gene mutations is the *Arg403Gln* mutation. Several studies have reported the *Arg403Gln*, *Arg719Gln*, and *Arg453Cys* mutations as those most commonly associated with sudden cardiac death [17,18]. The mutation in *alpha actinin-2* [19], the *Ala26Val* and *Arg719Trp* mutations in *MYH7* [20], and the *S297X* mutation in the *myosin binding*

*protein C* gene [21] are also associated with HCM. The screening for mutations associated with the risk of sudden death (*the Arg403Gln*, *Arg453Cys*, *Arg719Trp* and *Arg719Gln* mutations in *MYH7* Exons 13, 14, 19) revealed no mutations in any of the patients included in the study. We consider this to be a result of the limited number of patients included in this study and the genetic heterogeneity associated with the condition.

Electrocardiography was normal in 15-25% of the patients, particularly in those with localized hypertrophy. None of the electrocardiography alterations was specific for HCM. ST-T changes and prominent LV hypertrophy are the most common electrocardiography findings. Prominent Q waves were observed, particularly in inferolateral leads in 25-50% of the patients. Giant T-waves in mid-precordial leads are characteristic for apical HCM. The attempts to define the factors increasing the risk of sudden death in HCM led to several studies on QTc dispersion and heart rate variability [22,23]. QTc interval and dispersion may be clinical indicators of electrophysiological instability and ventricular arrhythmia. Some studies showed the association between increased QTc dispersion and the risk of sudden death in HCM [23,24]. Electrocardiography changes were present in 65% of the patients included in the present study, and in contrast to some other studies [25], comparison of patients with and without the LV outflow gradient above 30 mmHg deduced reveal a significant increase regarding QTc dispersion (Table 3). Furthermore, similar to the study by Yi et al. [23] there was no correlation between septum thickness and QTc dispersion. QT dispersion showed a positive correlation with the LV E/Ea while there was no correlation with the mass index-LA volume index - The septum thickness-Tei index.

B-type BNP is a protein released by the ventricles in the presence of myocytic stretch and has been associated with LV filling pressure and, independently, with other cardiac morphological abnormalities. BNP has high sensitivity but low specificity for the detection of elevated LV filling pressures. Given the respective strengths and limitations of BNP and mitral E/Ea, algorithms combining them can be used to estimate more accurately LV filling pressures in patients presenting with dyspnea. Some studies have demonstrated that elevated BNP levels are associated with the severity of LV hypertrophy rather than LV outflow tract obstruction [26] while other studies have shown further elevated BNP levels in patients with LV outflow tract obstruction [27]. We found that there was no difference regarding BNP levels between HCM patients with and without LV outflow obstruction.

At a cut-off point of 50 pg/mL, BNP had a positive predictive value of 93% and a negative predictive value of 80% for predicting E/Ea(s) >10. BNP levels correlate with non-invasive parameters of disease severity in children with HCM, including measures of raised LV filling pressures. For patients

in whom the evaluation of symptoms is difficult, BNP may be a useful additional tool in the assessment of disease severity [28]. Geske et al. found the 3-year survival to be 99.2% in patients with a BNP  $\leq$ 98 pg/mL [16]. In this study, tissue Doppler flow was found to be further disrupted in patients with BNP  $>$ 98 pg/mL.

In a study that was evaluated the 128 patients, extreme LV hypertrophy with interventricular septal wall thickness or posterior wall thickness Z-score  $>$ 6, sinus tachycardia, and supraventricular tachycardia were found to be independent risk factors for prediction of sudden death in patients with HCM [29]. In another study, Decker et al. determined that extreme LV hypertrophy (Z-score:  $>$ 6) and an abnormal blood pressure response to exercise were predictive of non-sudden cardiac death and Kaplan–Meier survival analysis predicts an 82% survival over a 20-year period [30]. As shown in these studies, the septum Z-score was significantly increased ( $6.05 \pm 0.49$ ) While the mitral lateral annulus and septal annulus Ea waves were decreased in patients with abnormal BNP levels compared to those with normal BNP levels.

Myocardial contraction and relaxation velocities, detected by TDI imaging, are reduced in HCM, including in those without LVH. Before and independently of LVH, TDI imaging is an accurate and sensitive method for identifying patients who are positive for HCM mutations [31]. Transmitral E/septal Ea ratio predicts children with HCM, who are at risk of adverse clinical outcomes including death, cardiac arrest, VT, and significant cardiac symptoms [32]. The transmitral E to lateral Ea ratio correlates with NYHA functional class and exercise capacity [33].

The septal E/Ea value was higher in patients who experienced cardiovascular events. Multivariate forward regression analysis revealed the septal E/Ea to be independent predictors of cardiovascular events [34]. A high septal E/Ea ratio, to a history of syncope and documentation of atrial fibrillation, was a significant predictor of combined end points. In contrast, plasma BNP levels were not a significant predictor of combined end points [35]. In this study, however, BNP levels of  $>$ 98 pg/mL were found significantly associated with septal thickness Z-score  $>$ 6 that was found an important risk factor for sudden cardiac death in various studies, and higher mitral and septal E/Ea ratios in TDI measurements.

Clinical course in patients with HCM is quite variable. Nevertheless, the majority of patients are asymptomatic in the childhood period. Severe signs and symptoms associated with HCM become evident in the adulthood in most of the patients since the disease usually shows a slow progression with age. Sample size in our study was limited due to these factors mentioned above, as our study group consisted of the pediatric patients only.

## CONCLUSION

In our study, we did not find any mutations that lead to the sudden cardiac death. Similar to the previously reported data, we found that BNP levels above the 98 pg/mL, septal thickness Z-score  $>$ 6 and higher mitral and septal E/Ea are associated with the increased mortality in patients with HCM. Therefore, we suggest that patients with HCM can be followed up cautiously according to life-threatening events by using these parameters.

## DECLARATION OF INTERESTS

The authors declare no conflict of interests.

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