Brain natriuretic peptide (BNP) belongs to the family of natriuretic peptides which counterbalances the renin-angiotensin-aldosterone system. BNP are produced in ventricular cardiomyocytes, and secreted in response to volume expansion or pressure overload. The BNP test can be used to detect preclinical heart disease or to confirm the cardiac etiology in symptomatic patients. Brain natriuretic peptide was investigated in several extra-cardiac conditions such as rheumatic diseases, pulmonary diseases [1, 2].

Hyperthyroidism can manifest with overt clinical symptoms of heart failure. The increase in pulse rate and cardiac output seen in untreated hyperthyroid patients also represents a situation of increased cardiac stretch, which theoretically could influence the secretion of BNP [3]. There are only a few studies investigating the influence of thyroid dysfunction on BNP measurements [4-6]. However there are controversial issues in this field.

The purpose of this study was to assess BNP levels in patients with hyperthyroidism before specific treatment for hyperthyroidism and after euthyroidism was achieved.

Methods

The study was performed in a prospective design. The study population consisted of 48 consecutive newly diagnosed untreated overt hyperthyroid patients who had not been treated any anti-thyroid medications before. Overt hyperthyroidism was diagnosed when patients simultaneously showed high serum concentra-
tions of T3 and/or T4 levels and suppressed TSH level.

Exclusion criteria were presence of diabetes, hypertension, coronary artery disease, systolic heart failure (EF<40%), pregnancy or lactation; hepatic or renal dysfunction, significant neurological or psychological diseases, inflammatory bowel diseases, and systemic autoimmune diseases such as systemic lupus erythematosus, rheumatoid arthritis, and scleroderma. Patients who had used any medications (within the previous six months) that might have affected study parameters such as antihypertensives, amiodarone, multivitamins, oral contraceptives, antidepressants, anti-serotonnergics, oral corticosteroids, antifolates, anticonvulsant agents, lipid-lowering agents, were also excluded from the study. The study protocol is in accordance with the Declaration of Helsinki. Local Ethics Committee approved the study protocol. All patients were given informed consent.

All subjects underwent an initial screening assessment that included a medical history and physical examination. All subjects underwent transthoracic echocardiographic examination before the onset of the treatment and after euthyroidism was achieved.

Treatment for hyperthyroidism was chosen according to American association of clinical endocrinologists medical guidelines for clinical practice for the evaluation and treatment of hyperthyroidism and hypothyroidism [7]. Anti-thyroid medication, radioactive iodine and surgery were treatment alternatives for the subjects. Since patients were achieving euthyroidism, subjects were followed every 4 weeks. Levels of fT3, fT4, TSH and BNP were measured before the onset of the treatment and after euthyroidism was achieved.

Fasting blood samples were obtained by the venipuncture of the large antecubital veins of the studied patients without stasis, after a 12-hour fast. The samples were then centrifuged immediately; the plasma was separated and stored at −80°C. In order to avoid variation, all samples were studied on the same day and the same kit.

TSH, free T3, and free T4 were determined by immunometric assays (Diagnostic Products Corporation, Los Angeles, USA). BNP was measured from the venous whole blood taken into a tube with EDTA.

**BNP determination:**

BNP concentrations were determined with a 2-site sandwich chemiluminescent immunoassay (Lot No: 22161145, reference no: 02816634) on the ADVIA® Centaur® platform (Siemens Healthcare Diagnostic, IL, USA). The limit of detection for this assay was 2 ng/L with total imprecision (as CV) <5% at concentrations of 29 to 1400 ng/L. The ADVIA Centaur BNP test measures the physiologically active form of B-type natriuretic peptide, the most clinically relevant form of BNP.

**Transthoracic echocardiography**

Transthoracic echocardiographic examination was performed to all subjects by using a System Three (GE Vingmed Ultrasound, Horten, Norway) cardiac ultrasound scanner and 2.5-3.5 MHz transducers.

**Echocardiography of the LV**

The following parameters were measured from cross-sectional echocardiographic images of the LV: 1) End-diastolic and end-systolic diameter (cm); 2) Fractional shortening (FS %); 3) Ejection Fraction (EF %).

**Doppler echocardiography**

Flow velocity indexes were obtained using pulsed and continuous wave Doppler from apical projections, and measurements were made utilizing the software of the ultrasound equipment. Mitral diastolic flow was obtained after the pulsed Doppler sample volume was positioned perpendicular to the tips of the mitral valve leaflets. The Doppler cursor was then moved toward the LV outflow position, and the sample volume was placed approximately 1 cm proximal to the aortic valve so that it would come in contact with the anterior mitral valve leaflet. Isovolumic relaxation time (IVRT) (ms) was measured as the interval between the end of the aortic click artifact and the onset of mitral inflow waveform.

The following indexes were measured from the mitral and tricuspid valve diastolic wave form: peak early (E) and atrial (A) flow velocities (m/s), E/A ratio of the LV diastolic filling. Heart rate (beats/min) was measured from simultaneous electrocardiogram recordings.

**Pulsed Doppler tissue echocardiography**

The myocardial velocities of the LV were measured sampling the mitral annulus excursion at lateral sites in
the four-chamber view. Care was taken to keep the ultrasound beam perpendicular to the plane of the annulus in order to minimize the angle between the beam and the direction of annular motion. The width of the sample volume was 3-5 mm. Measurements were focused on the systolic myocardial wave (Sa), the early-diastolic (Ea) and end-diastolic myocardial (Aa) waves. Usually, several cardiac cycles were acquired, and the best two consecutive ones were analyzed and averaged.

Statistical Methods

Distribution of the continuous variables was determined by the Kolmogorov-Smirnov test. Continuous variables with normal distribution were expressed as mean±SD, variables with skew distribution are expressed as median (minimum-maximum), and categorical variables are expressed as percentage. The paired sample t-test was used for normally distributed variables and the Wilcoxon rank-sum test for skew distributed variables. Pearson and Spearman analysis was used to identify correlations between study parameters. For all statistics, a two-sided p value < 0.05 was considered statistically significant. All analyses were performed with SPSS 10.0 for Windows.

Results

Forty-eight patients with newly diagnosed untreated overt hyperthyroid patients were examined. Demographic data, clinical characteristics, and medications were shown in Table 1. The mean age was 43.6±16.2 years. Majority of patients were female (40, 83%). Thirty-six (75%) patients were Graves’s disease, 7 (15%) patients were Toxic Nodular goiter (TNG) and 5 (10%) patients had Toxic Multi Nodular goiter (TMNG). Twenty-eight (58%) patients received radioactive iodine therapy, 10 (21%) patients underwent surgery, 10 (21%) patients received oral anti-thyroid drug.

All patients and control subjects had normal ejec-

### Table 1. Demographic characteristics of patients

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Patients (n=48)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (mean±SD)</td>
<td>43.6±16.2</td>
</tr>
<tr>
<td>Female/Male (no. - %)</td>
<td>40/8(83/17%)</td>
</tr>
<tr>
<td>Graves (no. - %)</td>
<td>36 (75%)</td>
</tr>
<tr>
<td>TNG (no. - %)</td>
<td>7 (15%)</td>
</tr>
<tr>
<td>TMNG (no. - %)</td>
<td>5 (10%)</td>
</tr>
</tbody>
</table>

### Medications and Treatments
- PTU (no. - %) 10 (21%)
- Radioactive Iodine 28 (58%)
- Surgery 10 (21%)

PTU: Propylthiouracil
TNG: Toxic Nodular goiter
TMNG: Toxic Multi Nodular goiter

### Table 2. Laboratory Characteristics of Subjects

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Hyperthyroidism</th>
<th>Euthyroidism</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BNP (ng/L)</td>
<td>102.5 (6.7-1769)</td>
<td>5.0 (0.1-87.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>fT3 (pmol/L)</td>
<td>8.2 (4.3-25.0)</td>
<td>3.0 (2.0-4.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>fT4 (ng/dL)</td>
<td>3.1 (1.8-10.0)</td>
<td>1.1 (0.5-2.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TSH (µIU/mL)</td>
<td>0.002 (0.001-0.086)</td>
<td>1.5 (0.4-3.9)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Free T3 (8.2 (4.3-25.0) pmol/L vs. 3.0 (2.0-4.8) pmol/L, p<0.001), fT4 (3.1 (1.8-10.0) ng/dL vs. 1.1 (0.5-2.1) ng/dL, p<0.001) levels significantly decreased, whereas TSH (0.002 (0.001-0.086) µIU/mL vs. 1.5 (0.4-3.9) µIU/mL, p<0.001) levels significantly increased after anti-thyroid treatment.

A significant decrease in BNP (102.5 (6.7-1769) ng/L vs. 5.0 (0.1-87.0) ng/L p< 0.001) levels were observed, after euthyroidism was achieved. Distribution of BNP level before and after treatment was shown in figure 1. There was no significant differences in BNP levels of hyperthyroid stage between Graves patients (102.0 (6.7-1643)), TNG patients (105 (60.1-1769)), and TMNG patients (83.2 (79.3-460.2)). The decrease in BNP levels was positively correlated with the decrease in fT3 ($r=0.298; p=0.049$) and fT4 ($r=0.313; p=0.030$). There was no correlation between BNP levels and TSH levels ($p=NS$).
In our previous cross-sectional study we investigated the relation between BNP and different thyroid dysfunctions such as overt hypothyroidism, overt hyperthyroidism, subclinical hypothyroidism and subclinical hyperthyroidism [5]. BNP level of the hyperthyroid group was found to be significantly higher than the other groups. BNP level of the hypothyroid group was significantly lower than the euthyroid group. The present study supported the findings of our previous study showing that BNP levels were higher in hyperthyroid status. Kato et al. found a significant elevation of BNP and atrial natriuretic peptide levels in patients with thyrotoxicosis [8].

In vitro studies showed that T3 and T4 can stimulate the release of BNP from both cultured atrial and ventricular myocytes in a dose-dependent manner and triiodothyronine is able to increase BNP gene transcription [6, 9]. Liang et al. previously showed that the BNP gene is a target of T3 action. BNP secretion was increased 6-fold, BNP mRNA levels 3-fold, and BNP promoter activity 3–5-fold following T3 treatment. As T3 can directly stimulate BNP promoter, heart failure may not be required for BNP hypersecretion during clinical hyperthyroidism [9]. Several clinical studies investigated the role of BNP and NT-pro BNP in hyperthyroidism with contradictory results. Kohno et al. have previously found increased levels of BNP in a group of untreated hyperthyroid patients and rats [6]. Wei et al. have measured the BNP levels and left ventricular functions of 67 hyperthyroid patients and 32 healthy subjects [10]. Plasma BNP in patients with left ventricular dysfunction was significantly higher than patients with normal left ventricular function.

### Discussion

In this study, we determined how hyperthyroidism influences BNP measurements. We measured BNP levels in hyperthyroid state and after euthyroidism was achieved with the treatment of hyperthyroidism. We also used echocardiography to evaluate left ventricle function and exclude other possible cardiac diseases. This study showed that BNP levels were significantly higher in hyperthyroid than euthyroid status of the same patients. We found that decrease in BNP levels was positively correlated with the decrease in fT3 and fT4. However we did not observe a similar correlation between the change of BNP and TSH levels.

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#### Table 3. Transthoracic Echocardiographic parameters of Subjects

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Hyperthyroidism</th>
<th>Euthyroidism</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVEDD (cm)</td>
<td>4.6±0.5</td>
<td>4.6±0.5</td>
<td>NS</td>
</tr>
<tr>
<td>LVESD (cm)</td>
<td>2.9±0.4</td>
<td>2.9±0.4</td>
<td>NS</td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>67.2±3.3</td>
<td>66.5±3.9</td>
<td>NS</td>
</tr>
<tr>
<td>Left atrium diameter</td>
<td>3.3±0.5</td>
<td>3.1±0.4</td>
<td>0.02</td>
</tr>
<tr>
<td>Mitral E peak (m/s)</td>
<td>0.79±0.13</td>
<td>0.78±0.14</td>
<td>NS</td>
</tr>
<tr>
<td>Mitral A peak (m/s)</td>
<td>0.63±0.13</td>
<td>0.62±0.14</td>
<td>NS</td>
</tr>
<tr>
<td>Mitral IVRT (ms)</td>
<td>88.1±16.1</td>
<td>85.3±10.9</td>
<td>NS</td>
</tr>
<tr>
<td>Mitral Early diastolic annular velocity of Lateral wall (m/s)</td>
<td>13.6±2.5</td>
<td>13.6±3.9</td>
<td>NS</td>
</tr>
<tr>
<td>Mitral Late diastolic annular velocity of Lateral wall (m/s)</td>
<td>9.1±2.1</td>
<td>9.7±2.7</td>
<td>NS</td>
</tr>
<tr>
<td>Mitral Peak systolic annular velocity of Lateral wall (m/s)</td>
<td>10.4±2.1</td>
<td>9.7±2.4</td>
<td>NS</td>
</tr>
</tbody>
</table>
They found the average BNP between healthy subjects and hyperthyroid patients with normal left ventricular function were not significantly different. In our study we only examined BNP levels in newly diagnosed hyperthyroid patients and after euthyroidism was achieved in the same population. We did not measure BNP levels in normal control subjects. This may explain the disparity between our findings and the results of Wei et al.

N-terminal-pro-B-type natriuretic peptide (NT-proBNP), another member of the natriuretic peptide family, is produced and released from cardiac ventricles. In a recent study serum NT pro-BNP levels were measured in 21 patients with hyperthyroidism and in 24 patients with hypothyroidism and compared with 20 healthy control subjects. The researchers found that NT pro-BNP levels were higher in hyperthyroid patients than in hypothyroid patients and control subjects. Moreover, there was a significant positive correlation between NT pro-BNP and fT4 levels [11]. Schultz et al. showed that serum levels of NT-pro-BNP were strongly influenced by thyroid function and treatment of the dysthyroid state resulted in a significant increase in NT-pro-BNP in hypothyroid patients and a decrease in hyperthyroid patients [12]. In this study echocardiographic examination was not used to assess possible cardiac diseases.

In this study we did not observe a significant correlation between BNP and TSH levels. TSH test method that we used in this study can not measure TSH values beyond 0,01mU/L, and this may be one reason for the lack of correlation between BNP and TSH in this study. Another reason for this discrepancy may be that BNP reaction to physiological stimuli is very fast; on the other hand TSH level normalization is delayed up to months. This delay can also explain the lack of correlation between these parameters in our study [13].

**Conclusions**

We conclude that hyperthyroidism may cause high BNP measurements which can lead to misdiagnosis of congestive heart failure. The treatment of hyperthyroidism is quite different than the treatment of heart failure. We suggest that thyroid hormones should be checked in patients with high levels of BNP.

**Declaration of interest**

There is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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