Salivary B-type natriuretic peptide: a new method for heart failure diagnosis and follow-up

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Abstract
Background: Frequent hospital admissions and reduced quality of life are the main complications of heart failure (HF). Plasma B-type natriuretic peptide (BNP) levels have been considered as a cost-effective method of screening for left ventricular dysfunction. Studies regarding BNP-guided therapy revealed reduction in death or hospital stay for HF.

Aim: As saliva has fewer limitations than blood in regard to sampling, the aim of the present study was to test if salivary BNP concentration might be a new biomarker in patients with chronic HF.

Methods: This pilot study involved 35 admitted patients with decompensated HF diagnosis and 35 HF patients who had come for a check-up at the Department of Cardiology. The control group consisted of 25 people with no history of cardiac events. Saliva and plasma samples of all the participants were collected.

Results: Mean plasma NT-proBNP was found at higher levels in admitted HF patients compared to outpatient HF (9.37 vs. 6.62 pg/mL, p < 0.001) and control groups (9.37 vs. 4.69 pg/mL, p < 0.001). Also, mean salivary BNP levels were higher in admitted patients with HF (6.50 ng/L, p < 0.001); and outpatient HF group (5.87 ng/L, p = 0.02) compared to the control group (5.64 ng/L).

Conclusions: Our study demonstrated that BNP could be detected in saliva and that the level is higher in HF patients, especially symptomatic ones. This means that salivary BNP may be useful in the diagnosis and follow-up for patients with HF, especially in emergency settings.

Key words: B-type natriuretic peptide, heart failure, saliva

INTRODUCTION
Heart failure (HF) is the final stage of many cardiac diseases [1]. HF with reduced ejection fraction (EF) and HF with preserved EF are the main types of this disease on the basis of EF. The first type is defined as EF ≤ 40% with signs and symptoms of HF; and in the second one manifestations are associated with normal EF (≥ 50%) [2]. Despite improvements in survival rate, the mortality of HF has been estimated as approximately 50% within five years of diagnosis. Frequent hospital admissions and reduced quality of life are the main complications of HF. Early diagnosis and treatment could improve the prognosis [3].

Along with chest X-ray and electrocardiogram examinations, the European Society of Cardiology recommends the analysis of B-type natriuretic peptide (BNP) and the N-terminal part of the propeptide of BNP (NT-proBNP) in evaluating patients with suspected HF [4]. These cardiac natriuretic hormones are produced and secreted by cardiomyocytes and have diuretic and vascular smooth muscle relaxing effects [5]. High concentrations of these hormones are associated with poor prognosis in myocardial infarction or HF and are useful in guiding therapy [6, 7]. Both peptides provide the ability to distinguish HF from non-HF subjects [8].

Twenty per cent of blood plasma proteins are secreted in saliva via the gingival crevicular fluid. Saliva has fewer limitations in comparison to blood for sampling because it does not clot and its collection is noninvasive [9]. In addition to being...
Table 1. Characteristics of participants

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Admitted HF patients (n = 35)</th>
<th>Outpatient HF (n = 35)</th>
<th>Control group (n = 25)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age [year]</td>
<td>64 ± 13 (45–87), 65</td>
<td>63 ± 14 (42–84), 65</td>
<td>56 ± 11 (42–77), 58</td>
<td>0.09</td>
</tr>
<tr>
<td>Gender (male/female)</td>
<td>18/17</td>
<td>18/17</td>
<td>13/12</td>
<td>0.9</td>
</tr>
<tr>
<td>Systolic BP [mm Hg]</td>
<td>131 ± 20 (100–185), 130*</td>
<td>130 ± 25 (100–160), 130*</td>
<td>112 ± 10 (110–150), 110</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Diastolic BP [mm Hg]</td>
<td>78 ± 6 (60–95), 80</td>
<td>80 ± 3 (80–100), 85*</td>
<td>75 ± 6 (60–95), 80</td>
<td>0.01</td>
</tr>
<tr>
<td>Heart rate [bpm]</td>
<td>86 ± 5 (80–96), 86*#</td>
<td>75 ± 6 (55–90), 72</td>
<td>76 ± 10 (58–95), 74</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Ejection fraction [%]</td>
<td>25 ± 2 (10–35), 25*</td>
<td>25 ± 3 (10–40), 25*</td>
<td>55 (50–60), 55</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>NT-proBNP [pg/mL] (logarithm)</td>
<td>9.37 ± 0.68 (4.19–10.46),</td>
<td>6.62 ± 0.19 (6.18–7.02),</td>
<td>4.69 ± 0.31 (4.19–5.35),</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>(logarithm)</td>
<td>6.69*#</td>
<td>6.65*</td>
<td>4.7</td>
<td></td>
</tr>
<tr>
<td>Salivary BNP [ng/L] (logarithm)</td>
<td>6.50 ± 0.22 (5.84–6.85),</td>
<td>5.87 ± 0.23 (5.33–6.38),</td>
<td>5.64 ± 0.28 (4.96–6.02),</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>(logarithm)</td>
<td>6.51*#</td>
<td>5.94*</td>
<td>5.73</td>
</tr>
<tr>
<td>Creatinine [mg/dL]</td>
<td>0.94 ± 0.24 (0.6–1.5), 90</td>
<td>0.87 ± 0.16 (0.6–1.2), 90</td>
<td>0.85 ± 0.17 (0.5–1.2), 90</td>
<td>0.21</td>
</tr>
<tr>
<td>Glomerular filtration rate</td>
<td>88 ± 7.8 (58–98), 90</td>
<td>91 ± 4.5 (80–98), 92</td>
<td>92 ± 4.4 (80–100), 90</td>
<td>0.09</td>
</tr>
</tbody>
</table>

Data are presented as [mean ± standard deviation (maximum-minimum), median]. Data were analysed by ANOVA, and complementary Bonferroni test was performed, significances were considered as p < 0.05. *Represents the difference with the control group; #Represents the difference with outpatient HF group; BNP — B-type natriuretic peptide; BP — blood pressure; HF — heart failure; NT-proBNP — N-terminal of the prohormone B-type natriuretic peptide.

Measurement of salivary BNP

Saliva samples of all the participants were collected while fasting, in a sterile tube (2–3 cc). The samples were placed on ice and then transported to the laboratory on dry ice. Collected samples were centrifuged (1000 rpm) to separate mucus strands and solid deposits. If the saliva contained a high concentration of mucus, the centrifugation was repeated as necessary. After centrifugation, the supernatant was transmitted to a clean tube and was frozen at −20°C (human BNP ELISA Kit, Shanghai, China, Shanghai Crystal Day Biotech Co., Ltd. — Cat. No: E1287Hu), and samples were removed from the refrigerator and were left for about an hour in the temperature of the laboratory. Interassay variation and intra-assay variation for this kit were coefficient of variability (CV) < 10% and CV < 12%, respectively. The assay range was 5 ng/L → 2000 ng/L, and sensitivity was reported at approximately 2.51 ng/L. Kit calibrator vial was available with a concentration of 2400 ng/L. This solution was serially diluted to concentrations of 1200 ng/L, 600 ng/L, 300 ng/L, 150 ng/L, and 75 ng/L. 50 ng of streptavidin-HRP solution was added into the wells of the calibrator. 40 ng of the saliva and 10 ng of BNP antibody (available in kit) were added to the well samples. 50 ng streptavidin-HRP was added again to that collection. Samples were transmitted to the incubator (37°C) for 1 h. The wells were washed five times with washing solution. Then 50 ng chromogen A and 50 ng chromogen B were added and the solutions were left for 10 min at 37°C in darkness. 50 ng of STOP solution was added to all wells, and the samples were read by ELISA reader at a wave length of 450 nm.

Statistical analysis

Kolmogorov-Smirnov statistics were performed on clinical characteristics in order to test for normal distribution.
before statistical analyses. To compare the groups, post hoc test for ANOVA was done. Statistical significance for differences between HF and healthy subjects was considered to be p < 0.05. Data was analysed using IBM SPSS Statistics 19.0 (IBM, Armonk, New York, USA). Data are presented in mean ± standard deviation (median) form, and the receiver-operating characteristic (ROC) curve analysis for salivary BNP in the admitted HF group was done. The equality of variances for a variables were assessed by Tamhane’s T2 test. Because based on Kolmogorov-Smirnov statistic test the salivary BNP and plasma NT-proBNP levels did not have normal distribution, the logarithm forms of these variants were calculated for analysis (Table 1). The units of reporting for these two variables were the same as their concentrations because the units did not change by logarithm conversion (pg/mL for plasma NT-proBNP and ng/L for salivary BNP).

**RESULTS Participants**

Ninety-five people were enrolled in this study: 35 patients with symptomatic HF, 35 stable HF patients, and 25 controls. The mean age of admitted HF patients was higher compared to outpatient HF (64 vs. 63, p = 1) and control groups (64 vs. 56, p = 0.09), and the mean age of the outpatient HF group was higher compared to the control group (63 vs. 56, p = 0.34), but these correlations were not significant based on statistical analysis. There was no significant difference between mean systolic (131 mm Hg vs. 130 mm Hg, p = 1) and diastolic pressures (80 mm Hg vs. 78 mm Hg, p = 0.67) of the two HF groups, but mean systolic pressure was significantly higher in HF patients compared to the control group (130 mm Hg vs. 112 mm Hg, p < 0.001). Heart rate was significantly higher in HF patients that were admitted to the hospital in comparison with the other two groups (86 bpm vs. 75 bpm and 76 bpm, p < 0.001). EF was less in the HF group compared to the control group (25% vs. 55%, p < 0.001), but there was no significant difference between admitted and outpatient HF groups (25% vs. 25%, p = 1). Obviously, admitted patients were more symptomatic and had higher class based on New York Heart Association (NYHA) classification. Creatinine (Cr) levels of all participants were measured and glomerular filtration rates (GFR) calculated. There was no significant correlation between mean Cr level of admitted HF patients compared to the outpatient HF (0.94 mg/dL vs. 0.87 mg/dL, p = 0.47) and control groups (0.94 mg/dL vs. 0.85 mg/dL, p = 0.34). Also, no significant differences were noticed for mean GFR between the admitted HF patients compared to the outpatient HF (88 mL/min vs. 91 mL/min, p = 0.1) or control groups (88 mL/min vs. 92 mL/min, p = 0.09). There was no significant difference between the outpatient HF and control group either in mean Cr level (0.87 mg/dL vs. 0.85 mg/dL, p = 1) or GFR levels (91 mL/min vs. 92 mL/min, p = 1).

**Plasma NT-proBNP concentrations**

NT-proBNP plasma concentrations differed between all three groups. Mean plasma NT-proBNP was found at higher levels in the admitted HF patients compared to the outpatient HF (9.37 pg/mL vs. 6.62 pg/mL, p < 0.001) and control groups (9.37 pg/mL vs. 4.69 pg/mL, p < 0.001). Also, mean plasma NT-proBNP was found at higher levels in the outpatient HF patients compared to the control group (6.62 pg/mL vs. 4.69 pg/mL, p < 0.001).

**Salivary BNP concentrations in the healthy control subjects and HF patients**

Mean salivary BNP levels were higher in admitted patients with HF compared to the outpatient HF (6.50 ng/L vs. 5.87 ng/L, p < 0.001) and control groups (6.50 ng/L vs. 5.64 ng/L, p < 0.001). Also, mean salivary BNP of the outpatient HF patients was significantly higher than in the control group (5.87 ng/L vs. 5.64 ng/L, p = 0.02) (Fig. 1).

ROC curve analysis for the admitted HF group and salivary BNP was performed. The area under the curve (AUC) was 0.98 with a confidence interval (CI) of about 95–100. It shows that for admitted HF patients (symptomatic group) log salivary BNP at the level of 5.84 ng/L had about 100% sensitivity and 53.3% specificity for decompensated HF diagnosis (p < 0.001). Positive predictive value (PPV) and negative predictive value (NPV) were about 92.8% and 83.3%, respectively (Fig. 2).

There was a significant negative correlation between salivary BNP level and EF (p < 0.001, r = -0.45). This means
that salivary BNP is at a higher level with lower EF (Fig. 3). There were no differences between salivary BNP levels in male and female genders (p = 0.27). There was a significant correlation between NYHA class and salivary BNP (p < 0.001), which means that salivary BNP was significantly higher in symptomatic patients with NYHA class three or four. Salivary BNP levels were higher in older patients (p < 0.001), but this finding may be a result of the fact that the participants in the HF group were older than the control group.

**Salivary BNP versus plasma NT-proBNP concentrations in the HF population**

There was a significant correlation between salivary BNP and plasma NT-proBNP concentrations (Pearson correlation was significant, p < 0.001, r = 0.459).

**DISCUSSION**

Our study showed that salivary BNP could be detected in the control group and HF patients. There was a significant relationship between salivary BNP and plasma NT-proBNP. Moreover, salivary BNP was significantly higher in symptomatic HF patients. As symptoms improved—for instance, in patients who visited the clinic—the level of BNP diminished. Thus, the level of BNP in saliva allowed us to differentiate HF patients in the decompensated phase, while decreased levels of BNP were associated with improved symptoms and decreased congestion.

Previous studies have assessed other factors in saliva. In one study, the level of NT-proBNP was assessed in saliva and showed that levels were higher in HF patients when compared with healthy control subjects. In this study, saliva samples were collected from healthy controls (n = 40) without underlying heart conditions, and from HF patients (n = 45). They demonstrated that the salivary NT-proBNP immunoassay has a clinical sensitivity of 82.2% and specificity of 100%, PPV of 100%, and NPV of 83.3%, with an overall diagnostic accuracy of 90.6% [11]. Another study revealed that higher daily salivary alpha-amylase activity output correlated with increasing levels of NT-proBNP. In this study, 24 patients with established chronic HF in NYHA class I to III and 24 controls were included. This study showed that salivary alpha-amylase activity has potential as a non-invasive index of adrenergic activity in HF [12].

B-type natriuretic peptide is secreted by ventricles and many studies have assessed the role of plasma BNP level in chronic HF [13–16]. Plasma BNP level is decreased by many drugs such as valsartan, carvedilol, and spironolactone. These drugs are the main components of HF treatment [17, 18]. Studies regarding BNP-guided therapy revealed reduction in mortality and hospital stay for chronic HF. This effect was mainly due to an increase in angiotensin converting enzyme inhibitor and beta-blocker dosages [19, 20]. Plasma BNP levels have been considered as a cost-effective way of screening for left ventricular dysfunction. There is a logical relation between the severity of chronic HF and circulating BNP levels [21]. Despite the wealth of efforts to develop relevant algorithms based on symptoms or haemodynamic variables for risk stratification of HF patients, these algorithms have failed to alter the prognosis due to the heterogeneous aetiology of different forms of HF [22, 23]. In another study, plasma BNP provided important prognostic information in patients with chronic HF, independently of the cause of the disease.
In comparison to lower levels, high circulating levels of BNP were associated with high incidence of death or attacks of decompensation [24].

The aforementioned findings demonstrated that BNP monitoring is useful for diagnosis and follow-up of HF patients. Currently, blood sampling is the most common technique for detecting BNP. However, blood sampling frequently leads to discomfort and bruising at the site. It is an invasive technique and is sometimes complicated by early clotting. In addition, the risk of blood-borne infectious transmission leads many patients to fear needles. By contrast, saliva sampling is noninvasive, is not complicated by clotting, is easier to handle and process, and bears no risk of contracting blood-borne infections. Roughly 27% of the whole-saliva proteins are found in plasma, but nearly 40% of the proteins that have been suggested to be candidate markers for diseases such as cancer, cardiovascular disease, and stroke could be found in whole saliva [25].

These similarities between saliva and plasma proteins, along with the valuable role of BNP in HF diagnosis, follow-up, and treatment encouraged us to measure this factor in saliva. To our knowledge this is the first study that detects BNP in saliva in HF and healthy control groups. The noninvasive nature of the procedure and its lower costs could shift plasma sampling to more accessible sampling such as saliva. It is important to note that salivary BNP assay kits may cross-react to salivary NT-proBNP or BNP metabolites, and therefore the findings of this study should be further investigated using advanced methods such as gel-filtration or reverse-phase high performance liquid chromatography analysis in order to characterise the immunoreactivity of salivary BNP. This paper is limited by the fact that we measured plasma NT-proBNP rather than BNP. It is well known that BNP and NT-proBNP are correlated, but it would have been nice to compare salivary and plasmatic BNP directly. Another limiting factor of our study was the age of the control group. The mean age of our control group was 56 vs. 63 and 64 years in outpatient and admitted HF patients, respectively. The mean age was lower in the control group, but there was no significant difference based on statistical analysis among our three groups. If other studies confirm our finding, salivary BNP could represent a cost-effective approach to HF diagnosis and treatment by avoiding the need for phlebotomy and sample processing.

CONCLUSIONS
Our study showed that BNP could be detected in saliva and its level is higher in HF patients, especially symptomatic ones. These findings point to the role of salivary BNP in the diagnosis and follow-up of patients with HF. Nevertheless, results of larger, controlled trials are needed before recommendations can be made for broad clinical application.

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Conflict of interest: none declared

References

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Stężenie peptydu natriuretycznego typu B w ślinie: nowa metoda diagnozowania i monitorowania niewydolności serca

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Streszczenie

Wstęp: Głównymi powikłaniami niewydolności serca (HF) są częste hospitalizacje i pogorszenie jakości życia. Stężenia peptydu natriuretycznego typu B (BNP) w osoczu uważa się za kosztowo efektywną metodę badań przesiewowych w kierunku niewydolności zaburzeń czynności lewej komory. W badaniach oceniających leczenie na podstawie monitorowania stężenia BNP wykazano zmniejszenie liczby zgonów i hospitalizacji z powodu HF.

Cel: Ponieważ łatwiej jest pobrać próbkę śliny niż próbkę krwi, autorzy badania postanowili sprawdzić, czy stężenie BNP w ślinie może być nowym biomarkerem u chorych z przewlekłą HF.

Metody: To pilotowe badanie objęło 35 chorych przyjętych do szpitala z rozpoznaniem niewyrównanej HF oraz 35 pacjentów z HF, którzy zgłosili się na wizytę kontrolną do poradni kardiologicznej. Grupę kontrolną stanowiło 25 osób bez zdarzeń sercowych w wywiadzie. Od wszystkich uczestników badania pobrano próbki śliny i krwi.

Wyniki: Średnie stężenie NT-proBNP w osoczu było wyższe u hospitalizowanych chorych z HF niż u pacjentów z HF leczonych ambulatoryjnie (9,37 pg/ml vs. 6,62 pg/ml; p < 0,001) oraz u osób z grupy kontrolnej (9,37 pg/ml vs. 4,69 pg/ml; p < 0,001). Również stężenia BNP w ślinie były wyższe u chorych z HF, zarówno hospitalizowanych (6,50 ng/l; p < 0,001), jak i leczonych w domu (5,87 ng/l; p = 0,02), niż u osób z grupy kontrolnej (5,64 ng/l).

Wnioski: W niniejszym badaniu wykazano, że można oznaczać stężenie BNP w ślinie i że jest ono wyższe u pacjentów z HF, zwłaszcza tych, u których występują objawy. Oznacza to, że stężenie BNP w ślinie może być przydatne w diagnozowaniu i monitorowaniu chorych z HF, zwłaszcza w stanach nagłych.

Słowa kluczowe: peptyd natriuretyczny typu B, niewydolność serca, ślin

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