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Gender differences in platelet brain derived neurotrophic factor in patients with cardiovascular disease and depression

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ABSTRACT

Women have a higher prevalence of depression compared to men. Serum levels of Brain-derived neurotrophic factor (BDNF) are decreased in depression. BDNF may also have a protective role in the pathogenesis of coronary artery disease (CAD) or events. We examined whether there are gender differences in BDNF levels in patients with stable CAD and comorbid depression. We enrolled 37 patients (17 women) with stable CAD with and without depression from a single medical center. All patients had depression assessment with the Beck Depression Inventory-II questionnaire. Both plasma and platelet BDNF were measured in all patients using a standard ELISA method. Platelet BDNF levels were higher than plasma BDNF levels in the entire group (5903.9 ± 1915.6 vs 848.5 ± 460.5 pg/ml, $p < 0.001$). Women had higher platelet BDNF levels than men (6954.2 ± 1685.6 vs. 5011.2 ± 1653.5 pg/ml, $p < 0.001$). Women without depression (BDI-II < 5, $n = 8$) had higher platelet BDNF than men without depression ($n = 8$, 7382.8 ± 1633.1 vs 4811.7 ± 1642.3 pg/ml, $p = 0.007$). Women with no or minimal depression (BDI < 14, $n = 14$) had higher platelet BDNF levels than men with no or minimal depression ($n = 18$, 6900.2 ± 1486.6 vs 4972.9 ± 1568.9 pg/ml, $p = 0.001$). The plasma BDNF levels were similar between men and women in all categories of depression. In conclusion, women with stable CAD have increased platelet BDNF levels when compared to men with stable CAD regardless of their level of depression. Sex specific differences in BDNF could possibly indicate differences in factors linking platelet activation and depression in men and women.

1. Introduction

Gender differences in depression have been well documented (Piccinelli and Wilkinson, 2000). The prevalence, incidence and morbidity risk of depressive disorders are higher in women than in men (Kessler et al., 2007). Given this difference, investigators have examined whether gender differences in health are due to risk factors associated with depression. Patients with untreated depression have decreased serum levels of Brain derived neurotrophic factor (BDNF) and increased plasma and platelet levels. BDNF is a member of the nerve growth factor family. It promotes neural and synaptic growth and plays a critical role in the survival, differentiation, neuronal strength, and morphology of neurons (Duman, 1998). BDNF is a neurotransmitter and is also stored in large amounts in platelets. It is thought that stored BDNF is released by platelets in response to neuronal injury, where it plays a role in nerve repair (Fujimura et al., 2002). BDNF also plays a critical role in cardiovascular viability and function and has been suggested to play a protective role in the pathogenesis of CAD or CAD events (Kaess et al., 2015). Serum and platelet BDNF appear to mirror neuronal BDNF, and therefore due to ease of sampling, serum and platelet BDNF levels have been used as a surrogate measure of BDNF levels in the brain (Karege et al., 2002). BDNF levels are increased in platelet-poor plasma and decreased in serum as well as washed platelets in patients with untreated depression (Karege et al., 2002; Yu and Chen, 2011; Serra-Millas et al., 2011). Healthy women have been found to have lower platelet BDNF levels than men (Lommatzsch et al., 2005).

Depression has been correlated with the development of CAD and poor outcomes among patients with an acute coronary

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syndrome (Ford et al., 1998; Lichtman et al., 2014; Carney et al., 2003). Given that heart disease is the leading cause of death in both men and women (Kochanek et al., 2011), we sought to determine whether men and women with CAD would also have different BDNF levels in platelets and platelet-poor plasma and if levels in individuals without depression differ from those with depression. Understanding sex specific differences in factors such as BDNF that could possibly link platelet activation and depression may lead to novel predictors of CAD disease within women or men with depression.

2. Materials and methods

We enrolled 37 patients (17 women) with stable CAD from a single out-patient cardiology practice presenting for routine follow-up. CAD was defined as diagnostic cardiac catheterization demonstrating ≥50% obstructive coronary lesion, electrocardiographic evidence of previous myocardial infarction, or stress testing revealing myocardial ischemia or infarction without an acute event in the past year (Lloyd-Jones et al., 2009).

Patients were excluded if they were less than 21 years-old, were recently (within 14 days) treated with a glycoprotein IIb/IIIa inhibitor, had an acute coronary syndrome within the past 12 months, were active narcotic users by report or by laboratory testing, were unable to give informed consent, were on antidepressant medication, had chronic disease with anticipated death within one year, or who had a baseline platelet count below 100,000/μl. There were no restrictions in regard to race or socioeconomic status. The study was approved by the Johns Hopkins Institutional Review Board and all patients provided written informed consent. After providing written informed consent, blood (10 ml) was drawn between the hours of 8:30 a.m. and 10:30 a.m. to minimize the impact of potential circadian rhythm alterations on BDNF concentrations. Demographic data were recorded including age, race, gender, history of hypertension, family history of early CAD in first degree relatives (men before the age of 55, women before the age of 65), smoking status, history of hypercholesterolemia, history of type I or type II diabetes, medication use, prior history of myocardial infarction and date, education, body mass index (BMI), marital status, and history of depression.

All patients had depression assessment with the Beck Depression Inventory-II questionnaire. Prior studies used BDI scores of <5 as the "no depression" reference group and found that even scores of 5–9, which represented minimal symptoms, were associated with increased mortality (Bush et al., 2001). Individuals were categorized as no depression (<5) or as minimal (5–13), mild (14–19), moderate (20–28), or severe (≥29) depression (Beck et al., 1996; Cameron et al., 2011) In order to have an equal representation of men and women with and without depression we stratified our recruitment according to BDI-II scores of <5 or ≥ 5. We screened 39 potential participants and enrolled 37 who met inclusion criteria.

2.1. Blood collection

From each patient in the study, 2 samples of 5 ml of blood were obtained in tubes containing citrate/phosphate/dextrose.

2.2. Plasma and platelet BDNF samples processing

Platelet count was taken from whole blood using Beckman/Coulter MD II System Hematology Analyzer (Beckman Coulter, CA, USA). Plasma poor in platelets (PPP)- (plasma BDNF) was obtained by centrifugation at 2200 rpm (15 min, 22 °C) and platelets were obtained by centrifugation at 1100 rpm (15 min, 22 °C) to obtain the platelet-rich plasma.

Platelets were washed as previously described with equal volume of citrate/citric acid/dextrose (Diaz-Ricart et al., 2002). Platelet count was normalized to 0.8×10^6 platelets/μl, and then were resuspended in a Hank's balanced salt solution supplemented with dextrose and $NaHCO_3$ and incubated for 30 min at 37 °C as described by Serra-Millas et al (Serra-Millas et al., 2011). In order to obtain the platelet-BDNF sample, the washed platelets were diluted with lysis buffer (100 mM Tris–HCl, pH 7.4, 2% Triton X-100) containing 10 mM ethylene glycol bis (β-aminoethylether)-N,N,N′,N'-tetra-acetic acid, 4 mM ethylenediaminetetraacetic acid, 2 mM phenylmethyl sulfonyl fluoride, 2 mM benzamidine, 2 μg/ml leupeptin, 2 μg/ml pepstatin and 2 mM sodium orthovanadate, to yield a final platelet concentration of 0.2×10^6 platelets/μl. Platelet suspensions were treated with lysis buffer for 30 min at 4 °C. Acid treatment (1N HCl for pH ≤ 3) was performed on both plasma-BDNF and Platelet-BDNF, then neutralized with NaOH (pH ~ 7.6) as indicated by BDNF Emax® ImmunoAssay Systems (Promega, Madison, WI) ELISA kit before freezing them at −20 °C.

2.3. Plasma-BDNF and platelet-BDNF assay

ELISA method was used to determine BDNF concentrations using BDNF Emax® ImmunoAssay Systems with an analytical CV of 8.8 (Promega, Madison, WI) ELISA kit followed by the immediate reading of the plate at 450 nm BioRad, Microplate manager 6, version 6.1. Results are reported in pg/ml.

2.4. Statistical analysis

Distributions of demographic and clinical characteristics of the study sample are described as mean and standard deviation for continuous variables and as frequency (%) for categorical variables. The differences of platelet BDNF versus plasma BDNF levels were tested using paired *t*-tests. Difference in BDNF levels between men and women were tested using 2-sample *t*-tests. The effect of body mass index (BMI) on the association between gender and BDNF was assessed using multiple linear regression. Association with a 2-sided p-value of 0.05 was considered statistically significant. Subgroup analyses were carried out comparing BDNF levels in men vs women with BDI score of <14, (i.e., patients with less than mild depression). Analyses were also performed in the subgroup with no depression (BDI < 5). In addition to comparing mean BDNF levels in traditionally defined BDI categories between the gender groups, nonparametric locally weighted scatterplot smoothing (lowess) fits and their corresponding 95% confidence bounds were also used to explore potential difference in patterns of association of BDNF measures with levels of BDI score between men and women. Because the only man with BDI score of ≥20 had a score of 25, which is very far away from the rest of the BDI data points in men (all with BDI ≤ 14), we excluded this man, and thus also excluded the only female patient with BDI score of ≥20 (BDI = 22), from this set of exploratory analyses.

3. Results

Table 1 shows the demographic and clinical profile of our patient sample. The median BDI for women in the sample was 5 with interquartile range (IQR) of 8 (3–11), for men the median BDI was 5 with IQR of 5.5 (3–8.5). Almost one half (47%) of women had BDI < 5 while 40% of men had BDI < 5. A total of 18% of women had BDI ≥14 compared to 10% of men. All but one patient in each gender group had BDI <20.

Platelet BDNF levels were higher than plasma-BDNF levels in the entire group see Table 2.

Mean platelet BDNF levels were significantly higher for women