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FOCUS: NURSING

The Role of Platelet Serotonin and Depression in the Acute Coronary Syndrome Population

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Platelet serotonin has been associated with depression and coronary artery disease. Understanding the association between platelet serotonin and depressive symptoms during acute coronary syndrome (ACS†) may explain some of the ACS events seen in depressed individuals. The objectives were to evaluate whether levels of platelet serotonin during an ACS event differ between individuals who screen positive or negative for depressive symptoms and to determine if a linear relationship exists. In this cross-sectional study, data were collected on 51 patients with ACS. Multiple linear regression models were examined. Platelet serotonin levels were not significantly different between the depressed and non-depressed groups ($\beta = -4.093$ and $p = .293$); a linear relationship was not found ($\beta = -.254$ and $p = .250$). In conclusion, a relationship between platelet serotonin and depressive symptoms was not found. It remains unclear if an association exists between platelet serotonin levels and depressive symptoms during hospitalization for ACS.

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†Abbreviations: ACS, acute coronary syndrome; BDI-II, Beck Depression Inventory-II; CAD, coronary artery disease; AMI, acute myocardial infarction; ELISA, enzyme-linked immunosorbent assay; EDTA, ethylene diamine tetra acetic acid; PRP, platelet-rich plasma; LVEF, left ventricular ejection fraction; WBS, whole blood serotonin.

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INTRODUCTION

The American Heart Association estimates that coronary artery disease (CAD) is the number one cause of death worldwide [1]. CAD and depression have been identified as common comorbid disease states, with disproportionately high prevalence of depression found in individuals with CAD ranging from 25.2 percent to 40.3 percent [2-4]. Approximately 20 percent to 40 percent of individuals hospitalized for acute coronary syndrome (ACS) experience depression and encounter subsequent major adverse coronary events twice as often as those individuals without depression [5-7]. Despite evidence that depression may be an independent risk factor for ACS, the relationship that underscores this significant association remains poorly understood [5,8].

In some individuals with CAD, a disruption in an atherosclerotic plaque and resulting platelet activation and aggregation leads to the development of a coronary thrombosis and an acute cardiovascular event [9]. Activated platelets release multiple platelet agonists, including stored platelet serotonin that binds to serotonergic receptors throughout the cardiovascular system and to 5-HT_{2A} receptors located on the membrane surface of platelets [10]. Platelet-released serotonin mediates some of its hemostatic effects by activating available platelet surface 5-HT_{2A} receptors leading to further platelet aggregation and thrombosis [10]. Heightened platelet activation and aggregation in response to atherosclerotic plaque disruption has been documented in some individuals with depression, suggesting that some depressed individuals may be vulnerable to the effects of serotonin-mediated platelet aggregation [11-12]. These biologically distinct depressed individuals may have higher levels of stored platelet serotonin that when released mediate some of its hemostatic effects by stimulating available platelet serotonin receptors and enhancing thrombosis [8,13]. Researchers have found changes in platelet aggregation and platelet serotonin uptake, decreased platelet serotonin transporters in central nervous system and platelet membranes, and alterations in platelet serotonin binding in depressed individuals [8,11-12]. This may indicate the existence of a subgroup of CAD patients who, when depressed, have a distinct underlying biological response to serotonin regulation in both the brain and periphery, compared to depression in otherwise healthy individuals [8].

There is a paucity of literature on the cardiovascular effects of altered platelet serotonin levels in individuals experiencing depressive symptoms. Serotonin-mediated platelet aggregation may be one potential mechanism underlying the link between depression and ACS, placing these depressed individuals at a higher risk for an ACS event. Understanding the association between platelet serotonin levels and depressive symptoms may lead to earlier identification of individuals experiencing depressive symptoms who are at a higher risk for ACS as well as earlier identification of patients with ACS who are at risk for depressive symptoms. The purpose of this cross-sectional study of patients with ACS was to determine whether levels of platelet serotonin, during an ACS event, differ between individuals who screen positive (BDI-II $\geq$ 14) or negative (BDI-II $<$ 14) for depressive symptoms and to determine if a linear relationship exists between platelet serotonin levels and depressive symptoms among patients hospitalized for ACS.

MATERIALS AND METHODS

Setting and Sample

A cross sectional sample of 24 ACS patients with a BDI-II $\geq$ 14 and 27 ACS patients with a BDI-II $<$ 14 were analyzed as part of their participation in an ongoing prospective study of patients hospitalized for ACS [14]. The parent study is a cohort of English- and Spanish-speaking adults hospitalized for ACS in two large teaching and research hospitals in a Houston, Texas medical center. Participants recruited under the parent study who met the following criteria were included in the present study: 1) adults

18 years of age or older, 2) final provider discharge diagnosis of ACS, 3) completed BDI-II, and 4) blood sample available for platelet serotonin analysis [15]. ACS was defined under the parent study as hospitalization for chest pain or symptoms suggestive of ACS lasting for more than 15 minutes, with an ultimate diagnosis of acute myocardial infarction (AMI) or angina requiring revascularization.

In order to meet the requirements for the standardized Enzyme-Linked Immunosorbent Assay (ELISA) laboratory protocol for platelet serotonin analysis, blood samples collected under the parent study were excluded from the present study if immediate transportation to the research laboratory for platelet serotonin processing after blood sample collection could not occur [16]. Because antidepressant medications may alter platelet serotonin levels, six patients currently taking anti-depressants, defined as any anti-depressants taken within the last 30 days, were excluded from this study [17]. In order to control for potential seasonal variations and effects of freezer storage time prior to platelet serotonin analysis, for every qualified BDI-II $\geq$ 14 ACS patient enrolled, the next qualified BDI-II < 14 ACS patient was enrolled from January 2010 to December 2010. After applying the inclusion and exclusion criteria for the 340 ACS patients recruited under the parent study, a final sample size of 51 was available for the current study. Study approval was obtained by the university's Committee for the Protection of Human Subjects Internal Review Board, and all participants provided written informed consent under the parent study.

Platelet Serotonin Processing

Blood samples for platelet serotonin analysis were collected from a peripheral venous site during hospital admission for ACS and prior to any cardiac intervention. A sample of 4 mL of blood was collected in an ethylene diamine tetra acetic acid (EDTA) tube and gently inverted to ensure mixture. The EDTA tube was placed in a 4º C refrigerator until transfer to the university's School of Nursing laboratory. Upon arrival to the laboratory, the 4 mL EDTA was mixed 20 times by inverting. The EDTA tube was then centrifuged for 10 minutes at 200 x g at ambient temperature. A sample of 300 µL of plasma supernatant was then removed from the 4 mL EDTA tube and added to a 15 mL conical tube for platelet purification. Physiological saline solution (1.5 mL) was then added to the platelet-rich plasma (PRP), and the 15 mL conical tube was centrifuged at 4,500 x g for 10 minutes at 4º C. The supernatant was then discarded, with care taken not to disturb the platelet pellet, and 300 µL of distilled water added. After vortex of the platelet pellet to ensure thorough mixture, the 300 µL platelet pellet was transferred into one aliquot and placed in a -70º C freezer for storage until time of assay. *In vitro* diagnostic quantification of platelet serotonin levels (ng/109 platelets) in human plasma was determined using Immuno-Biological Laboratories ELISA [16].

Depression Screening Instrument

Depressive symptoms, the independent predictor variable, was measured using the BDI-II, which asks individuals to consider each statement as it relates to the way they have felt for the past two weeks [15]. The BDI-II was administered, within 1 to 3 days of hospital admission, by trained research nurses, in order to measure depressive symptoms occurring during the time period that corresponds to the current ACS event. The BDI-II is a self-report 21-item screening instrument used to screen for and assess the severity of depressive symptoms, which reflects the American Psychiatric Association's *Diagnostic and Statistical Manual of Mental Disorders Fourth Edition* criteria for diagnosing depression and has been used in cardiovascular populations [18]. The BDI-II instrument was scored as a dichotomous variable, with a depression score <14 considered negative for depressive symptoms and a score between 14 and 63 considered positive for depressive symptoms. The BDI-II was also assessed as a continuous measure. The total BDI-II score is the sum of all items and ranges from 0 to 63.

Table 1. Demographic and Clinical Characteristics of Acute Coronary Syndrome Patients by Beck Depression Inventory-II Group.

	BDI-II ≥ 14 (n=24)	BDI-II < 14 (n=27)	p-value
Age *M* (year) ± *SD*	60.38 ± 15.6	59.7 ± 13.0	.868
Male	13 (54.2)	20 (74.1)	.138
Race			.669
White	14 (58.3)	16 (59.3)	
Black	6 (25)	5 (18.5)	
Hispanic	2 (8.3)	5 (18.5)	
Other	2 (8.3)	1 (3.7)	
Marital Status			.417
Married	16 (66.7)	15 (55.6)	
Single	8 (33.3)	12 (44.4)	
Education			.725
≤ High school diploma	12 (50)	12 (44.4)	
Some college or vocational	4 (16.7)	7 (25.9)	
College Degree	8 (33.3)	8 (29.6)	
BMI *M* ± *SD*	30.13 ± 5.7	31.52 ± 4.6	.342
Current Alcohol Use	6 (25)	8 (29.6)	.712
Current Cigarette Use	4 (16.7)	7 (25.9)	.422
BDI-II Score *M* ± *SD*	20.88 ± 5.7	5.26 ± 3.8	.000*
Platelet Serotonin (ng/109platelets)			
Platelet Serotonin M ± SD	942.10 ± 461.3	1192.41 ± 764.3	.231
Time to laboratory processing M (minutes) ± SD	115.63 ± 53.85	113.19 ± 50.08	.868
Current Hospitalization			
Diagnosed AMI	9 (37.5)	20 (74.1)	.008*
LVEF M ± SD	45.48 ± 14.3	53.16 ± 9.4	.050
Medically treated only	8 (33.3)	7 (25.9)	.562
Invasive cardiac procedure	16 (66.7)	20 (74.1)	.562
Coronary Angioplasty/Stent	14 (58.3)	15 (55.6)	.842
CABG	2 (8.3)	4 (14.8)	.671
Self-Report Medical History			
CABG	8 (33.3)	3 (11.1)	.054
AMI	11 (45.8)	7 (26.9)	.164
AMI Males <50 years of age	3 (12.5)	1 (3.7)	.331
AMI Females <55 years of age	2 (8.3)	1 (3.7)	.595
Coronary Angioplasty	3 (12.5)	0	.097
Coronary Stent	12 (50)	4 (14.8)	.007*
CAD	12 (50)	9 (33.3)	.227
Diabetes	12 (50)	10 (37)	.351
Hypertension	19 (79.2)	14 (51.9)	.042*
Stroke	2 (8.3)	2 (7.4)	1.00
History of depression diagnosis or treatment	5 (21.7)	3 (11.1)	.444
Familial History of CAD	18 (75)	22 (81.5)	.574

a. p-value=2-tailed level of significance; b. percentages in parenthesis unless indicated otherwise; c. table abbreviations: M=mean; SD= standard deviation; BMI= body mass index; BDI-II= Beck Depression Inventory-II; AMI= acute myocardial infarction; LVEF= left ventricular ejection fraction; CABG= coronary artery bypass surgery; CAD= coronary artery disease.

Table 2. Multiple Regression Analyses Predicting Platelet Serotonin Levels from Depressive Symptoms.

	Model 1* R^2 *(df), F, p* .049 (7, 34), 248, 969			Model 2** R^2 *(df), F, p* .055 (7, 34), 281, 957		
Variables	β	*SE_ β*	p	β	*SE_ β*	p
Depressive symptoms	-4.09	3.83	.293	-.254	.217	250
Age	-.052	.116	.654	-.060	.116	.607
LVEF	-.018	.147	.901	-.011	.144	.940
Medication only vs. invasive	1.69	4.04	.678	2.33	4.08	.571
History of hypertension	2.48	3.77	.515	2.83	3.83	.465
History coronary stent	2.45	3.78	.521	2.87	3.85	.461
Current diagnosed AMI	-1.15	3.53	.746	-1.13	3.53	.718

a. *Model 1 depression is analyzed as an independent dichotomous variable; b. **Model 2 depression is analyzed as an independent continuous variable; c. Depressive symptoms = Beck Depression Inventory-II score ≥ 14; c. table abbreviations: AMI= acute myocardial infarction. LVEF= left ventricular ejection fraction.

Statistical Analyses

Statistical power was determined for a multiple linear regression model using nQuery Advisor 7.0 [19]. Data from Schins et al. was used to determine three of the covariates included in the final model that account for an estimated 27 percent of the variation ($R^2 = 0.2700$) in platelet serotonin levels: age, left ventricular ejection fraction (LVEF), and medication only versus invasive treatment [8]. Variables associated with platelet serotonin levels including gender, history of depression, nicotine, and alcohol were also examined between the BDI-II ≥ 14 and BDI-II < 14 groups. Basic descriptive statistics were tabulated to characterize the sample. The available sample size of 51 (24 BDI-II ≥ 14 and 27 BDI-II < 14) has a power of 80 percent to detect, at an alpha level of 0.050, an increase in R^2 of 0.1010 due to including depression level (BDI-II ≥ 14 or BDI-II < 14) into the final model. The power analyses based on an increase in R^2 of 0.1010 accounting for $R^2 = 0.2700$ in the linear regression model using nQuery Advisor Version 7.0. SPSS Statistics 19 was used to perform all analyses [20]. Statistical tests were two-tailed with an alpha level of 0.05. Initial comparisons between BDI-II ≥ 14 ACS individuals and BDI < 14 ACS individuals were conducted using unpaired t tests for continuous variables and chi-square tests for categorical variables. Because platelet serotonin levels were not normally distributed, a square root transformation was applied to the platelet serotonin levels of the participants. Multiple linear regression models were computed to determine if depressive symptoms (positive depressive symptoms BDI-II ≥ 14 and negative depressive symptoms BDI < 14) were associated with higher levels of platelet serotonin after adjusting for potential confounding variables. Potential confounding variables included in the multiple regression models were available under the parent study via participant self-report and medical record abstraction.

RESULTS

Demographic and clinical characteristics according to BDI-II status are presented in Table 1. Compared to BDI-II ≥ 14 participants (37.5 percent), BDI-II < 14 participants (74.1 percent) were more likely to be currently diagnosed with an AMI (p = .008) and were more likely to have a lower LVEF (p = .050). BDI-II ≥ 14 participants were more likely than BDI-II < 14 participants to report a medical history of coronary stent placement (p = .007) and hypertension (p = .042). Mean platelet serotonin levels were not significantly different between individuals who screened positive for depressive symptoms with BDI-II scores ≥ 14 (942.10

± 461.3) and individuals who screened negative for depressive symptoms with BDI-II scores <14 (1192.41 ± 764.3) (p = .231). Multiple linear regression models were computed with all of the potential confounders analyzed as predictors and platelet serotonin as the dependent variable. When depressive symptoms were analyzed as a dichotomous variable (BDI-II ≥ 14 versus BDI-II < 14), the analysis resulted in a non-significant model (F = .248, df = 7, p = .969, R^2 = .049) indicating that depressive symptoms were not a significant predictor of platelet serotonin levels (β = -4.09, p = .293). None of the potential confounders were significantly related to platelet serotonin levels (Table 2). When depressive symptoms were analyzed as a continuous variable (M = 12.61 ± 9.172), the analysis also resulted in a non-significant model (F = .281, df = 7, p = .957, R^2 = .055), indicating that depressive symptoms were not a significant predictor of platelet serotonin levels (β = -.254, p = .250). None of the potential confounders were significantly related to platelet serotonin levels (Table 2).

DISCUSSION

The present study revealed that platelet serotonin levels did not differ between patients who screened positive for depressive symptoms (BDI-II ≥ 14) and those who screened negative for depressive symptoms (BDI-II < 14), and a linear relationship was not found between depressive symptoms and platelet serotonin levels. To our knowledge, only two other studies have evaluated the association of depressive symptoms with platelet serotonin levels in cardiovascular patients, both finding higher platelet serotonin levels in patients with depression [8,21]. Schins et al. collected whole blood serotonin levels from a cohort of 25 depressed and 21 non-depressed stable outpatients with CAD after a mean period of 5.6 months after AMI [8]. Wulsin et al. collected whole blood serotonin levels on 791 stable outpatients with documented CAD [21]. Both studies measured whole blood serotonin levels in stable CAD outpatients, whereas the present study examined platelet serotonin in platelet-rich plasma (PRP) in hospitalized patients during ACS.

Because heightened platelet activation and aggregation to atherosclerotic plaque disruption has been documented in some individuals with depressive symptoms, it is important to not only study platelet serotonin levels in stable CAD outpatients, but also during an acute cardiovascular "clotting" event (ACS) [11-12]. Although our findings were not statistically significant, those with higher depression scores had lower platelet serotonin levels that were the opposite direction of what was expected. While our findings are discrepant with both studies finding associations of diagnosed depression with higher platelet serotonin levels, they are consistent with the Wulsin et al. findings regarding the lack of any association between depressive symptoms and whole blood serotonin levels [21]. The Wulsin et al. results suggest that the relationship between higher platelet serotonin levels and depressive symptoms may be limited to diagnosed major depression and may not apply to depressive symptoms alone or even to diagnosed mild to moderate depression [21].

Although our findings are different, it is important to note that the previous studies measured whole blood serotonin (WBS), as opposed to the current study that measured platelet serotonin in PRP. The degree to which platelet serotonin in PRP compares to WBS is uncertain. In the present study, using PRP and an expected fixed platelet count to derive platelet serotonin levels (compared to other methods using WBS and actual platelet counts) does not take into account individual differences in platelet counts and may be one possible explanation for the lack of association between platelet serotonin levels and depressive symptoms in the current study. Since platelet counts at the time of sample collection were not available, this may be a critical point in explaining the negative findings in the present study.

Studies have shown that standard values for platelet serotonin levels show wide variation due to the sensitivity of platelets

[22,23]. Calculating platelet serotonin levels, even under stable conditions, presents a challenge when considering all aspects of platelet biology. Therefore, altered platelet counts or platelet functioning, due to clot formation during ACS, may have altered our platelet serotonin levels. Blood samples available for platelet serotonin analysis, under the parent study, were obtained as soon as possible after hospital admission and prior to any cardiac intervention (stent, coronary artery bypass graft). This may have controlled for some of the variables that may disrupt platelet function and platelet serotonin levels due to cardiac intervention, but future research should focus on gaining a better understanding of the variables that may influence the variation in platelet serotonin levels. One possible explanation for the absence of increased platelet serotonin in the depressed group as compared to the non-depressed group may be disrupted platelet function and platelet serotonin levels due to serotonin's cardiovascular response to a stressful ACS event. Studies have reported wide variability in the range of values for platelet serotonin levels due to the influence of many different variables (for example, intake of serotonin rich foods, stress, nicotine, medications, and phlebotomy technique and specimen preparation) [24]. Previous studies have reported varied results, some finding higher platelet serotonin levels in younger individuals [22], females [25], and those who smoke [26], while other studies have reported lower platelet serotonin levels with alcohol intake [25]. In healthy depressed individuals (including individuals with a history of depression), results have varied with an increase [11], decrease [27], or no difference [28] found in platelet serotonin levels. While it was not possible to test the influence of all potential variables that may lead to altered platelet serotonin levels, the present study did consider many potential variables that may influence platelet serotonin levels (for example, anti-depressants, nicotine, alcohol, history of depression, age, gender, race, and "time to laboratory processing"). While there were no significant differences in these variables between the BDI-II $\geq$ 14 and BDI-II < 14 groups, these variables may have altered overall platelet serotonin levels in both BDI-II groups, impacting study findings. For example, we found no significant differences between self-reported current (within the last year) nicotine and alcohol use between the BDI-II $\geq$ 14 and BDI-II < 14 groups, but patients were not instructed to abstain from nicotine or alcohol before blood sample collection for platelet serotonin analysis; therefore, the impact of recent nicotine or alcohol intake on the current blood sample for platelet serotonin analysis cannot be determined.

One limitation of the present study is the inability to generalize study results to ACS patients actively taking anti-depressants. Because anti-depressants may alter platelet serotonin levels, we excluded any patients taking anti-depressants defined as any anti-depressants taken within the last 30 days. It is possible that some patients actively being medicated for depression have experienced a greater number of depressive symptoms, experienced depressive symptoms more frequently, or have been diagnosed with major depression, all of which requires further investigation. As previously suggested, the relationship between platelet serotonin levels and depressive symptoms may be limited to a diagnosis of major depression and may not apply to depressive symptoms alone as measured by the BDI-II.

Several conceivable explanations for the lack of association between platelet serotonin levels and depressive symptoms in individuals with ACS may exist. First, clinical diagnoses for depression were not available. However, the BDI-II does reflect the American Psychiatric Association's *Diagnostic and Statistical Manual of Mental Disorders Fourth Edition* criteria for diagnosing depression and asks individuals to consider each symptom as it relates to the past two weeks, including the current day, which measures depressive symptoms during the time period that corresponds to the current ACS event [15,18]. Second, there may have been insufficient numbers in both the BDI-II $\geq$ 14 group (n = 24) and BDI-II < 14 group

(n = 27) to have the power to identify an association between platelet serotonin levels and depressive symptoms. Only 51 out of the 340 ACS patients available under the parent study met the criteria for the current study. Third, while the mean "times to laboratory processing" (calculated in minutes) were not significantly different between the BDI-II ≥ 14 group (115.63 ± 53.85) and the BDI-II < 14 group (113.19 ± 50.08) (p = .868), research has shown that platelet serotonin levels are dependent on the time to laboratory processing [29]. Although some researchers have found that delayed ELISA laboratory processing may result in a wide range of unpredictable platelet serotonin levels, the definition of "delayed" laboratory processing remains unclear [29]. In the present study, several variables delayed "time to laboratory processing" in both the BDI-II ≥ 14 and BDI-II < 14 groups including: 1) the time needed to obtain written informed consent before specimen transport; 2) the use of an off-site research laboratory; and 3) specimen processing restricted to laboratory hours. This delayed "time to laboratory processing" may be one possible explanation for the lack of association between platelet serotonin levels and depressive symptoms. In addition, a potential selection bias may have occurred as the current study excluded patients if platelet serotonin laboratory processing could not occur within 3 hours of blood sample collection. Finally, only 37.5 percent of the BDI-II ≥ 14 patients had a documented AMI during the current hospital stay compared to 74 percent of the BDI-II < 14 patients (p = .008). Patients diagnosed with ACS and ultimately an AMI may differ from those patients who are diagnosed with ACS but do not go on to have an AMI during that hospital stay.

While previous research findings suggest that platelet serotonin levels clearly impact platelet aggregation and is likely to influence acute coronary syndrome, it remains unclear what the association is between platelet serotonin levels and depressive symptoms during acute coronary syndrome. Researchers need to further explore the potential association between platelet serotonin levels and depressive symptoms in a defined group of ACS patients with hospitalization for AMI and defined depression groups (diagnosed mild, moderate, major depression). Future research should focus on gaining additional insight into the association between platelet serotonin levels and depression and ultimately its impact on cardiovascular outcomes.

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