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Serotonin and Cortisol as Suicidogenic Factors in Patients with PTSD

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A B S T R A C T

Post-traumatic Stress Disorder (PTSD) frequently occurs in commorbidity with different mental disorders, including suicidal behaviour. Group of biological factors, including serotonergic system, HPA axis and some genetic factors, are being studied as potential markers, able to differentiate suicidal and non-suicidal behaviour across the group of PTSD patients. This study is examining statistical relation between platelet serotonine concentration and serum cortisole concentration, within the group of PTSD patients with and without attempted suicide, treated at »Sveti Ivan« Psychiatric Hospital in Zagreb. The hypothesis of this study is that periferal biochemical markers are different accross the groups of PTSD patients with and without attempted suicide and the group of healthy controls. Our results have shown significantly lower platelet serotonine concentration in PTSD patients with and without suicide behaviour, compared to healthy controls. There are no statisticaly significant differences of the serum cortisole concentration accross observed groups. Our results correspond with those reported by other authors in this area of research, suggesting that platelet serotonine level might be used as potential periferal marker to detect risk of suicidal behaviour in PTSD patients.

Key words: *cortisol, attempted suicide, Post-traumatic Stress Disorder, serotonin, suicidogenic factors*

Introduction

Post-traumatic stress disorder (PTSD), even fifteen years upon the Croatian War of Independence, still presents significant public health, diagnostic and therapeutical challange, as well as the social problem. PTSD often appears in comorbidity with various psychiatric disorders, and is know to be associated with suicidal behaviour. The involvement of the serotonergic system, hypotalamic-pituitary-adrenal axis (HPA axis), as well as genetic factors, within the group of biological factors, is being reserched for their potential marker role in the process of distinguishing psychiatric patients with and without suicidal behaviour. Biological factors of suicidal behaviour are present in number of psychiatric disorders, while being very specific for some psychiatric disorders, which leads to the research hypothesis about distinction of biological and/or biochemical behavioural factors in the group of PTSD patients with and without attempted suicide[1].

Both the increased understanding of relationship among the platelet serotonine concentration changes and blood cortisol level changes, in PTSD patients with and without suicidal behaviour, with application of the timely and efficient suicide prevention measures and procedure, might contribute to the increased overall eficacy of suicide prevention in PTSD patients.

The most significant PTSD feature is development of specific psychopatological symptoms, as response to the stressor event, or any other situation of extremely threatening or chatastrofic nature. The stressor experience that occurs prior to PTSD development, might be the consequence of both witnessing and/or participating in the traumatic events, such as war-related crimes, torture, terorism, rape, criminal act, severe traffic accidents, etc. PTSD presents itself with the involuntary re-experience of the stressor event, where patient is consequently avoiding all the reminders of the trauma, while suffering from the numerous symptoms, such as: anxiety, sleep disorders, irritability, anger outbursts, difficulty concentrating, depresive mood, difficulties with controlling agressive impulses, with the increased risk of autodestructive and heterodestructive behaviour[2–4].

PTSD is multisystemic disorder that is clinicaly manifested both on psychological and somatic level. It is consequence of the complex interrelationship and interac-

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tions of the biological and psychological predispositions, as well as premorbidity of the personality on one side, with the exposure to the psichological and sociocultural factors, such as stress type and intensity, attitude of the community towards the traumatized person, on the other side[5,6]. All the research until now, have underlined the fact that hypothalamus, amygdaloid nuclei, as well as frontal and orbitofrontal cortex, play significant role in the patophysiology of PTSD[7,8]. In the PTSD patients, most authors have found low levels of urine cortisol, with the unchanged or reduced plazma cortisol levels, which presents neuroendocrinological paradox by itself[9,10]. Such findings in the PTSD patients are explained with the chronic adaptation of the hypotalamic-pituitary-adrenal axis to the stress situation[10,11]. Involvement of this system in the suicidal behavioural biology, as well as the interaction to the other neurotransmitter systems, have not been sufficiently clarified until now[12].

Patophysiologically, serotonine is related to the PTSD symptoms, as it is considered that serotonine controls symptoms such as hyperarrousal, increased impulsivity and aggression, as well as sleep disturbance and mood disorders[13]. Blood platelets are considered as periferal serotonergic markers, since platelets share both structural similarities with the serotonin neurons (similarities in the serotonine uptake, storage and release), as well as binding characteristics between serotonin and 5-HT2 receptors. Studies that have researched concentration of the platelet serotonin concentration as well as platelet MAO, in the patients with PTSD, have presented various results, from the values that have not changed, to the values that have been either increased or dicreased[14–17].

Prevalence of the PTSD in the general population is less than 1%[18]. Prevalence of the PTSD in the high risk groups (i.e. war veterans, volcanic eruption victims and crime victims) is considered to be from 3 to 58%[18,19].

Suicidal behaviour is the ultimate way of desctructive and agressive behaviour in the patients with PTSD. While PTSD is associated with a high suicide rate, suicidal behaviour often remains unrecognized and unreachable to preventive measures, due to the overall complexity of the clinical features[20]. In the research of serotonergic system in patients with suicidal behaviour, numerous findings presented disrupted neurotransmitter systems[11,17]. Reduced liquor concentration of 5-HIAA (5-hydroxyindoleacetic acid) is known as the most important biological marker to predict the risk of suicidal behaviour[22]. The research has shown the reduced values of liquor 5-HIAA, in the group of PTSD patients with the suicidal behaviour. The role of cortisol in the suicide etiology remains obvious, although the mechanisms have not been fully clarified[23].

Subjects and Methods

Subjects

The study included 46 subjects diagnosed with PTSD and attempted suicide, 41 subject diagnosed with PTSD without attempted suicide and 50 phenotipically healthy patients. The patients diagnosed with PTSD, were diagnosed using the diagnostic criteria of the ICD-10 (International Classification of Mental and Behavioural Disorders ICD-10; Clinical descriptions and diagnostic guidelines, 10th revision) and Diagnostic and Statistical Manual of Mental Disorders (DSM-IV)[18,24], produced by the American Psychiatric Association, 4th revision. Patients were not diagnosed with any other mental disorder, according to the criteria of the above mentioned classifications. The suicide attempt has been diagnosed in accordance to the ICD-10, classified by the Beck classification of suicidal behaviour[25].

Methods

Blood samples have been taken on the second day of hospitalisation, in the »Sveti Ivan« Psychiatric Hospital, on an empty stomack, during the usual laboratory evaluation, according to the standardized procedure, in accordance with the ethics principles (with the approval of the Ethics Committee of the Hospital and with the Informed Consent of the patient). Blood samples of the phenotypically healthy patients, have been taken in the Croatian Institute for Transfusion Medicine, and delivered to the Medical Biochemical Laboratory of the »Sveti Ivan« Psychiatric Hospital, to be analysed, with the written consent of the voluntary blood donor.

Platelet serotonin concentration was determined by the competitive enzyme immunoassay (enzyme-linked immunosorbent assay test-ELISA test, produced by BioSource, Belgium), on immunoanalyser Elysis Uno (Human, Germany), while the serum cortisol level concentration, has been determined by the enzyme immunoassay test, produced by Human, Germany, on Elysis Uno immunoanalyser.

All conclusions have been reached upon conduct of statistical tests and results have been interpreted at 5% significance level. The statistical evaluation of the data have applied descriptive statistics methods, t-test, ANOVA test, Mann-Whitney U, Kruskal-Wallis test, ROC (Receiver operating characteristic) curve. Statistical Package for Social Sciences Software (SPSS) 13.0 for Windows has been used for all of the above statistical evaluation.

Results

Therapy has been provided to both groups of PTSD patients, with and without suicidal behaviour. Proportion and quantitative share of specific medicaments have been presented for both groups. The application of the Fisher's exact test has shown no difference among the groups, with regards to the proportion of the particular medicament (Table 1) Odds ratio (OR) for the suicide attempt, with regards to the SSRI therapy (antidepressants from the group of selective serotonin reuptake inhibitors), was not statistically significant at 5% significance level (OR= 0.459; 95 % CI=0.191–1.104; p=0.082).

TABLE 1
MEDICATION ADMINISTERED TO PATIENTS PRESENTING WITH PTSD

Therapy		PTSD with attempted suicide		PTSD without attempted suicide		Total		p*
		N	(%)	N	(%)	N	(%)	
SSRIs	No	32	(69.6)	21	(51.2)	53	(60.9)	
	Yes	14	(30.4)	20	(48.8)	34	(39.1)	0.123
Total		46	(100.0)	41	(100.0)	87	(100.0)	
New	No	43	(93.5)	34	(82.9)	77	(88.5)	
Antipsychotics	Yes	3	(6.5)	7	(17.1)	10	(11.5)	0.180
Total		46	(100.0)	41	(100.0)	87	(100.0)	
Classical Antipsychotics	No	42	(91.3)	33	(80.5)	75	(86.2)	
	Yes	4	(8.7)	8	(19.5)	12	(13.8)	0.214
Total		46	(100.0)	41	(100.0)	87	(100.0)	
Anxiolytics	No	20	(43.5)	18	(43.9)	38	(43.7)	
	Yes	26	(56.5)	23	(56.1)	49	(56.3)	>0.999
Total		46	(100.0)	41	(100.0)	87	(100.0)	
Hypnotics	No	39	(84.8)	29	(70.7)	68	(78.2)	
	Yes	7	(15.2)	12	(29.3)	19	(21.8)	0.128
Total		46	(100.0)	41	(100.0)	87	(100.0)	
Other medicaments	No	36	(78.3)	32	(78.0)	68	(78.2)	
	Yes	10	(21.7)	9	(22.0)	19	(21.8)	>0.999
Total		46	(100.0)	41	(100.0)	87	(100.0)	

Upon testing the normality of distribution with Kolmogorov-Smirnov test (K-S test), where the result has shown normal Gauss distribution curve ($p=0.269$) of the serum cortisol concentration, the ANalysis Of VAriance (or ANOVA) has presented no statistically significant difference in the cortisol concentration accross all of the patient groups ($F=2.578$; $df=2$; $p=0.080$). For the group of patients that have been taking SSRI therapy, equal variance T-test (test for homogeneity of variance)has shown no statistically significant difference in the average cortisol concentration ($t=–0.488$; $df=31$; $p=0.629$), among the group of PTSD patients with and without suicide attempt. In the presented group of patients with PTSD and attempted suicide, there was only one male patient included in the group, the results of the statistical analysis are related to the female patients only.

Upon testing the normality of distribution with Kolmogorov-Smirnov test (K-S test), it was shown that the platelet serotonin concentration of female patients does not differ from the normal (Gauss) distribution curve ($p=0.098$), in the way that would present statistically significant difference. The analysis of the variance has shown that within the group of female patients, there was statistically significant difference of the average platelet serotonin concentration among the groups ($F=9.152$; $df=2$; $p=0.001$). Tamhane-T2 post hoc test for inhomogeneous variances were applied for comparisons among the groups. It was shown that female PTSD patients without attempted suicide, have statistically significant decrease of the average platelet serotonine concentration, compared to the female PTSD patients with attempted suicide ($F= 9.152$; $df=2$; $p=0.001$), as well as compared to the helthy female population ($p<0.001$). Values of the average platelet serotonin concentration in the groups of female patients, have been shown in the Table 2.

T-test for inhomogeneous variances applied to the group of patients taking SSRIs (selective serotonin reuptake inhibitors) has shown no statistically significant difference of the average platelet serotonine concentration, amongst suicidal PTSD patients and nonsuicidal PTSD

TABLE 2
PLATELET SEROTONIN CONCENTRATION (ng/10^9 PLATELETS) IN FEMALE PATIENT GROUPS

Patient Group	$\overline{X}$	SD (+/–)	Median	Q_1–Q_3	N
PTSD with attempted suicide	787.77	675.02	527.03	263.16–1223.98	17
PTSD without attempted suicide	182.60	147.09	172.21	66.00–241.84	13
Healthy Control Group	1105.26	660.90	880.36	497.61–1721.74	14
Total	709.99	667.07	470.67	175.82–910.88	44

patients (t=–1.746; df=3; p=0.178). Although there is obvious big difference of the average platelet serotonine concentration, this is not statistically significant for two reasons: small number of patients in one of the groups and inhomogeneous variance of the groups. Results are presented in Table 3.

ANOVA test and SNK post hoc test (N=44, F=8.966, p<0.001), presented the difference in the platelet serotonin concentration accross all the groups of female patients, while the nonsuicidal PTSD female patients with SSRI therapy, have shown statistically significant reduction of the platelet serotonine concentration, compared to the nonsuicidal PTSD female patients with no SSRI therapy (152 ng/10^9 platelets *vs.* 216 ng/10^9 platelets).

The same analysis has been shown statistically lower platelet serotonin concentration in the group of the suicidal PTSD patients with SSRIs therapy, compared to the group of PTSD patients without SSRI therapy (716 ng/10^9 platelets *vs.* 891 ng/10^9 platelets).

ROC analysis has been used in order to evaluate diagnostic accuracy of the platelet serotonin concentration in the group of PTSD patients with and without attempted suicide, compared to the phenotypically healthy control group. Out of 44 patients analysed, with the cut-off platelet serotonine concentration of 594.43 (ng/10^9 platelets), optimizing specificitiy/sensibility pair, the value of the area under the ROC curve was 79% (p=0.002), with test specificity of 71.4%, which means that 71.4% women from the group of healthy controls, have platelet serotonine concentration greater then or equal to 594.43 ng/10^9 platelets and test sensitivity of 73.3%, which means that 73.3% women from group of PTSD patients, have the platelet serotonin concentration less then or equal to 594.43 (ng/10^9 platelets). Results are shown in the Table 4. Positive predictive value (PPV) of the platelet serotonin concentration is 84.6%, meaning that 84.6% of women, with the platelet serotonine concentration less then or equal to 594.43 ng/10^9platelets, do belong to the group of PTSD patients. Negative predictive value (NPV) of the platelet serotonin concentration is 55.7%, meaning that 55.7% of women with the platelet serotonine concentration greater then or equal to 594.43 ng/10^9 platelets, belongs to the group of healthy controls.

Discriminatory efficacy of the platelet serotonin concentration has been tested, in order to determine belonging to the group of women with PTSD and attempted suicide, in relation to the group of women with PTSD without attempted suicide. The value of the area under the curve was 82% (p=0.003), with the cut-off platelet serotonine concentration of 303.12 (ng/10^9 platelets).

The sensitivity of platelet serotonin concentration used as diagnostic test is 76.5%, which means that 76.5%

TABLE 3
PLATELET SEROTONIN CONCENTRATION (ng/10^9 PLATELET) RELATED TO SSRIs IN PTSD PATIENT GROUPS

Analit	Group	$\overline{X}$	SD (+/–)	Median	Q_1–Q_3	N
Platelet serotonin concentration	PTSD without attempted suicide	152.53	102.81	130.43	96.15–226.93	15
	PTSD with attempted suicide	716.01	643.36	621.29	149.50–1377.23	4

TABLE 4
ROC ANALYSES RESULTS IN THE GROUP OF FEMALE PTSD PATIENTS (PTSD WITH/WITHOUT ATTEMPTED SUICIDE) IN RELATION TO THE PHENOTYPICALLY HEALTHY CONTROL GROUP

		Healthy control group		PTSD with and without attempted suicide		Total	
		N	(%)	N	(%)	N	(%)
Platelet serotonine concentration	≥594.43 ng/10^9 platelet	10	(71.4)	8	(26.7)	18	(40.9)
	≤594.43 ng/10^9 platelet	4	(28.6)	22	(73.3)	26	(59.1)
Total		14	(100.0)	30	(100.0)	44	(100.0)

TABLE 5
ROC ANALYSES RESULTS IN THE GROUP OF FEMALE PTSD PATIENTS WITH ATTEMPTED SUICIDE IN RELATION TO THE GROUP OF FEMALE PTSD PATIENTS WITHOUT ATTEMPTED SUICIDE

		PTSD without attempted suicide		PTSD with attempted suicide		Total	
		N	(%)	N	(%)	N	(%)
Platelet serotonin concentration	≤303.12 ng/10^9 platelets	11	(84.6)	4	(23.5)	15	(50.0)
	≥303.12 ng/10^9 platelets	2	(15.4)	13	(76.5)	15	(50.0)
Total		13	(100)	17	(100)	30	(100)

of women from the group of PTSD patients with attempted suicide, have the platelet serotonin concentration greater then or equal to 303.12 ng/10^9 platelets.

The specificity of platelet serotonin concentration used as diagnostic test is 84.6%, which means that 84.6% of women from the group of PTSD patients without attempted suicide, have the platelet serotonin concentration lesser then or equal to 303.12 ng/10^9 platelets.

Positive predictive value of the platelet serotonin concentration (PPV) is 86.7%, meaning that 86.7% of women, with the platelet serotonine concentration greater then or equal to 303.12 ng/10^9 platelets, do belong to the group of PTSD patients with attempted suicide.

Negative predictive value of the platelet serotonin concentration (NPV) is 73.3%, meaning that 73.3% of women, with the platelet serotonine concentration lesser then 303.12 ng/10^9 platelets, do belong to the group of PTSD patients without attempted suicide.

Discussion

Suicidal behaviour often remains unrecognised and remains outside of prevention measures, due to teh complexity of the PTSD clinical presentation. Research of both the risk of suicide and the exposure to the traumatic event, with and without subsequent PTSD, it has been established that PTSD is clearly related to the increased risk of suicide and that PTSD is independent predictor of the suicidal risk[26,27]. Numerous biological studies have been conducted in order to identify the biological supstrate of suicidality. Suicidal behaviour is believed to involve alterations in different neurotransmitter systems, and most extensive evidence of the neurotransmitter disfunction have been obtained from the serotonergic system research[17]. Studies that have been researching suicide and serotonergic neurotransmitter system, have unonimously documented that decreased level of 5-HIAA (5-hydroxyindoleacetic acid – key metabolic substance in the serotonic decomposition) in liquor together with the previous attempt of suicide, may be considered as suicide predictors[28]. According to the results of this research, platelet serotonin concentration is statistically significantly lower in the group of PTSD patients without suicide attempt (182.60 ng/10^9 platelets) and with suicide attempt (787.77 ng/10^9 platelets), when compared to the group of phenotipically healthy controls (1105.26 ng/10^9 platelets).

Number of studies have delt with the possibility of increased suicidal risk during antidepressive therapy, including SSRIs, especially within the group of children and adolescents[29]. In this resaserch, odds ratio (OR) for the suicide risk in the group of patients on SSRIs, is not considered to be statistically significant at 5% significance level (OR=0.459, 95% CI=0.191–1.104, p=0.082).

In relation to decreased platelet serotonin concentration in the group of PTSD patients without attempted sucide (182.60 ng/10^9 platelets), compared to the group of PTSD patients with attempted suicide (787.77 ng/10^9 platelets), as well as the fact that 68% of patients have been on therapy, possible impact of SSRIs therapy to the platelet serotonin concentration has been researched. PTSD patients with and without attempted suicide, have been analysed according to SSRIs intake. ANOVA test and SNK post hoc test, presented reduced platelet serotonin concentration in the group that has received SSRIs, both in the group of PTSD patients with and without suicide attempt.

When taking antidepressant therapy from the SSRI group (39% patients have been taking SSRIs), there was no statisticaly significant difference amongst the groups (Fisher exact test p=0.123). The same was observed with other kinds of therapy (anxiolytics and hypnotics), there was no statisticaly significant difference amongst the patient groups (p>0.05). All of the presented data, both from the literature, as well as results obtained through our research, we may conclude that response to the antidepressant therapy was better in the group of the PTSD patients with the suicide attempt.

In order to evaluate platelets serotonin concentration diagnostic accuracy, ROC analysis has been used in female PTSD patient groups, with and without suicide attempt (as there was no difference in the proportions related to the use of medicaments), compared to the group of the phenotipically healthy controls. Platelet serotonin concentration discriminatory efficacy has been examined as well, in order to distinguish female PTSD patients with attempted suicide, from female PTSD patients without attempted suicide. ROC analysis have shown very high diagnostic reliability. According to the obtained results, it is possible that platelet serotonin concentration is potential marker of the suicidal behaviour in the patients with PTSD. For potential marker values, it would be possible to use two discriminatory platelet serotonin concentrations: 1. Platelet serotonin concentration <594 ng/10^9 platelets, with 73% sensitivity, showing characteristics of the PTSD patient group with and without suicide (marker of the possible suicidal risk) and i 2. Serotonin concentration >303 ng/10^9 platelets with 77% sensitivity, showing characteristics of teh PTSD patient group with suicide attempt (marker of the probable suicide risk). Platelet serotonin concentration in the range from 303 to 594 ng/10^9 platelets, may serve as the suicide risk predictors.

However, the limitation of this research is the fact that basic platelet serotonin concentrations were not known. This is the reason we can not say if the reduction is related to the efficacy of the antidepressants, and/or it is included within the pathophysiology of the serotonin neurotransmission. In addition, only female patients with PTSD have participated in the research of the platelet serotonin concentration. Further research is needed, on the larger patient pool, in order to clarify the gender impact on the platelet serotonin concentration.

Prospective biological studies point out that disfunction of HPA axis has predictive values for suicide[20]. In this research, the conducted ANOVA variance analysis has shown lack of statistically significant difference in

the cortisol concentration, accross all patient groups ($F=2.578$; $df=2$; $p=0.080$). The above mentioned results are confirming HPA axis chronic dysfunctionality, where even at the point of the acute stressor exposure (the attempt of suicide), cortisol concentration remains unchanged. Such findings are corresponding to the cortisol level findings presented in former research. Accordingly, the results achieved through ANOVA test, present no statistically significant difference in the cortisol concentration, across all patient groups ($N=83$; $F=0{,}069$; $p=0{,}976$), distributed in accordance to SSRI therapy.

Platelet serotonin concentration values presented in this research, have confirmed our basic hypothesis about serotonin as the suicidal risk factor. Together with all other clinical indicators, especially with attempt of suicide, it can have predictive value in determining high risk group for the attempt of suicide, within the group of PTSD patients. The results presenting cortisol concentration, present confirmation of the chronic disfuncionality of the HPA axis, as found in previous studies, but can not be considered as the predictors for the risk of suicide.

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SEROTONIN I KORTIZOL KAO SUICIDOGENI FAKTORI KOD OBOLJELIH OD PTSP-A

SAŽETAK

Posttraumatski stresni poremećaj često se javlja u komorbiditetu s različitim psihijatrijskim poremećajima, a povezan je i sa suicidalnim ponašanjem. Serotoninski sustav, hipotalamo-hipofizno-adrenalni sustav i genetski čimbenici u skupini su bioloških faktora koji se istražuju kao potencijalni biljezi koji bi općenito mogli omogućiti razlikovanje psihički oboljelih osoba sa suicidalnim ponašanjem, od psihički oboljelih osoba bez suicidalnog ponašanja. U ovom radu

ispitana je statistička povezanost koncentracijâ serotonina u trombocitima i koncentracijâ kortizola u serumu i pokušaja suicida kod oboljelih od PTSP-a koji su liječeni u PB »Sveti Ivan«. Glavna hipoteza je bila da postoje razlike u perifernim biokemijskim pokazateljima u bolesnika s PTSP-om s pokušajem suicida u odnosu na bolesnike s PTSP-om bez pokušaja suicida, i u odnosu na fenotipski zdravu kontrolnu skupinu ispitanika. Utvrđene su statistički značajno snižene koncentracije serotonina u skupinama oboljelih od PTSP-a sa i bez pokušaja suicida u odnosu na fenotipski zdravu skupinu, te da nema statistički značajne razlike u koncentraciji kortizola između svih skupina. Rezultati su u skladu s prethodnim istraživanjima te govore u prilog mogućnosti da je koncentracija serotonina u trombocitima potencijalni biljeg suicidalnog ponašanja u oboljelih od PTSP-a.