Platelet serotonin and magnesium concentrations in suicidal and non-suicidal depressed patients

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Abstract. The pathophysiology and treatment of depression involves monoamine neurotransmitters and the magnesium (Mg)-modulated monoaminergic pathway. Serum and platelet Mg concentrations and platelet serotonin concentrations were measured in 79 depressed patients who had attempted suicide, and 101 patients without suicidal behaviour, according to the ICD-10 diagnoses F 33.2 and F32.2, with or without intentional self-harm (X60-X84). The control group consisted of 77 voluntary blood donors. The platelet serotonin concentration was determined using the competitive enzyme immunoassay test: Mg concentrations in platelets and serum were determined by atomic absorption spectrophotometry. The ANOVA test showed significantly lower serum Mg in the group of depressive patients who had attempted suicide ($N = 257$, $F = 8.32$, $p<0.001$), compared to depressive patients who had not, and the control group. Serum albumin was lower in the group of depressive patients who had attempted suicide and showed a significant, positive correlation with serum Mg concentrations. Platelet Mg concentrations were found to be higher in depressive patients who had not attempted suicide ($N = 257$, $F = 3.90$, $p = 0.012$) compared to the control group, with no difference compared to depressive patients who had attempted suicide. The Kruskal Wallis test ($N = 257$, $H = 48.54$, $p<0.0001$) showed the lowest concentration of platelet serotonin in the groups of depressed patients with and without suicidal behaviour, compared to the healthy control group. A positive correlation was found between platelet Mg and serotonin concentrations only in the healthy control group. In conclusion, differences were observed in serum and platelet Mg concentrations, which represent progress in the study of Mg status and its relation to serotonin.

Key words: magnesium, platelet serotonin, depression, suicidal behaviour

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Magnesium (Mg) is an essential cofactor for many enzymatic reactions, including energy production, synthesis of essential biomolecules, active ion transport across cell membranes, cell signalling and cell migration [1]. In addition, the Mg ion is an essential cofactor for the activation of tryptophan hydrolase [2], for which there is evidence of its presence in human platelets [3], and for binding of serotonin to its receptor [4]. It has been well established that at

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physiological concentrations, Mg blocks N-methyl-D-aspartate (NMDA) receptors in neurons [5], modulates GABAergic neurotransmission, and affects numerous transduction pathways [6, 7]. The pathophysiology and treatment of depression involves monoamine neurotransmitters and substances that reduce NMDA function, including the Mg modulated, monoaminergic pathway [8]. Several studies have demonstrated antidepressant and anti-anxiety-like effects of Mg in animal models [9-11] while an Mg-deficient diet increased depression and anxiety-related behaviour in mice [12, 13]. Epidemiological studies have demonstrated an inverse relationship between Mg intake and depressive symptoms in community-dwelling, middle-aged and older adults [14]. The majority of investigations determined the concentration of Mg in serum or plasma; however, this does not reflect the true state of the Mg supply in the organism, as only 1% of Mg is found in blood plasma and therefore, blood levels may not reflect total body stores [15]. Caution should be taken in interpreting results from patients with low total Mg and low albumin, as they may have normal concentrations of ionised Mg. With regard to ionised Mg, an increased albumin concentration was accompanied by a decreased ionized fraction [16].

With regard to cells, it is considered that different types of cells have different kinetic interchanges of Mg and other electrolytes, and it is still not completely clear which type of cell best reflects the status of Mg in the organism [15, 17, 18]. The determination of intracellular Mg levels from muscle biopsy, lymphocytes and red blood cells via nuclear magnetic resonance spectroscopy or ion-specific electrode measures is a much better predictor of magnesium status [19]. These tests are of value in basic research; however, they are expensive and impractical. The advantages of measuring platelet Mg *versus* red blood cells and lymphocytes, relates to the fact that platelets are relatively easy to isolate and they have certain features that resemble neuronal cells [20]. Loading tests appear to be the gold standard for Mg status, although there is also a need to reach a consensus on a standardized protocol to be used [19]. On the whole, some reports indicate higher plasma, serum and erythrocyte concentrations of Mg in patients with mood disorders [21-26], some report lower Mg concentrations [27-30], while some studies indicated no differences compared with normal subjects [31]. Banki *et al.* [32, 33] demonstrated lower cerebrospinal fluid (CSF) Mg in patients with depression and attempted suicide, and that CSF Mg concentrations correlated significantly with CSF serotonin. Studies of a possible association between major depression and platelet serotonin reported no changes [34-36], a decrease [37-40] or an increase [41] in platelet serotonin compared to healthy control subjects. Only a few studies have examined platelet serotonin in patients with depression and attempted suicide, compared to platelet serotonin of non-suicidal patients with depression. In these studies, platelet serotonin was lower in patients with depression and attempted suicide, compared to patients with depression and no attempted suicide [41-43], or to healthy control subjects [44, 45]. Platelets were chosen in these studies because of several commonalities they have with neuronal cells with respect to serotonin, monoamine and glutamate metabolism [20].

With regards to the considerable interest in investigating the connection between Mg and serotonin, and the lack of data on platelet Mg concentrations, the objective of this study was to examine the potential differences in platelet and serum Mg and platelet serotonin concentrations in groups of patients with a depressive episode, (F32.2 severe depressive episode without psychotic symptoms and F33.2 recurrent depressive disorder, current episode severe without psychotic symptoms), and a control group of phenotypically healthy subjects. Because an association between plasma Mg disturbances (either higher or lower) and the severity of clinical disturbances has been shown [27], and the serotonin neurotransmitting system is the decisive factor in the pathophysiology of depression in people with suicidal behaviour, our depressive patients were also stratified according to whether or not they had attempted suicide.

Subjects and methods

Subjects

A cross-sectional study was carried out using three groups used for comparison. Data were collected for the period August 2005 to January 2009. Initial psychiatric interviews were conducted within the period of 1 h to 24 h upon admission. The evaluation consisted of a psychiatric and medical

history review, data on current and previous medications, and alcohol intake. This was followed by a semi-structured interview, with the purpose of establishing the ICD-10 (International Classification of Diseases and Related Health Problems, Tenth Revision, 2004) criteria for diagnosis of F32.2 and F33.2, with or without intentional self-harm (X60-X84).

The first group consisted of 79 patients with a diagnosis of F32.2 and F33.2 who had attempted suicide (D-S), and who had been admitted to the Sveti Ivan Psychiatric Hospital shortly after the suicide attempt. The second group consisted of 101 patients who were consecutively admitted to the same hospital during the same period. Upon admission, the ICD-10 diagnosis (F32.2 and F33.2) was established and it was confirmed that there was no history of attempted suicide in this group of patients (D). The patients examined had no other comorbid, psychiatric disorders.

The 77 phenotypically healthy people in the control group (C) consisted of voluntary blood donors from the Croatian Institute for Blood Transfusion. These people self-reported that they had no psychiatric or medical disorders. The criteria for blood donation excluded numerous somatic diseases and pharmacological treatment. In all three groups, the exclusion criteria were the same, including: BMI >30 kg/m^2, use of diuretic drugs or Mg supplement, pregnancy, lactation, alcohol and reported substance abuse or dependence, cardiovascular disease and hypertension. *Table 1* presents the features of the study subjects according to diagnosis, psychopharmacological treatment, age and gender. The Chi-square test indicated no differences between the three groups, with respect to gender ($\chi^2 = 2.831$, DF = 2, $p = 0.243$). One-way analysis of variance (ANOVA test) showed that the lower age of phenotypically healthy control subjects ($p<0.001$, $F = 30.57$) was statistically significant. The age median of healthy controls was lower than in the patient groups, although all the study subjects were within the same age range. The correlation between serum and platelet Mg and age, and platelet serotonin and age, for all three groups was not significant.

Ongoing and previous antidepressant treatment status and dosage were determined for all patients. It was found that 97% (77/2) patients who had attempted suicide and 82.4% (84/17) with no history of attempted suicide were currently receiving therapy, in accordance with the current algorithms for the treatment of depression [46, 47]. All patients had been continuously under stable psychopharmacological treatment for the three months prior to inclusion in the study. Fisher's exact test found no differences in psychopharmacological treatment between the patient groups, apart from a group of other

Table 1. Depressive patients who attempted suicide (D-S), depressive patients without attempted suicide (D) and phenotypically healthy control (C) presented according to ICD-10 diagnosis, age, gender and psychopharmacological treatment.

	D-S N_{tot} **= 79**	**D** N_{tot} **= 101**	**C** N_{tot} **= 77**
ICD-10 diagnosis	F 33.2 (N = 38) F 32.2 (N = 41)	F 33.2 (N = 60) F 32.2 (N = 41)	
Age (years): median, mean (min-max)	48, 44 (22-57)	45, 44 (20-59)	33, 36 (21-59)
Gender (M/F)	30/49	40/61	38/39
Duration of disorders (years)	3.9 ± 3.1	4.6 ± 3.7	
Psychopharmacological therapy N (%),			
Fisher's exact test (p)			
Anxiolytics p = 0.224	55 (67)	59 (58)	
Tricyclic antidepressants p = 0.835	11 (13)	15 (14)	
SSRI p = 0.453	30 (37)	43 (42)	
Other antidepressants p = 0.001	5 (6)	25 (24)	

antidepressants seen in the group of depressed patients without attempted suicide, although this was of weak intensity, as tested by Cramer's V test (V = 0.181) .

Blood collection and biochemical measurements

Patient blood samples were taken for analysis, in all cases, on the second, fasting day ufollowing hospitalization at the Sveti Ivan Psychiatric Hospital. Blood samples were collected using a standardised process, and in accordance with the ethical principles of the Helsinki Declaration (verified by the Hospital Ethical Committee, and an informed consent was signed accordingly by each patient). Blood samples from blood donors were collected at the Croatian Institute for Blood Transfusion, and transferred according to the standardised, supervised procedure to the Department of Laboratory Diagnostics, where laboratory analysis was conducted.

The total Mg concentration in platelets and serum was determined using atomic absorption spectrophotometry on the AAnalyst 200 (Perkin Elmer, USA). Platelet serotonin concentrations were determined using an enzyme-linked immunosorbent assay (ELISA), and the immunoanalyser Elysis Uno (Human, Germany) using BioSource, Belgium reagents, following platelet isolation from the blood, as recommended by the reagent manufacturer. The serum albumin concentration was determined using the bromcresol green assay on the Cobas Integra analyser (Roche, Germany).

Platelet isolation from the blood was prepared, as follows: in order to obtain platelet-rich plasma (PRP), all samples were centrifuged for 10 min at room temperature (200 × g). The supernatant was transferred to another tube and the platelets counted. The platelet pellet was obtained by adding 800 μL of physiological saline to 200 μL of PRP and centrifuged (4,500 × g, 10 min at 4 °C); 200 (L of distilled water was added to the pellet and mixed on a vortex mixer. This suspension was stored frozen until analysis.

Statistical analysis

In the case of all variables analysed, descriptive statistics were determined with a 5% level of significance as statistically significant. The Kolmogorov-Smirnov test was used to test the normality of Mg and platelet serotonin distribution within the groups. As the test for normality of the Mg in serum and platelet distribution was satisfied, ANOVA was applied for the testing of differences between groups. The Student-Newman-Keuls (SNK) *post hoc* test was used to establish the groups that constituted that particular difference. In order to establish differences in the platelet serotonin concentrations between the examined groups, due to the abnormal distribution of variables, the Kruskal-Wallis and Mann-Whitney tests were applied. Spearman's rank coefficient of correlation was applied to correlate platelet Mg and serotonin, and Pearson's coefficient of correlation was applied to correlate serum Mg and albumin concentrations in the subject groups examined. All analyses were performed using the statistical package SPSS version 18.

Results

Concentrations of serum Mg

The one-way ANOVA test showed a statistically significant difference in serum Mg concentrations (N = 257, F = 6.90, p = 0.001). With the application of the SNK *post hoc* test, a significantly lower serum Mg was found in the group of depressive patients with attempted suicide (0.89 mmol/L, SD ± 0.16) when compared to the group of depressive patients without attempted suicide (0.96 mmol/L, SD ± 0.12) and the phenotypically healthy control group (0.94 mmol/L, SD ± 0.07). Approximately 30% of serum Mg is bound to protein, primarily albumin. Since the serum Mg concentration is dependent on albumin concentrations, which is is usually lower in hospitalized patients, we examined the concentration of albumin and correlated it with the serum Mg concentration in the entire examined group. The ANOVA test (N = 257, F = 66.0, $p<0.001$) and SNK *post hoc* test showed lower albumin concentrations in depressive patients who had attempted suicide (42 g/L SD ± 6.00) compared to depressive patients without attempted suicide (45 g/L, SD ± 3.91) and the phenotypically healthy control group (50 g/L, SD ± 3.03). A statistically significant positive correlation was found between Mg and albumin concentrations (N = 257, Pearson coefficient correlation, r = 0.297,

$p<0.0001$).The distribution of serum Mg and albumin concentrations in all three groups is shown in *table 2*.

Concentrations of platelet Mg

One-way analysis of variance (N = 257, F = 4.52, p = 0.012) and the SNK *post hoc* test showed higher concentrations of platelet Mg (0.19 μmol/10^9 platelet, SD ± 0.09) in depressive patients without attempted suicide compared to the phenotypically healthy control group (0.15 μmol/10^9 platelet, SD ± 0.07), with no difference compared to depressive patients with attempted suicide (0.18 μmol/10^9 platelet, SD ± 0.07). *Table 2* presents the distribution of platelet Mg concentrations in all three groups. There was no difference in platelet numbers in the platelet pellet suspension (ANOVA test, F = 2.53, p = 0.082) between depressive patients with attempted suicide (346×10^9/L), depressive patients without attempted suicide (372×10^9/L) and the phenotypically healthy control group (329×10^9/L).

Platelet serotonin concentration

Due to the abnormal variable distribution confirmed by the Kolmogorov-Smirnov test, the Kruskal-Wallis test indicated a statistically significant difference among the tested groups (N = 257, H = 34.92, $p<0.001$). The application of the Mann-Whitney test established a lower platelet serotonin in the group of patients with depression and no suicide attempt (median 176 ng/10^9 platelet, 95%CI 97-309) and in the group of patients with depression and attempted suicide (median 227 ng/10^9 platelet, 95%CI 147-710), compared to the phenotypically healthy control group (median 502 ng/10^9 platelet, 95% CI 420-591). The platelet serotonin levels for all groups examined are presented in *table 2*. A positive Spearman's coefficient of rank correlation was found between serotonin and Mg concentrations in platelets for the healthy controls (ρ = 0.548, $p<0.001$), but not for the group of patients with depression and attempted suicide (ρ = 0.142, p = 0.406) or patients with depression and no attempted suicide (ρ = 0.220, p = 0.115).

Discussion

The study showed the following:

– statistically significant lower serum Mg in patients with depression and attempted suicide, compared to patients with depression and no suicide attempt and phenotypically healthy participants. The lower serum Mg is connected with the lower albumin concentrations in patients with depression and attempted suicide and with the positive correlation between albumin and serum Mg concentration in all groups examined,
– a higher concentration of platelet Mg and lower platelet serotonin in depressive patients with

Table 2. Distribution of serum Mg and albumin concentrations, and platelet Mg and serotonin concentrations in all three groups examined.

Analyte	D	D-S	C
Serum Mg (mmol/L) Mean ± SD	0.96 ± 0.12	*0.89 ± 0.16	0.94 ± 0.07
Serum albumin (g/L) Mean ± SD	45 ± 3.91	**42 ± 6.00	50 ± 3.03
Platelet Mg (μmol/10^9 plt) Mean ± SD	***0.19 ± 0.09	0.18 ± 0.07	0.15 ± 0.07
Platelet serotonin (ng/10^9 plt) Median 95%CI	176 : 97-309	227 : 147-710	•502 : 420-591

D-S: depressive patients with attempted suicide; D: depressive patients without attempted suicide; C: phenotypically healthy control group; D-S* p = 0.001 *versus* non-suicidal, depressive patients and healthy control group tested using the SNK *post hoc* test. D-S** $p<0.001$ versus non-suicidal, depressive patients and healthy control group tested using the SNK *post hoc* test. D*** p = 0.012 versus suicidal, depressive patients and healthy control group tested using the SNK *post hoc* test. C•, $P<0.001$ versus suicidal, depressive patients and non-suicidal, depressive patients tested using the Mann-Whitney test.

or without attempted suicide compared to the phenotypically healthy control group. A positive correlation was found between platelet serotonin and Mg concentrations only in the phenotypically healthy group.

In the literature, there are no data on Mg concentrations in patients with depression and attempted suicide, as most studies focus only on patients with depression. Only one study [32] pointed to a CSF Mg deficiency in patients with depression and attempted suicide. In this study, CSF Mg correlated significantly with CSF serotonin, and the authors propose that Mg might play a role in central indoleamine metabolism that is possibly related to suicidal behaviour. Nechifor [28, 48] reported reduced Mg concentrations in serum and erythrocytes of patients with more severe depression, whereas no change was observed for less severely affected subjects. In this study, an increase in Mg concentrations in erythrocytes was observed following treatment with antidepressants. Zieba *et al.* [30] reported lower Mg concentrations in the serum of patients with depression compared to the control group, although there was no correlation with the severity of depression. In these studies, no data were given on the concentrations of serum albumin, which could have affected the results. In hospitalised patients, serum Mg concentrations show a broader range of values than in healthy people [49]. Albumin and Mg are linearly related at albumin concentrations ranging from 45-50 g/L and 26-39 g/L, but not for the range of 39-45 g/L [50]. The present study showed lower Mg and albumin concentrations and a good positive correlation between Mg and albumin concentrations in all subjects groups, with the widest range of albumin values in patients with depression and attempted suicide. The resulting higher concentration of Mg in platelets of patients with depression and no attempted suicide in the present study could be compared to the results of other studies, in which higher Mg concentrations were obtained in the erythrocytes of patients with depression, compared to phenotypically healthy subjects [24-26]. With regard to Mg, the literature also points to the influence of antidepressants on increased Mg in the erythrocytes of patients with depression [48]. The differences in platelet Mg concentrations with respect to classes of antidepressants were analysed between patients treated with SSRIs, and those treated with tricyclic antidepressants. These two classes of drugs were chosen as they were found to be the most common treatment of depression in this study. No differences were found in the platelet Mg of patients without attempted suicide (0.18, SD $\pm$ 0.07 receiving SIPSS treatment *versus* 0.21, SD $\pm$ 0.11 receiving tricyclic antidepressant treatment), nor in the group of patients with attempted suicide (0.16, SD $\pm$ 0.05 receiving SIPSS treatment *versus* 0.19, SD $\pm$ 0.08 receiving tricyclic antidepressant treatment). In the present study, lower platelet serotonin was found in patients with depression with or without attempted suicide compared to the platelet serotonin of phenotypically healthy examinees, but with no differences between suicidal and non-suicidal patients. These result differs in relation to our previous research where lower platelet serotonin was established in depressed, non-suicidal patients compared to depressed, suicidal patients [51] and with other studies reporting lower platelet serotonin in depressed, suicidal patients compared to depressed, non-suicidal patients [41-43]. Lower platelet serotonin was also seen in suicidal patients compared to non-suicidal patients with different psychiatric diagnoses [52]. Furthermore, in other studies, higher platelet serotonin was a significant predictor for attempted suicide [53]. This discrepancy is presumably due to the fact that patients on treatment were included, and a wider range of values of platelet serotonin was observed in suicidal patients than in phenotypically healthy participants and in patients without attempted suicide. In addition, although significantly lower mean platelet serotonin values were observed in suicidal patients than in non-suicidal patients, in other studies, patients were drug-free for several weeks, and the variability of the platelet serotonin concentration was higher in the depressive, suicidal patient groups than the healthy controls or in the group of patients without attempted suicide [42, 43]. Most of the patients in this study received drug therapy, i.e. 97% (77/2) patients who attempted suicide, and 82.4% (84/17) with no history of attempted suicide. According to the published data on the efficacy of antidepressants, tricyclic antidepressants and SSRI can lower platelet serotonin to varying degrees [36-39]. We decided to include all patients without a washout period, and to explore the connection between Mg and platetlet serotonin with attempted suicide admitted to an acute psychiatric ward. The usual washout period (between one and three weeks) is insufficient for metabolic recovery and

there could be the possibility of residual effects of previous antidepressants on platelet serotonin content [54].

Recently, higher loudness dependence of auditory evoked potentials (LDAEP) was observed in patients with prior suicide attempts [55], which corresponds to another study where LDAEP was lower in acutely suicidal patients, although it increased within one week following the attempted suicide and the patient being on psychotropic agents over the course of the study [56].Taking the above into account, it can be assumed that the biochemical heterogeneity or general instability of the serotonergic system are connected with suicidality, and not simply lower platelet serotonin or deficient serotonergic neurotransmision. We believe that other biological and psychosocial covariates, not included in our research, can also influence suicidal behaviour.

A statistically significant correlation was found between platelet serotonin and Mg in phenotypically healthy participants, but not in the group of depressed patients with attempted suicide or depressed patients without attempted suicide. This positive correlation between platelet Mg and serotonin concentration points to the involvement of Mg in the functioning of the serotonergic system. Lack of correlation in both patients groups suggests an altered Mg and serotonin ratio in platelets.

Limitations of the study

When considering these results, it must be borne in mind that it is not possible to distinguish whether the results observed in medicated, depressed patients is an effect of the treatment or indeed a feature of the disorder.

Conclusions

This study showed a higher concentration of platelet Mg and lower platelet serotonin in both group of depressive patients, with or without attempted suicide, presumably because they were receiving drug treatment. In addition, a positive correlation between platelet Mg and serotonin concentration was obtained only in phenotypically healthy participants. The lower serum Mg concentrations in patients with depression and attempted suicide are connected to lower albumin concentrations. Differences were observed in serum and platelet Mg concentrations; this represents progress in our quest to establish the relationship between Mg status and serotonin.

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