

## ACTIVITY OF THE HYPOTHALAMIC-PITUITARY-ADRENAL AXIS AND INFLAMMATORY MEDIATORS IN MAJOR DEPRESSIVE DISORDER WITH OR WITHOUT METABOLIC SYNDROME

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received: 7.11.2016;

revised: 13.2.2017;

accepted: 28.2.2017

### SUMMARY

**Background:** The aim of the present study was to explore the differences in serum CRP, IL-6, TNF- $\alpha$ , ACTH and cortisol among patients with major depressive disorder with or without metabolic syndrome (MS) compared to a healthy control group.

**Subjects and methods:** The MDD study group consisted of 80 patients (mean age of 50.03 $\pm$ 9.55 years). The control group was recruited from the hospital personnel and it consisted of 40 examinees (mean age of 47.20 $\pm$ 7.99 years). All patients who participated in the study were diagnosed with depressive disorder using MINI questionnaire, and Hamilton rating scale for depression. Diagnosis of the metabolic syndrome was set by NCEP ATP III criteria.

**Results:** Examinees with depression but without MS had significantly more cortisol concentration when compared to the control group. CRP was significantly higher in the MDD group when compared to the control group and in MDD+MS group when compared to the control group. IL6 serum levels were significantly higher in the MDD group when compared to the healthy control group, and in MDD+MS group when compared to the healthy control group. ACTH had significant independent predictive values for abdominal obesity. Levels of TNF- $\alpha$  were statistically significant independent predictors for hyperglycaemia. Statistically significant predictive values for MDD were found for cortisol, and IL-6.

**Conclusion:** Results shown here emphasise the importance of neuroendocrine and inflammatory factors in pathogenesis of depressive disorder and MS. Further prospective research is necessary to clarify possible causal relationship between depression and MS. It is necessary to investigate the possibility of a joint biological mechanism in pathogenesis of these two disorders with the special attention given to the disturbances in the immune system.

**Key words:** depressive disorder - metabolic syndrome - inflammatory mediators - HPA axis - cortisol

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### INTRODUCTION

Depression and metabolic syndrome pathogenesis is complex and not well studied. However, it is known that chronic stress, psychotrauma, hypercortisolism and disrupted immunological functions can contribute to the development of these disorders (Jakovljević 2007, Lakka 2002, Mokdad 2003, Takeshita 2002, Perlmutter 2000, Anderson 2000, Martinac 2014, Babić 2007, Babić 2013). Previous research suggests that, in comparison to other psychiatric patients and general population, patients suffering from depression have a higher prevalence of obesity, are more likely to develop cardiovascular diseases, hypertension, diabetes and metabolic syndrome (MS) (Räikkönen 2002, Roberts 2003, Adamis D & Ball C 2000, Jonas 1997, Eaton 1996, Eaton 2002, Kawakami 1999, Penninx 2001, Brown 2005, Whooley 2006, Weber-Hamann 2006, Timonen 2005, Adriaanse 2006, McCaffery 2003, Herva 2006, Kinder 2004, Raikkonen 2007, Martinac 2016, Jakovljević 2007).

Approximately half of the patients suffering from depression have a high level of cortisol (Barden 2004, Khairova 2009). Chronic stress is related to the disrupted regulation of the hypothalamic-pituitary-adrenal (HPA) axis and it is also believed to contribute to the development of depression (Vitaliano 2002, Holsboer 2001, Rosmond 2005). Prolonged exposure to stress and high cortisol levels may induce such harmful consequences as atrophy or the loss of hippocampal cells and reduced proliferation of glial cells (Barden 2004, Khairova 2009, Sapolsky 2000, McEwen & Magarinos 2001, McEwen 2001). The mechanisms of negative feedback preventing hyperactivity of the HPA axis induced by stress are disrupted in depression, but the answer to the mentioned endocrine anomaly remains unresolved (Young 1991, Brown 2004). On the other hand, high cortisol levels are linked with the metabolic syndrome (MS) symptoms such as abdominal obesity and glucose intolerance and in this way depression can have an indirect effect on the MS development (Bjorn- torp & Rosmond 1999, Roberge 2007, Gold & Chrousos

2002, Tsigos & Chrousos 2002, Bruner 2002, Goldbacher & Matthews 2007, Everson-Rose 2004, Martinac 2014).

There is a lot of evidence which suggests that depression is followed by alterations in immune system, especially relating to the imbalance between pro-inflammatory and anti-inflammatory cytokines (Elenkov 2008, Hayley 2005, Smith 1991, Karlović 2012, Yirmiya 2000, Kim 2007, Sperner-Unterweger 2005, Lespérance 2004, Silić 2012). Characteristics of immune system activation in depressive disorder include increase in circulating lymphocytes and phagocytes, increased serum concentration of acute phase proteins such as CRP and increased production of proinflammatory cytokines. Proinflammatory cytokines, such as IL-6 and TNF- $\alpha$  reduce availability of tryptophan which in turn decreases 5-HT synthesis, causes positive regulation of serotonin transporter, downregulation of postsynaptic receptors, weakness, lethargy, fatigue, loss of appetite and reduced libido, suggesting potential correlation between depression and activation of inflammatory response (Schiepers 2005, Elenkov 2008, Adler 2008, Khairova 2009, Hayley 2005, O'Brien 2004, Kim 2007, Panagiotakos 2004, Ford 2005, Peitl 2015). Additionally, proinflammatory cytokines, TNF- $\alpha$ , IL-1 and IL-6 serve as a primary stimulator of the HPA axis and have a vital role in activating the HPA axis in depression (Schiepers 2005, Elenkov 2008). The concentration of proinflammatory cytokines increases during chronic stress and depression despite the hypersecretion of cortisol. This suggests that glucocorticoid receptors on immunological cells are hypofunctional in depressed patients and that cortisol is unable to suppress numerous components of cellular immunity (Elenkov 2008).

Many somatic disorders characterized by the activation of immune system are followed by depression such as acute and chronic infections and noninfective conditions related to inflammation. Symptoms of the immune system activation in these conditions are weakness, loss of appetite, weight loss, fatigue, sleeping disorders, psychomotor retardation, low interest in physical and social environment, loss of libido, disrupted cognitive abilities, dysphoria, irritability, anhedonia and depression (Schiepers 2005, Adler 2008, Khairova 2009, Hayley 2005). Furthermore, presented depression symptoms often occur in patients diagnosed with oncological, autoimmune and infective diseases followed by the increase in proinflammatory and antiviral cytokines (IL, TNF- $\alpha$ , IFN- $\alpha$ ) (Schiepers 2005, Elenkov 2008, Adler 2008, O'Brien 2004).

Pathological obesity is related to the low-degree systemic inflammation and immunological activation (Rydén & Arner 2007, Brandacher 2007, Weiss 2004, Festa 2000, Han 2002). Visceral fat produces many inflammatory mediators, and adipocytes and macrophages situated in the adipose tissue could be the main source of C reactive protein (CRP), TNF- $\alpha$  and IL-6 production (Eckel 2005, Moller & Kaufman 2005, Hotamisligil 1993, Weisberg 2003, Santos 2005, Wellen

& Hotamisligil 2003, Grundy 2002, Mertens 2001). A direct correlation between the increased synthesis of TNF- $\alpha$  in adipose tissue and resistance to insulin was found in obese people (Moller & Kaufman 2005, Rydén & Arner 2007, Brandacher 2007, Arner 2005, Maes 1997, Fernandez 2001). CRP represents an independent risk factor on all levels of metabolic syndrome severity (Han 2002, Ridker 2004), and some research suggest that there is a positive correlation between IL-6 and obesity, insulin resistance and hypertension (Weiss 2004, Glund & Krook 2008, Stenlof 2003, Maes 1997, Fernandez 2001).

Symptoms of the depressive disorder are common in patients with MS, (Everson-Rose 2004, Weber-Hamann 2006, Kinder 2004, Empana 2005, Heiskanen 2006, Saari 2005, Koponen 2008, Skilton 2007, Takeuchi 2009, Capuron 2008, Martinac 2014). Mechanisms responsible for development of depression symptoms in the patients with MS are still not explained, however the results of some studies show that chronically activated inflammatory processes in MS possibly participate in mood changes (Brandacher 2007, Capuron 2001, Capuron 2002, Capuron & Miller 2004, Musselman 2001, Capuron 2008).

In summary, the aim of the present study was to explore the differences in serum CRP, IL-6, TNF- $\alpha$ , ACTH and cortisol among patients with major depressive disorder with or without metabolic syndrome compared to a healthy control group.

## SUBJECTS AND METHODS

The MDD study group consisted of 80 patients. The research included patients of both sexes without somatic (with the exception of the metabolic syndrome), neurological or any other psychiatric disorders in comorbidity with MDD. The MDD group consisted of 46 men and 34 women with a mean age of 50.03 $\pm$ 9.55 years. Mean age of depression onset was 44.6 $\pm$ 9.17 years with the mean length of depression being 6.08 $\pm$ 4.92 years, the mean number of episodes 2.21 $\pm$ 1.98, and the mean score achieved on HDRS-17 26.65 $\pm$ 9.72. According to presence of MS, the MDD group was therefore divided into two subgroups, MDD without MS (mean HDRS was 27.50 $\pm$ 9.58), and MDD with MS (mean HDRS was 25.30 $\pm$ 9.95). The study included inpatients participants who were not given psychiatric medications for at least three months prior to the start of the study. They had no history of alcohol or other substance abuse. They were screened for acute infections by measuring the body temperature, erythrocyte sedimentation rate, and leukocyte count. All patients who were qualified for participation in the study were introduced to the objective and the aim of the study and were properly consented. The control group consisted of healthy volunteers, without somatic, neurologic or psychiatric diseases, with no history of alcohol or other substance abuse. Both sexes also gave their informed consent for participation in the study. They were screened for acute

infections by measuring the body temperature, erythrocyte sedimentation rate, and leukocyte count. The control group was recruited from the hospital personnel and it consisted of 40 examinees, 15 of which were men and 25 women, with mean age of 47.20±7.99 years, and a mean result on HDRS-17 of 3.44±2.06. The study did not include examinees who did not give their informed consent for the participation, those who have other comorbid psychiatric disorders, alcohol abuse or any other kind of psychotropic addiction, any other somatic disorder (which is not an MS component), infective disease, and those who used anti-inflammatory medicines. The study was approved by the Hospital's Ethics Committee.

Basic sociodemographic characteristics of the sample population are presented in Table 1. There were no significant differences between groups regarding age. The group which consisted of the MDD examinees without MS had more male examinees, while the group of MDD examinees with MS had more women. The control group had a higher degree of education and marriage when compared with the MDD examinees. MDD examinees with metabolic syndrome were more often unemployed or retired in comparison with examinees without MS. There were no differences in the smoking habit between the groups, but the examinees from the control group consumed alcoholic drinks more frequently.

### Diagnostic instruments

A structured questionnaire was used for the purpose of this research and it consisted of sociodemo-

graphic and anamnestic variables such as: gender, age, education, working status, marital status, and the place of residence. Education was subcategorized to elementary school, high school and university; marital status to single, married, divorced and widowed; working status to unemployed, employed and pensioner and place of residence to rural and urban. Smoking tendency was subcategorized to smoking, not smoking, and abstinent, alcohol to does not drink, drinks occasionally, drinks frequently and abstinent. Sociodemographic categorical variables were then compressed in a binary form for the purpose of logistic regressive analysis (smokes-does not smoke, drinks-does not drink, married-not married, etc.)

All patients who participated in the study were diagnosed with depressive disorder, and all other psychiatric diagnostic categories were excluded using MINI questionnaire (Mini International Neuropsychiatric Interview) (Sheehan 1998). Hamilton rating scale for depression (HDRS-17) was used as an additional diagnostic tool in diagnosing and severity of depressive disorder (0-7 no depression, 8-18 mild depression, 19-25 moderate depression, and >25 severe depression) (Hamilton 1960). The diagnosis of MDD was given if the patients fulfilled two criteria: indicative result on the HDRS-17, the total score of 8 or more and a sufficient number of symptoms shown in the structured clinical interview based on the Mini International Neuropsychiatric Interview (MINI). Both scales were performed by a trained psychiatrist.

**Table 1.** Sociodemographic characteristics in patients with major depression without metabolic syndrome (MDD), major depression with metabolic syndrome (MDD-MS), and healthy control group

| Variable             | Participants |               |                      | P       |
|----------------------|--------------|---------------|----------------------|---------|
|                      | MDD (N=49)   | MDD+MS (N=31) | Control group (N=40) |         |
| Age (M±SD)           | 49.43±8.34   | 50.97±10.84   | 47.20±8.99           | 0.204†  |
| Gender N (%)         |              |               |                      | 0.010‡  |
| Male                 | 33 (67.3)    | 13 (41.9)     | 15 (37.5)            |         |
| Female               | 16 (32.7)    | 18 (58.1)     | 25 (62.5)            |         |
| Education N (%)      |              |               |                      | <0.001* |
| Elementary           | 7 (14.3)     | 4 (12.9)      | 0 (0)                |         |
| High school          | 31 (63.3)    | 22 (71)       | 9 (22.5)             |         |
| University           | 11 (22.4)    | 5 (16.1)      | 31 (77.5)            |         |
| Marital status N (%) |              |               |                      | 0.001*  |
| Single               | 10 (16.7)    | 3 (9.7)       | 0 (0)                |         |
| Married              | 35 (71.4)    | 21 (67.7)     | 37 (92.5)            |         |
| Divorced             | 3 (6.1)      | 2 (6.5)       | 3 (7.5)              |         |
| Widowed              | 1 (2)        | 5 (16.1)      | 0 (0)                |         |
| Working status N (%) |              |               |                      | <0.001* |
| Unemployed           | 7 (14.3)     | 6 (19.4)      | 0 (0)                |         |
| Employed             | 30 (61.2)    | 11 (35.5)     | 40 (100)             |         |
| Pensioner            | 12 (24.8)    | 14 (45.2)     | 0 (0)                |         |
| Living place         |              |               |                      | 0.003‡  |
| Urban                | 30 (63.8)    | 13 (46.4)     | 34 (85)              |         |
| Rural                | 17 (36.2)    | 15 (53.6)     | 6 (15)               |         |
| Smoking N (%)        | 17 (36.2)    | 9 (31)        | 12 (32.4)            | 0.884‡  |
| Alcohol N (%)        | 8 (17)       | 5 (17.2)      | 20 (50)              | <0.001‡ |

†One way ANOVA; ‡Chi square test; \*Fisher's exact test

Diagnosis of the metabolic syndrome was set by NCEP ATP III criteria (NCEP ATP III 2001), as it was used in most of the previous research (Guy 1976). According to that definition, MS is diagnosed in examinees that had three or more of the following:

- central obesity (waist circumference >102 cm for men, >88 cm for women);
- high triglycerides ( $\geq 1.7$  mmol/L or using fibrates in therapy);
- high blood pressure (systolic  $\geq 130$  or diastolic  $\geq 85$  mm Hg or pharmacological treatment of hypertension);
- high morning level of glucose ( $\geq 6.11$  mmol/L or a previous diagnosis of type 2 diabetes);
- low HDL cholesterol (<1.04 mmol/L for men, <1.3 mmol/L for women or using fibrates).

### Clinical examination and anthropometric measurements

All examinees had their systolic and diastolic blood pressure measured with a mercury pressure gauge on their upper arm after 30 minutes of rest. Height and weight were measured with a hospital scale in light clothes without shoes. Waist circumference was measured with a tape measure on bare skin during expiration between the lower edge of the 12<sup>th</sup> rib and cristae iliace. All measurements were recorded three times and the mean was calculated for each parameter. General medical and neurological examination excluded all somatic and neurologic diseases.

### Biochemical tests

Blood samples were collected from the cubital vein into vacuum test tubes without any anticoagulant. The samples were collected around 8 AM after 12 hours of fasting, and 30 minutes of rest prior to the specimen collection. After the blood coagulated, the specimens were centrifuged at 3000 rpm, and the serum was extracted and stored at -20°C until analysis. Concentrations of glucose, cholesterol, HDL-cholesterol and triglycerides were determined using the commercial kits (Olympus diagnostics). The LDL-cholesterol concentrations were calculated from the measured cholesterol, triglycerides and HDL-cholesterol values. ACTH and cortisol levels in the serum were determined by lumimunochemical method (Roche Diagnostics GmbH, Germany). Levels of IL-6 and TNF- $\alpha$  were determined by standard ELISA procedures using commercial kits (DRG Instruments GmbH, Germany). Serum CRP was determined by using immunoturbidimetric method (BC, Co.Clare, Ireland commercial kits). Since smoking can influence the values of CRP and other biochemical parameters, we indicated the number of smokers in all three groups. We found no statistically significant difference in the number of smokers among the experimental groups (Table 1).

### Statistical analysis

Data were processed using descriptive and inferential statistical methods. Categorical variables are presented as a frequency and percentage while continuous variables, depending on the data distribution, were presented as arithmetic mean and standard deviation or as median and interquartile range. Distribution of the sample population was tested using Kolmogorov-Smirnov test. We assessed the normality of the distribution for all measures and for each group, and normal distribution could not be confirmed for the majority of variables (ACTH, cortisol, CRP, IL6, TNF- $\alpha$ ). Differences in sociodemographic variables for categorical variables were tested with a chi square test and Fisher's exact test where necessary, while one-way analysis of variance (ANOVA) was used for continuous variables. Since normal distribution for ACTH, cortisol, CRP, IL-6 and TNF- $\alpha$  could not be confirmed, one-way analysis of the variance, Kruskal-Wallis test and post hoc Kruskal-Wallis Z-test for pair-wise comparisons between groups were used as an alternative. The Bonferroni correction was used for multiple tests, using the total number of pair comparison as a correction factor, with the level of significance set at  $\alpha=0.003$ . The correlation between metabolic syndrome, depression, and metabolic and inflammatory parameters was analysed using nonparametric Spearman's rank correlation. Predicted values for the levels of ACTH, cortisol, CRP, IL-6 and TNF- $\alpha$  as continuous variables in relation to the presence of MS as dichotomous dependent variable, and predicted value of the already mentioned parameters in relation to depression as dependent binary variable, were tested using the multivariate logistic regression analysis. After conducting the nonadjusted logistic regression analysis, adjustment for confounding variables was conducted and the results were shown in the form of adjusted odds ratios (OR). According to the information from the available literature, age and gender, smoking and alcohol as determinants of lifestyle, and level of education, working and marital status as determinants of socioeconomic status were used as possible confounding variables (McCaffery 2003, Herva 2006, Kinder 2004, Skilton 2007, Takeuchi 2009, Capuron 2008, Dunbar 2008, Miranda 2005, Niskanen 2004, Wamala 1999, Miettola 2008, Viinamäki 2009, Hildrum 2009, Toker 2008, Bonnet 2005, Vogelzangs 2008). The p-value of <0.05 was taken as statistically significant for all measurements.

SPSS statistic software, version 11, was used for all statistical analyses (SPSS Inc., Chicago, IL).

## RESULTS

### Differences in levels of hormonal and inflammatory parameters between groups

Differences in serum levels of hormonal and inflammatory parameters are shown in Table 2. The Kruskal-Wallis test was used to determine the differences in

**Table 2.** Concentrations [median (interquartile range)] of adrenocorticotrophic hormone (ACTH) cortisol, C-reactive protein (CRP), interleukin-6 (IL-6), and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), in patients with major depression without metabolic syndrome (MDD), major depression with metabolic syndrome(MDD+MS), and healthy control group

| Variables             | Participants |               |                      | $\chi^2$ | p*     |
|-----------------------|--------------|---------------|----------------------|----------|--------|
|                       | MDD (N=49)   | MDD+MS (N=31) | Control group (N=40) |          |        |
| ACTH (pmol/L)         | 3.6 (2)      | 2.9 (1.8)     | 4 (2.6)              | 1.921    | 0.383  |
| Cortisol (nmol/L)     | 748.6 (549)  | 577 (351)     | 476 (152)            | 21.880   | <0.001 |
| CRP (mg/L)            | 1.4 (1.1)    | 1.5 (1.3)     | 0.7 (0.4)            | 32.149   | <0.001 |
| IL6 (pg/ml)           | 2 (0.5)      | 2 (1)         | 1 (1)                | 40.440   | <0.001 |
| TNF- $\alpha$ (pg/ml) | 5.9 (3)      | 6.5 (2)       | 5 (3)                | 2.840    | 0.134  |

\*Kruskal Wallis test

**Table 2.1.** Kruskal-Wallis Multiple-Comparison Z-Value Test (Dunn's Test)

| Cortisol      | MDD    | MDD+MS | Control group |
|---------------|--------|--------|---------------|
| MDD           | 0.0000 | 2.2900 | 4.6679        |
| MDD+MS        | 2.2900 | 0.0000 | 2.0407        |
| Control group | 4.6679 | 2.0407 | 0.0000        |

Bonferroni Test: Medians significantly different if z-value >2.3940

**Table 2.2.** Kruskal-Wallis Multiple-Comparison Z-Value Test (Dunn's Test)

| CRP           | MDD    | MDD+MS | Control group |
|---------------|--------|--------|---------------|
| MDD           | 0.0000 | 0.2508 | 5.0163        |
| MDD+MS        | 0.2508 | 0.0000 | 4.7295        |
| Control group | 5.0163 | 4.7295 | 0.0000        |

Bonferroni Test: Medians significantly different if z-value >2.3940

**Table 2.3.** Kruskal-Wallis Multiple-Comparison Z-Value Test (Dunn's Test)

| IL6           | MDD    | MDD+MS | Control group |
|---------------|--------|--------|---------------|
| MDD           | 0.0000 | 0.3259 | 5.5662        |
| MDD+MS        | 0.3259 | 0.0000 | 5.3728        |
| Control group | 5.5662 | 5.3728 | 0.0000        |

Bonferroni Test: Medians significantly different if z-value >2.3940

endocrine and inflammatory parameters between experimental groups (MDD, MDD+MS, control group). Statistically significant differences in cortisol, CRP and IL6 were found. There were no significant differences in ACTH and TNF- $\alpha$  level between the experimental groups. Kruskal-Wallis Z-test was used to determine differences between the experimental groups. Multiple comparisons showed that examinees with depression but without MS had significantly more cortisol concentration when compared to the control group (Table 2.1). There was no difference between groups of depressive examinees with or without MS, or between depressive examinees with MS and the control group. CRP was significantly higher in the MDD group when compared to the control group and in MDD+MS group when compared to the control group. There were no differences between MDD and MDD+MS groups (Table 2.2). IL6

serum levels were significantly higher in the MDD group when compared to the healthy control group, and in MDD+MS group when compared to the healthy control group. There were no differences between MDD and MDD+MS groups (Table 2.3).

### Correlation of levels of hormonal and inflammatory parameters with the level of depressiveness and metabolic syndrome

Spearman correlation test was used to determine the correlation between endocrine and inflammatory parameters with depression severity and metabolic syndrome. As shown in Table 3, there was no significant correlation between level of depression and ACTH serum level. A significant correlation was found between levels of cortisol, CRP, IL6, TNF- $\alpha$  and depression. Spearman's correlation coefficients show a moderately positive correlation between depression severity and CRP and cortisol levels. A strong positive correlation was found between depression severity and IL 6 level. A positive correlation between TNF- $\alpha$  and depression severity was on the level of lower expressed correlation. There were no significant correlations between present diagnosis of MS and ACTH levels and cortisol. Statistically significant positive correlations were found between MS and serum levels of CRP, IL6 and TNF- $\alpha$  were on level of low correlation (Cohen 1988).

**Table 3.** Spearman's rank correlation coefficients between score on Hamilton rating scale for depression (HDRS-17), presence of the metabolic syndrome (MS) and serum concentrations of adrenocorticotrophic hormone (ACTH), cortisol, C-reactive protein (CRP), interleukin-6 (IL-6), and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), in studied sample

|               | HDRS-17 |        | MS     |       |
|---------------|---------|--------|--------|-------|
|               | $\rho$  | p      | $\rho$ | p     |
| ACTH          | 0.058   | 0.556  | -0.088 | 0.354 |
| Cortisol      | 0.471   | <0.001 | -0.066 | 0.485 |
| CRP           | 0.532   | <0.001 | 0.304  | 0.001 |
| IL6           | 0.611   | <0.001 | 0.280  | 0.002 |
| TNF- $\alpha$ | 0.199   | 0.039  | 0.233  | 0.012 |

### Predictive values of endocrine and inflammatory parameters for metabolic syndrome and depression

Binary logistic regression analysis for determining independent predictive values of endocrine and inflammatory parameters for metabolic syndrome and depression was used. Statistically significant predictive values for metabolic syndrome were those of TNF- $\alpha$  (Wald=6.187; p=0.013). ACTH, cortisol, CRP and IL-6 did not have an influence. As shown in Table 4, the level of TNF- $\alpha$  was significantly correlated with the MS diagnosis in the unadjusted model. However, after the covariances of age, gender, alcohol use, smoking, education level and marital and working status were entered, the influence of TNF- $\alpha$  was lost (Wald=1.583; p=0.208). ACTH and TNF- $\alpha$  had significant predictive values of abdominal obesity. Cortisol, IL-6 and CRP did not have an influence. As shown in Table 6, levels of ACTH (Wald=10.806; p=0.001) and TNF- $\alpha$  (Wald=5.971; p=0.015) were significantly correlated with the abdominal obesity in the unadjusted model. After adjustment for age, gender, smoking, alcohol, educational level, marital and working status predictive values of ACTH stay on level of statistical significance (Wald=9.251; p=0.002) while the TNF- $\alpha$  influence is lost (Wald=0.565; p=0.452). Levels of TNF- $\alpha$  were statistically significant predictors for hyperglycaemia, while ACTH, cortisol, IL-6 and CRP did not have an influence. As shown in Table 8, levels of TNF- $\alpha$  (Wald=5.192; p=0.023) were significantly correlated with hyperglycaemia in the unadjusted model. After adjustment for age, gender, smoking, alcohol, educational level, marital and working status, TNF- $\alpha$  (Wald=5.9; p=0.015) influence remained statistically significant. Tested endocrine and inflammatory parameters did not show any statistically relevant predictive values for hypertension (Table 7), hypertriglyceridemia (Table 9) or for low level of HDL (Table 10).

Statistically significant predictive values for MDD were found for cortisol, CRP and IL-6. ACTH and TNF- $\alpha$  did not have an influence. Results of the analysis are shown in Table 5.

**Table 4.** Predictive values of hormonal and inflammatory variables for metabolic syndrome

| Variable        | Metabolic syndrome |       |                       |            |       |
|-----------------|--------------------|-------|-----------------------|------------|-------|
|                 | B                  | SE    | 95% CI for Odds Ratio |            |       |
|                 |                    |       | Lower                 | Odds Ratio | Upper |
| ACTH            | -0.104             | 0.122 | 0.710                 | 0.901      | 1.144 |
| Cortisol        | -0.002             | 0.001 | 0.996                 | 0.998      | 1.000 |
| CRP             | 0.080              | 0.074 | 0.937                 | 1.084      | 1.253 |
| IL-6            | 0.272              | 0.201 | 0.886                 | 1.313      | 1.947 |
| TNF- $\alpha$   | 0.339              | 0.136 | 1.074                 | 1.403      | 1.832 |
| TNF- $\alpha$ ‡ | 0.169              | 0.135 | 0.910                 | 1.184      | 1.542 |

R<sup>2</sup>=0.115 (Cox & Snell); 0.163 (Nagelkerke);  
Model:  $\chi^2=13.643$ , p=0.018;  
‡after adjustment for age, gender, smoking, alcohol,  
education level, marriage and working status

**Table 5.** Predictive values of hormonal and inflammatory variables for depressive disorder

| Variable      | MDD    |       |                       |            |        |
|---------------|--------|-------|-----------------------|------------|--------|
|               | B      | SE    | 95% CI for Odds Ratio |            |        |
|               |        |       | Lower                 | Odds Ratio | Upper  |
| ACTH          | -0.134 | 0.097 | 0.723                 | 0.875      | 1.058  |
| Cortisol      | 0.005  | 0.002 | 1.001                 | 1.005      | 1.008  |
| CRP           | -0.501 | 0.150 | 0.452                 | 0.606      | 0.812  |
| IL-6          | 2.773  | 0.670 | 4.310                 | 16.011     | 59.486 |
| TNF- $\alpha$ | 0.115  | 0.178 | 0.791                 | 1.122      | 1.591  |
| Cortisol‡     | 0.004  | 0.002 | 1.001                 | 1.004      | 1.007  |
| CRP‡          | 0.033  | 0.089 | 0.869                 | 1.034      | 1.231  |
| IL-6‡         | 1.575  | 0.514 | 1.764                 | 4.833      | 13.243 |

R<sup>2</sup>=0.391 (Cox & Snell); 0.547 (Nagelkerke); Model:  
 $\chi^2=55.521$ , p<0.001;  
‡after adjustment for age, gender, smoking, alcohol,  
education level, marriage and working status

Levels of cortisol (Wald=7.758; p=0.005), CRP (Wald=11.220; p=0.001) and IL-6 (Wald=17.152; p<0.001) were significantly correlated with the MDD in the unadjusted model. After adjustment for age, gender, smoking, alcohol, education level, marital and working status, cortisol influence remained statistically significant (Wald=5.517; p=0.019), and the predictive values of IL-6 remained statistically significant (Wald=9.383; p=0.002), while the CRP lost statistical significance after the adjustment (Wald=0.141; p=0.707).

**Table 6.** Predictive values of hormonal and inflammatory variables for Central obesity

| Variable        | Central obesity |       |                       |            |       |
|-----------------|-----------------|-------|-----------------------|------------|-------|
|                 | B               | SE    | 95% CI for Odds Ratio |            |       |
|                 |                 |       | Lower                 | Odds Ratio | Upper |
| ACTH            | -0.721          | 0.219 | 0.316                 | 0.486      | 0.747 |
| Cortisol        | 0.000           | 0.001 | 0.998                 | 1.000      | 1.002 |
| CRP             | 0.179           | 0.106 | 0.972                 | 1.196      | 1.472 |
| IL-6            | 0.108           | 0.227 | 0.714                 | 1.114      | 1.738 |
| TNF- $\alpha$   | 0.371           | 0.152 | 1.076                 | 1.449      | 1.951 |
| ACTH‡           | -0.626          | 0.206 | 0.357                 | 0.535      | 0.800 |
| TNF- $\alpha$ ‡ | 0.095           | 0.127 | 0.858                 | 1.100      | 1.409 |

R<sup>2</sup>=0.220 (Cox & Snell); 0.305 (Nagelkerke);  
Model:  $\chi^2=27.853$ ; p<0.001;  
‡after adjustment for age, gender, smoking, alcohol,  
education level, marriage and working status

**Table 7.** Predictive values of hormonal and inflammatory variables for Hypertension

| Variable      | Hypertension |       |                       |            |       |
|---------------|--------------|-------|-----------------------|------------|-------|
|               | B            | SE    | 95% CI for Odds Ratio |            |       |
|               |              |       | Lower                 | Odds Ratio | Upper |
| ACTH          | -0.028       | 0.063 | 0.858                 | 0.972      | 1.101 |
| Cortisol      | -0.001       | 0.001 | 0.998                 | 0.999      | 1.001 |
| CRP           | 0.017        | 0.071 | 0.886                 | 1.018      | 1.168 |
| IL-6          | -0.010       | 0.187 | 0.686                 | 0.990      | 1.430 |
| TNF- $\alpha$ | 0.218        | 0.117 | 0.989                 | 1.244      | 1.564 |

R<sup>2</sup>=0.040 (Cox & Snell); 0.053 (Nagelkerke);  
Model:  $\chi^2=4.556$ , p=0.472

**Table 8.** Predictive values of hormonal and inflammatory variables for Hyperglycaemia

| Variable        | Hyperglycaemia |       |                       |            |       |
|-----------------|----------------|-------|-----------------------|------------|-------|
|                 | B              | SE    | 95% CI for Odds Ratio |            |       |
|                 |                |       | Lower                 | Odds Ratio | Upper |
| ACTH            | 0.179          | 0.093 | 0.997                 | 1.196      | 1.434 |
| Cortisol        | -0.002         | 0.001 | 0.996                 | 0.998      | 1.001 |
| CRP             | -0.032         | 0.134 | 0.745                 | 0.968      | 1.259 |
| IL-6            | 0.280          | 0.243 | 0.822                 | 1.323      | 2.129 |
| TNF- $\alpha$   | 0.386          | 0.169 | 1.055                 | 1.471      | 2.050 |
| TNF- $\alpha$ ‡ | 0.501          | 0.206 | 1.102                 | 1.651      | 2.473 |

R<sup>2</sup>=0.106 (Cox & Snell); 0.201 (Nagelkerke);

Model:  $\chi^2=12.587$ , p=0.028;

‡after adjustment for age, gender, smoking, alcohol, education level, marriage and working status

**Table 9.** Predictive values of hormonal and inflammatory variables for Hypertriglyceridemia

| Variable      | Hypertriglyceridemia |       |                       |            |       |
|---------------|----------------------|-------|-----------------------|------------|-------|
|               | B                    | SE    | 95% CI for Odds Ratio |            |       |
|               |                      |       | Lower                 | Odds Ratio | Upper |
| ACTH          | 0.086                | 0.098 | 0.899                 | 1.090      | 1.322 |
| Cortisol      | 0.001                | 0.001 | 0.999                 | 1.001      | 1.003 |
| CRP           | 0.008                | 0.076 | 0.868                 | 1.008      | 1.170 |
| IL-6          | 0.359                | 0.221 | 0.929                 | 1.432      | 2.206 |
| TNF- $\alpha$ | 0.207                | 0.126 | 0.962                 | 1.230      | 1.575 |

R<sup>2</sup>=0.122 (Cox & Snell); 0.163 (Nagelkerke);

Model:  $\chi^2=14.594$ , p=0.012

**Table 10.** Predictive values of hormonal and inflammatory variables for low HDL level

| Variable      | Low HDL level |       |                       |            |       |
|---------------|---------------|-------|-----------------------|------------|-------|
|               | B             | SE    | 95% CI for Odds Ratio |            |       |
|               |               |       | Lower                 | Odds Ratio | Upper |
| ACTH          | -0.107        | 0.137 | 0.687                 | 0.898      | 1.175 |
| Cortisol      | -0.001        | 0.001 | 0.997                 | 0.999      | 1.001 |
| CRP           | -0.005        | 0.077 | 0.856                 | 0.995      | 1.157 |
| IL-6          | 0.381         | 0.201 | 0.988                 | 1.464      | 2.169 |
| TNF- $\alpha$ | 0.135         | 0.137 | 0.876                 | 1.145      | 1.496 |

R<sup>2</sup>=0.056 (Cox & Snell); 0.087 (Nagelkerke);

Model:  $\chi^2=6.508$ , p=0.260

## DISCUSSION

This study found significantly higher concentrations of cortisol in the MDD group, and significantly higher levels of CRP and IL6 in the MDD and MDD+MS groups when compared to the healthy control group. Significant intermediary positive correlation between cortisol levels and CRP with depression severity, and significant correlation of IL6 and low correlation of TNF- $\alpha$  with the depression severity were found. Statistically significant positive correlations were found between MS and serum levels of CRP, IL6 and TNF- $\alpha$  were on the level of low correlation. There were no significant predictors between tested hormonal and inflammatory variables for MS after adjustment for

sociodemographic variables. Analysing all components of MS in an adjusted model, there was a significant negative correlation between the development of abdominal obesity and ACTH. Hyperglycaemia was significantly correlated with TNF- $\alpha$  level. Cortisol and IL-6 levels were independent predictors for MDD.

Of all endocrine and inflammatory variables in this study, IL-6 showed the strongest predictive values for depression, ACTH was the strongest negative predictor for abdominal obesity, and TNF- $\alpha$  was independent positive predictor for hyperglycaemia. For MS, there were no significant predictive values. Pathophysiological basis for the correlation between MS and depression is complex and most likely involves numerous factors. Bjorntorp (2000) created a hypothesis that stated psychosocial factors, including depression, can activate HPA axis, producing hypersecretion of CRH, ACTH and cortisol, with consequent disposing of fat in visceral depots (Bjorntorp 1991) and secretion of inflammation mediators such as IL6 and TNF- $\alpha$  (Yudkin 2000, Hotamisligil 2003). Since this study shows no differences in ACTH levels between examined groups, while cortisol level is significantly higher in depressive groups without MS in comparison to the control group, it is possible that secretory response of ACTH to CRH is reduced because of constant high basal concentration of the cortisol in the depressive group. Constant hyperactivity of the HPA axis gradually comes to adrenocortical hyperplasia making the gland hypersensitive to ACTH. It is possible that there are other mechanisms of activation of cortisol secretion independent of ACTH with the depressive group. Regulation of HPA axis disturbed in this way can incur over activation of central noradrenergic system of stress induced by higher cytokine production and can contribute to dissociation between ACTH and cortisol in depression (Barden 2004, Khairova 2009, Chrousos 1995). In addition, cortisol can be synthesised locally by turning inactive cortisone into cortisol; this kind of local production arises primarily in visceral fat (Roberge 2007, Goldbacher 2007).

An alternative hypothesis for the relationship between MS and depression places development of central obesity and inflammatory process activation as an initial step (Hotamisligil 1993). Depression is viewed as a consequence of this immunological activation (Yirmiya 2000). In this model, the development of depression is analogue to a sick condition, which can be related to virus infections or some other causes of immune system activation (Dunbar 2008). Previous research shows that depression, similar to MS, can lead to immune system activation. Other studies report correlation between pathologic obesity and systemic, low-grade inflammation and immunologic activation (Rydén 2007, Brandacher 2007, Festa 2000). Studies show that macrophages of the fat tissue are responsible for almost complete synthesis of TNF- $\alpha$  and a significant amount of IL-6 (Eckel 2005, Moller 2005,

Weisberg 2003, Santos 2005, Wellen 2003, Grundy 2002, Mertens 2001). Other experiments deal with high levels of IL-6 in the presence of MS (Zeugmann 2010, You 2008, Reilly 2007). According to previous studies, the results of this study show high levels of CRP and IL-6 in MDD+MS in comparison to control group while there were no differences in the level of TNF- $\alpha$ . Proinflammatory cytokines were also connected with depression in several other studies (Dantzer 2008, Capuron 2008). Other experiments show high levels of IL-6 in depression (Schiepers 2005, Maes 1997, Sluzewska 1996), which raises the question of the immune system's involvement in neuropathological processes in the pathophysiology of depressive disorder (Schiepers 2005). Depressive patients show disturbances several different aspects of immunologic functioning (Hayley 2005). However, acute and chronic infections and noninfective conditions related with inflammation, as in the cytokine therapy (IL, TNF- $\alpha$ , IFN- $\alpha$ ), are correlated with depression (Schiepers 2005, Elenkov 2008, Adler 2008, Khairova 2009, O'Brien 2004). In this study, we found significantly higher values of CRP and IL6 in MDD groups when compared to the control group. There were no differences found in levels of TNF- $\alpha$ . Symptoms of mood disorders are often seen in patients with MS (Raikkonen 2002, Empana 2005), and MS is connected with high prevalence of depression (Skilton 2007, Vogelzangs 2008). Mechanisms linking depression and MS are still not clarified. According to our study, high levels of IL-6 were an independent risk factor for depression after the covariance influence was removed while the high levels of TNF- $\alpha$  were determined to be an independent risk factor for hyperglycaemia. This result supports a theory that inflammation can be at least partly responsible for the pathophysiologic processes in the complex relationship between depression and MS. According to Zeugmann et al. (2010) MS did not have an influence on any biomarkers except IL-6. We also determined that there were no differences in other immunologic parameters in MDD examinees with MS in comparison to the group without MS, but contrary to Zeugmann et al (2010) there was no difference in IL-6 levels either.

While Zeugmann et al (2010) found that there was no correlation between the severity of depression measured with the HDRS-17 and the level of inflammation, the results of our study suggest a significant positive correlation between the severity of depression measured with HDRS-17 and levels of cortisol, CRP, IL6, and TNF- $\alpha$ . Additionally, IL-6 was a significant predictor for depression using multiple regression analyses. Some other studies suggest that the hypercortisolemic depression was correlated with MS, and that the level of cortisol in depression was a predictor for MS, meaning that the hypercortisolemic depression represents a specific risk factor for MS (Weber-Hamann 2006, Vogelzangs 2008). As opposed to these studies, we found that cortisol level was not a predictor of MS

development in MDD patients but it was significantly correlated to the severity of depression in multiple regressive analyses.

The role of the HPA axis as a connection between depression and MS was stated in some other studies (Rosmond 2005, Bjorntorp & Rosmond 1999). However, these studies did not take into consideration the inflammatory parameters in the major depressive disorder. As previously stated, proinflammatory cytokines are a primary stimulator of HPA axis and they have a crucial role in activating HPA axis in depression. IL-6 is a powerful stimulator of CRH synthesis and it directly stimulates the secretion of ACTH and cortisol above the level that is reached by a maximum of CRH stimulation. During chronic stress and depression, concentrations of proinflammatory cytokines increase despite the hypersecretion of cortisol. This relates to the dysregulation of cortisol inhibitory feedback mechanism and explains why cortisol in depression is not able to suppress numerous components of cellular immunity. It is also important to note that 30-50% of MDD patients do not show any signs of HPA axis hyperactivity (Schiepers 2005, Elenkov 2008, Adler 2008). In this study, significantly higher levels of CRP and IL-6 have been found in both groups of MDD examinees in comparison to the control group. If taken into account that IL-6 is, according to this research, a significant predictive factor of depression, it can be inferred that chronic inflammation or systemic activation of the immune system is the most important common pathogenetic mechanism affecting depression and the MS.

Finally, it is necessary to look at some limitations of this study. Because of the cross sectional design of the study, the results do not directly indicate the mutual causative relationship between depression and MS, and its particular elements. They only indicate the presumable causal relationship after the elimination of influences from important sociodemographic and behavioural risk factors. Additionally, it was not possible to determine longitudinal correlation of proinflammatory condition, especially the high level of IL-6, and development of depressive symptoms and individual components of metabolic syndrome. It was also not possible to precisely determine a longitudinal relationship between functioning of the HPA axis and development of depression symptoms in the examined population. For more precise answers to these questions, further prospective research is necessary. Concentrations of cortisol were measured on a one-time basis, only in the morning, which is significantly inferior to the 24-hour cortisol monitoring in urine, especially when diurnal fluctuations in cortisol level are taken in consideration. Lastly, this study did not address the level of stress or the level of physical activity, although there is evidence that it can have an influence on inflammatory mediators (Khairova 2009, Glund & Krook 2008, Hayley 2005, Pitsavos 2005).



## CONCLUSION

Despite the stated limitations, the results of this research shine a new light on a complex relationship between depression and the metabolic syndrome and give guidelines for further studies. Results shown here emphasise the importance of neuroendocrine and inflammatory factors in pathogenesis of depressive disorder and MS. Further prospective research is necessary to clarify possible causal relationship between depression and MS. It is necessary to investigate the possibility of a joint biological mechanism in pathogenesis of these two disorders with the special attention given to the disturbances in the immune system.

**Acknowledgements:** None.

**Conflict of interest:** None to declare.

### Contribution of individual authors:

Marko Martinac, Dalibor Karlović and Miro Jakovljević have contributed to the concept of the study.

Marko Martinac and Dragan Babić were writing and drafting the work for intellectual content.

Milenko Bevanda and Ivan Vasilj participated in the design of the study.

Marko Martinac and Dalibor Karlović, participated in patient enrollment, questionnaire distribution, statistical analyses and interpretation of data.

Danijela Bevanda Glibo made literature search.

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