Potential Major Depressive Disorder Biomarkers in Pediatric Population – a Pilot Study

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Summary

Mental disorders affect 10-20 % of the young population in the world. Major depressive disorder (MDD) is a common mental disease with a multifactorial and not clearly explained pathophysiology. Many cases remain undetected and untreated, which influences patients' physical and mental health and their quality of life also in adulthood. The aim of our pilot study was to assess the prediction value of selected potential biomarkers, including blood cell counts, blood cell ratios, and parameters like peroxiredoxin 1 (PRDX1), tenascin C (TNC) and type IV collagen (COL4) between depressive pediatric patients and healthy peers and to evaluate a short effect of antidepressant treatment. In this study, 27 young depressive patients and 26 non-depressed age-matched controls were included. Blood analyses and immunological assays using commercial kits were performed. Platelet count was the only blood parameter for which the case/control status was statistically significant (p=0.01) in a regression model controlling for the age and gender differences. The results from ELISA analyses showed that the case/control status is a significant predictor of the parameters PRDX1 (p=0.05) and COL4 (p=0.009) in respective regression model considering the age and gender differences between MDD patients and controls. A major finding of this study is that values of platelet count, monocyte to lymphocyte ratio, white blood cell, and monocyte counts were assessed by the Random Forest machine learning algorithm as relevant predictors for discrimination between MDD patients and healthy controls with a power of prediction AUC=0.749.

Key words

Major depressive disorder • Adolescent depression • Potential biomarkers • Blood cell counts • Blood cell ratios

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Introduction

Globally, 10-20 % of the young population experience mental disorders. Half of the mental illnesses start by the age of 14 and 75 % by the age of 24 (WHO 2017). Depressive disorders affect 47 million children and adolescents worldwide (Polanczyk *et al.* 2015) and are among the leading causes of illness and disability in this group of patients. As many cases remain undetected and untreated or treated inappropriately, the disease influences patients' mental and physical health and their quality of life also in adulthood (WHO 2019).

A depressive pathology is multifactorial – it is a complex condition with many factors contributing to

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the course of the disease (Bernaras *et al.* 2019). One of the contributing factors for the development of depression is oxidative stress. As confirmed by a *post-mortem* analysis of depressed patients, higher levels of reactive oxygen species (ROS) and reactive nitrogen species (RNS) and altered levels of glutathione (GSH), essential nonenzymatic endogenous antioxidant, were found (Gawryluk *et al.* 2011, Maes *et al.* 2011). Peroxiredoxins are a family of peroxide reductases, the most important peroxide and peroxynitrite scavengers alongside GSH peroxidases and catalases (Poole and Nelson 2016, Adimora *et al.* 2010). Among the most vulnerable cerebral regions to oxidative stress have been included hippocampus and amygdala that together with prefrontal cortex attribute to behavioral and cognitive functions (Wang and Michaelis 2010, Patki *et al.* 2013). This enzyme family has also been proposed to play key roles in innate immunity and inflammation (Knoops *et al.* 2016).

Tenascin C was reported to be an essential factor in nervous system development, mediating neuron-glia interactions and thus contributing to the development of the hippocampal cells. As the depressive disorder is linked to hippocampal dysfunction, TNC might also contribute to the MDD pathophysiology (Ferhat *et al.* 1996, Mineur *et al.* 2013, Mokhtari-Zaer *et al.* 2017, Peng *et al.* 2018).

Recent studies show a strong association between the digestive tract and the central nervous system, also called a gut-brain axis (Peirce and Alviña 2019). Microbiota residing in the gut produce so-called short chain fatty acids. Reduction in their production, reported in mental diseases (Morris *et al.* 2018), could alter intestinal barrier function, thus compromise proper immune responses and even lead to brain dysfunction (Peirce and Alviña 2019). Type IV collagen (COL4) is a major component of basement membranes. It lies under epithelial and endothelial cells and acts as a barrier between tissue compartments (Sand *et al.* 2019).

Other hypotheses involved in the pathophysiology of the disease include neuroinflammation and neurodegeneration (Morris *et al.* 2018). Elevated proinflammatory cytokines lead to altered neurotransmitter metabolisms such as monoamine metabolism, over-expression of serotonin transporter and influence of neuroendocrine functions and synaptic plasticity (Bahrami *et al.* 2019, Morris *et al.* 2018). Blood cell counts and blood cell ratios, calculated from blood cell assays determined in simple laboratory conditions, are inexpensive and reproducible inflammation markers found to be increased in affective disorders (Mazza *et al.* 2019). Among others, neutrophil-lymphocyte ratio (NLR), platelet-lymphocyte ratio (PLR), mean platelet volume (MPV) and red blood cell distribution width (RDW) were demonstrated to be good indicators of inflammatory conditions (Uçar *et al.* 2018). The link between platelets and the disease has been investigated for many years. Platelets represent the greatest serotonin storage in a human body and even a platelet hypothesis of depression was questioned (Gialluisi *et al.* 2020, Williams 2012).

Identification of MDD biomarkers might bring deeper insights into depression pathology and improve its diagnostics, treatment and remission rate. There are only a few studies concerning potential biomarkers in adolescent depression. Some of them studied altered levels of growth factors such as brain-derived neurotrophic factor (Lee *et al.* 2020), others looked for association between inflammation and depression (Moriarity *et al.* 2020, Stumper *et al*. 2020), and there are also some metabolomics studies in children and adolescent depression (Zhou *et al.* 2019).

The aim of this study was to assess if the selected rarely used potential biomarkers based on the recent findings in research of depressive pathophysiology might be relevant predictors between the group of young MDD patients and the control group of healthy young volunteers. Another goal was to observe any pharmacotherapeutic effect on levels of these biomarkers during a short period of treatment.

Methods

Children and adolescent patients suffering from MDD were recruited from the inpatients admitted to Psychiatric Clinic of Jessenius Faculty of Medicine and University Hospital in Martin. The diagnosis of severe depression, single episode without psychotic symptoms (e.g. mood congruent or incongruent delusions, hallucinations) and possible presence of other comorbid psychiatric disorders (e.g. ADHD, anxiety disorders), was classified by thorough clinical investigation based on unstructured diagnostic interview by a staff child/ adolescent psychiatrist according to Diagnostic and Statistical Manual of Mental Disorders, DSM-V (American Psychiatric Association 2018) The severity of depression was evaluated according to profound clinical examination and Clinical Global Impression rating scale

(Busner and Targum 2007).

Two peripheral blood samples were taken at admission and during the treatment period (4^{th}-19^{th} day of antidepressant therapy in hospital) in the morning. Blood samples on the first day were collected with fasting blood before the medication administration and the second samples were withdrawn either before or after morning medicines and breakfast as the protocol changed during the study period. Fasting blood samples were also collected from young healthy volunteers in the morning hours. Immediately after the blood collection, the sample was analyzed by Mindray BC-5500 Hematology Analyzer (Mindray, United Kingdom) and plasma was then obtained and stored at -80 °C until the analysis. Blood parameters as white blood cells (WBC) and differential – neutrophils (NEU), lymphocytes (LYM) and monocytes (MONO); platelet count (PLT) plus mean platelet volume and red blood cell distribution width were evaluated, and blood cell ratios as NLR, PLR, and monocyte to lymphocyte ratio (MLR) were counted. The levels of other potential biomarkers – peroxiredoxin 1 (PRDX1), tenascin C (TNC), and type IV collagen (COL4) were measured by immunological analyses using commercial kits for enzyme-linked immunosorbent assays (Cloud-Clone Corp., USA). Hepatic functions, height and weight, and the levels of drug concentrations in blood were other clinical parameters that were observed.

Following exclusion criteria were applied: age over 19, underweight, overweight and obesity, history of cardiovascular, respiratory, endocrine, neurological, metabolic or infectious diseases, alcohol or drug abuse, smoking.

All the statistical analyses were performed in R (R Core Team, Vienna, Austria) ver. 3.5.2. $P<0.05$ was considered as statistically significant. The effect of potential influencing factors (age, gender) was considered by multivariate linear regression and the final model was selected by Akaike Information Criterion (AIC). To assess the diagnostic power of the predictors for discriminating between adolescents with MDD and controls, the data on blood parameters were fed into Random Forest (RF) machine learning algorithm. The nested cross-validation method with the graph depth as the objective function was applied for the feature selection; i.e. to discover and prioritize the relevant predictors and exclude the irrelevant ones. RF with the selected features was used to quantify the diagnostic power of the predictors *via* the ROC curve and the AUC (Area under ROC curve). The ROC curve was constructed from the out-of-bag data.

Results

In total, 27 drug-naïve hospitalized pediatric patients (19 females, 8 males) with the acute episode of the major depressive disorder were included in the study. Blood samples were taken from the patients and 26 healthy pediatric volunteers (15 females, 11 males). The median age was 15 and 17 years for MDD patients and healthy peers, respectively. The basic demographic data are displayed in Table 1. All the included patients were non-smokers and their hepatic functions were normal. The second blood sample of patients was collected on the 7^{th} day of the treatment on average. The patients were treated with a continually titrated appropriate and individual dose of either fluoxetine (a first-choice drug) or vortioxetine. Median dose on second blood collection day was 20 mg for fluoxetine and 10 mg for vortioxetine.

Table 1. Basic demographic data of the population in the study.

		MDD patients (27)	Controls (26)	Total (53)
Age	Mean	15.04	17.31	16.17
	SD	2.14	0.88	1.99
	Min	10	16	10
	Max	19	19	19
Sex	Female	19 (70 %)	15 (58 %)	34 (64 %)
	Male	8 (30 %)	11 (42 %)	19 (36 %)

Case/control status was statistically significant only in the multivariate regression model for platelet count (p=0.01, adj. R^2=0.46). Regression model predicts for a 16-year old depressed female the platelet count of 272×10^9 l^{-1} (95 % CI: 179-365), healthy female 264×10^9 l^{-1} (95 % CI: 234-294), depressed male 215×10^9 l^{-1} (95 % CI: 124-307), and healthy male 207×10^9 l^{-1} (95 % CI: 116-299). A prediction from the model is shown in Figure 1.

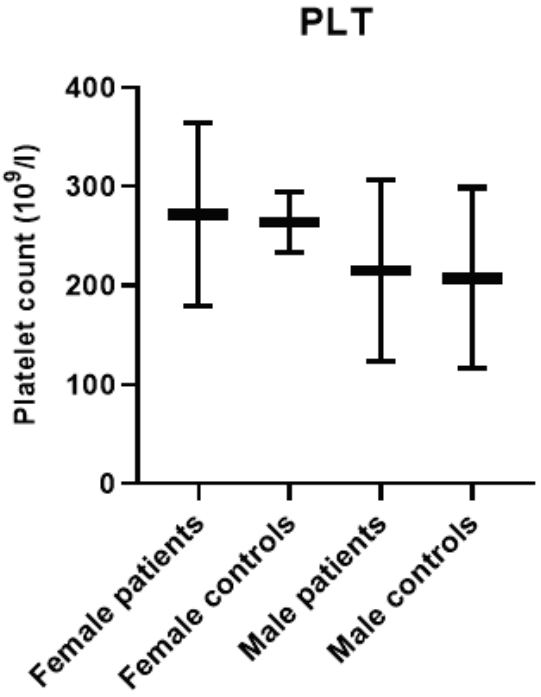

Fig. 1. Prediction from regression model of platelet count in a 16-year old adolescent.

There was a decrease in MPV during the treatment, which was highly dependent on the age of the individual – the younger patient or control, the bigger difference between MPV levels during the observed period. However, no significant difference was detected between the MPV levels of patients and controls.

According to the RF machine learning algorithm, four parameters from hemogram were selected to be relevant predictors – PLT, MLR, WBC and MONO with the diagnostic power AUC=0.749. There, the y axis gives a ranking of the predictors that were found to be important, implying that PLT is the 1st, MLR the 2nd, WBC the 3rd and MONO the 4th in the ranking. The x axis indicates the relative importance of the predictors (the smaller the minimal depth, the more important predictor), indicating that PLT and MLR are essentially equally important, and they are followed by WBC and MONO, which have substantively smaller importance. ROC curve is shown in Figure 2.

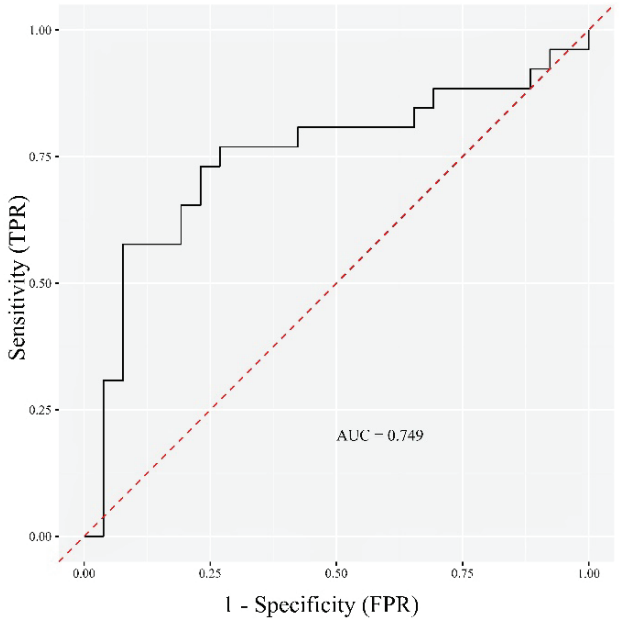

Fig. 2. Diagnostic power of the selected predictors for discrimination between MDD patients and controls by the RF algorithm depicted as a ROC curve.

The results from ELISA analyses show that the levels of parameters PRDX1 and COL4 are significantly different between the groups of patients and controls although the adjusted coefficient of determination is relatively poor – 0.08 and 0.14, respectively. Thus, PRDX1 (p=0.05) and COL4 (p=0.009) were found to be significant predictors when controlling for age and gender differences. Data are presented as a prediction from the regression model of a 16-year old adolescent in Figure 3 and Figure 4. There was not observed any significant difference between TNC levels of patients and controls nor any difference before and after antidepressant treatment in this parameter. As the levels of TNC were strictly dependent on the fact if the blood was taken before or after the meal, only the fasting blood samples were taken into consideration. Other parameters did not show any significance of before vs. after meal blood withdrawal.

Discussion

The aim of the present study was to assess if the selected potential biomarkers could be predictors between young MDD patients and healthy controls and to evaluate any difference in biomarker levels after a short antidepressant intervention during the stay in the hospital.

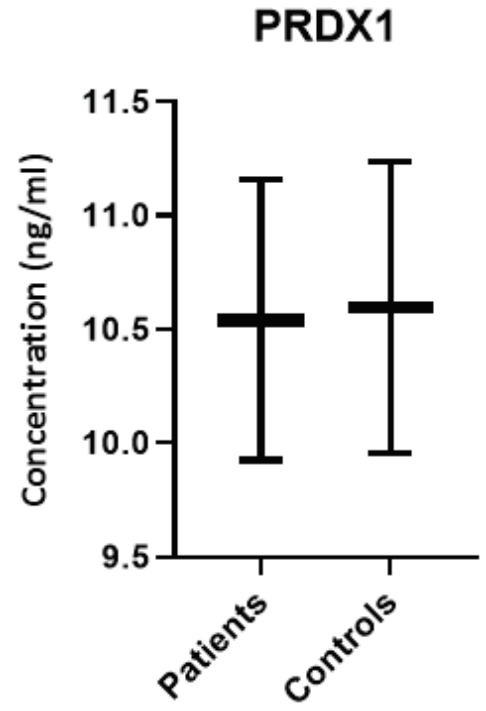

Fig. 3. Prediction from regression model of PRDX1 levels in a 16-year old depressed and non-depressed adolescent.

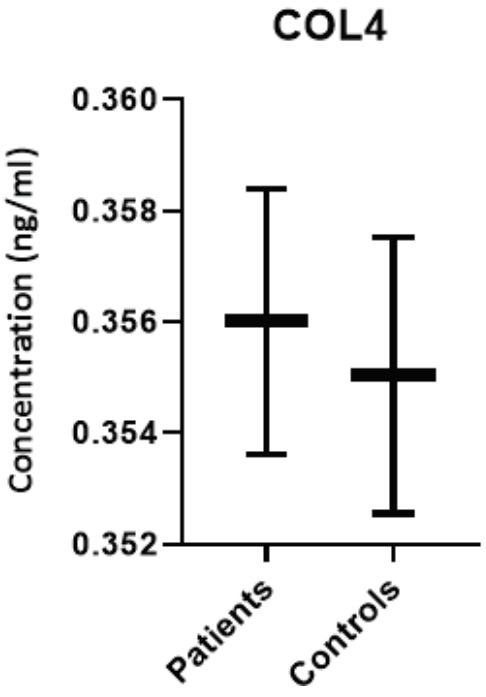

Fig. 4. Prediction from regression model of COL4 levels in a 16-year old depressed and non-depressed adolescent.

Platelets and depression

Our study reported that case/control status is a significant predictor (p=0.01) of platelet count in multivariate regression model that controls for differences in age and gender composition of the MDD and control cohorts. There is contrasting evidence of increase in platelet count levels in depressed and non-depressed individuals (Aleksovski *et al.* 2018, Bondade *et al.* 2018, Cai *et al.* 2017, Canan *et al.* 2012). However, these studies are focused also on the platelet activity related to depression, not just the platelet count itself (Aleksovski *et al.* 2018, Morel-Kopp *et al.* 2009). The study of Morel-Kopp *et al.* (2009) reported that a positive correlation exists between the level of platelet activation and severity of depression. Moreover, both antidepressant medication and psychotherapy reduced platelet activation, which denies a direct and sole effect of pharmacotherapy. This study also reported a higher platelet count of MDD patients at the beginning of observation, which is in accordance with our findings (prediction from regression model of a 16-year old adolescent). However, the check-up after 6 months did not reveal any further change in platelet count. Our study did not find any change in platelet count after a short treatment in hospital but a short observation period – on average 7 days – limits the value of these findings.

As platelet count association with MDD is controversial, other parameters were suggested as more probable biomarkers (e.g. MPV). In this study, we found a decrease in MPV after a short period of antidepressant treatment (p=0.01, adj. R^2=0.24), but it was significantly dependent on the age of an individual. Mean platelet volume is an indicator of platelet activity and it represents average platelet size (Canan *et al.* 2012). Larger platelets are enzymatically and metabolically more active than smaller ones (Karpatkin 1978). A negative association exists between MPV and PLT count that is explained as the maintenance of constant platelet mass (Canan *et al.* 2012, Thompson and Jakubowski 1988). Recent studies found elevated levels of MPV in depressed patients in comparison with non-depressed subjects (Aleksovski *et al.* 2018, Ataoglu and Canan 2009, Bondade *et al.* 2018, Cai *et al.* 2017, Canan *et al.* 2012, Öztürk *et al.* 2019). The first study also reported normalization of the levels of MPV after 8 weeks of citalopram treatment and a significant decline in platelet count. Study of Öztürk *et al.* (2019) reported a statistical decline in MPV after a period spent in the hospital. The studies suggest a strong and independent relationship between MDD and platelet activity expressed as MPV. Elevated MPV levels were also found in depressed adolescents compared to non-depressed controls (Uçar *et al.* 2018). The same authors also found significantly elevated NLR values and a positive correlation between these two factors and the

Children's Depression Scale scores.

Recently, a new potential marker related to platelets was suggested to play a role in the pathophysiology of depression. It is a platelet distribution width, a parameter reflecting the individual variation and heterogeneity of platelet size (Gialluisi *et al.* 2020). This factor was, however, not evaluated in the present study.

Discriminating predictors between MDD patients and healthy controls

In this study, relevant discriminating predictors between depressed young patients and non-depressed peers were evaluated. Among all the selected blood cell parameters and blood cell counts, four parameters are of importance in this prediction and their diagnostic power counted as AUC under ROC curve is 0.749. The most important of them is the platelet count; MLR is almost equally important and then white blood cell count and monocyte count have smaller importance. We have not found such a prediction model in the previous literature, but there were studies that used other discriminating predictors. For instance, a study of Dinga *et al.* (2018) predicted a remission course of depression with accuracy AUC=0.62 and the presence of the MDD diagnosis at follow-up with AUC=0.66 using various clinical, psychological, and biological characteristics. In the study of Köhler-Forsberg et al. (2017), low WBC counts were predictors of higher depression severity and thus an increased hospitalization rate.

Other potential biomarkers of depression

Peroxiredoxine enzymes act as cytoprotective antioxidants against oxidative stress, extracellular pathogen- or damage-associated molecular patterns, and modulate redox signaling (Knoops *et al.* 2016, Rhee 2016). Oxidative stress occurs when ROS and antioxidant systems are not in balance. This condition might be involved in neuropsychiatric diseases considering that the brain is particularly vulnerable to oxidative stress. The reason for that is a relatively low concentration of antioxidants, high level of polyunsaturated fatty acids and increased oxygen needs (Ogłodek *et al.* 2017, Salim 2017). Excessive ROS production results in protein and DNA oxidation, and lipid peroxidation; thus, cellular degeneration and functional damage may lead to altered synaptic plasticity and synaptic signaling (Salim 2017). In our study, the level of PRDX1 was a significant predictor of MDD diagnosis considering also age and gender of individuals. Ogłodek *et al.* (2017) found a significantly lower level of PRDX1 in MDD patients and they reported that with the severity of depression, the concentration of PRDX1 decreased. They also found that the levels of PRDX1 were significantly higher in female patients and controls; however, we did not confirm this in our study, probably due to small sample size. Moreover, the study of Weckmann *et al.* (2017) found out that after ketamine injection (antidepressant with a rapid effect) the levels of cytoplasmatic PRDX1 were significantly lower after 2 and 72 h. The results showed that ketamine decreased ROS, supporting the hypothesis that the main effectors in ketamine response play mitochondria energy metabolism and antioxidant defense system.

Another factor evaluated in this study as a potential MDD biomarker was tenascin C. A proteomic study of Bot *et al.* (2015) revealed that patients with current MDD diagnosis had higher serum levels of tenascin C compared to controls. Even before, higher levels of TNC had been linked to various cardiovascular diseases, tissue injury, and inflammation (Golledge *et al.* 2011). Another study suggested that TNC might be a diagnostic marker for MDD, but more clinical studies are required (Peng *et al.* 2018). They found out that significantly higher serum concentrations of TNC were measured in MDD patients compared to controls, significantly higher in patients who had attempted suicide compared to those who had not. They concluded that levels of TNC positively correlated with the risk of suicide attempts in depressive patients and also severity of depression. However, in this study, we did not reveal any significant difference between levels of TNC in depressed versus non-depressed individuals nor any significant change after a short treatment period. The small sample size and a short observation time might limit these results.

COL4 has been proposed to be a plasma marker of extracellular matrix in liver disease, especially in liver fibrosis (Santos *et al.* 2005, Veidal *et al.* 2011) and in chronic inflammatory diseases as inflammatory bowel syndrome (Koutroubakis *et al.* 2003). Our results showed that also for this parameter the case/control status is a significant predictor. To the best of our knowledge, there has not been reported any association of COL4 and depression so far. In our study, we found a positive correlation between COL4 levels in controls and increasing age ($p=0.0046$). Bigger studies are needed to confirm any linkage between type IV collagen and depressive disorder.

In conclusion, the major finding of this study is

that values of four parameters from easy and inexpensive blood analysis were assessed by the Random Forest machine learning algorithm as relevant predictors for discrimination between MDD patients and healthy controls. The most important were platelet count and MLR; WBC and monocyte count showed smaller importance. In regression modelling, the case/control status was found statistically significant predictor for PRDX1 and for COL4, though the effect size was rather small. The limitations of our study include small sample size, effect of pharmacotherapy was observed after a very short period, the fact that the second blood withdrawal was performed according to two different protocols and all the measured parameters were peripheral markers and might not reflect the mechanisms in the central nervous system.

Conflict of Interest

There is no conflict of interest.

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