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Original article

# Changes in the concentrations of inflammatory and oxidative status biomediators (MIP-1 α, PMN elastase, MDA, and IL-12) in depressed patients with and without posttraumatic stress disorder

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ABSTRACT

*Background:* Both proinflammatory cytokines and oxidative stress are considered an imbalance between the cellular production of reactive oxygen species and the antioxidant defense mechanisms. An inflammatory response that occurs in depression leads to a synergy between pro-inflammatory cytokines and oxidative stress. This synergy induces common signal transduction pathways that boost the inflammatory cascade. The object of this study was to assess the concentrations of inflammatory and oxidative status biomediators such as MIP-1α, PMN elastase, MDA, and IL-12 in depressed patients with and without posttraumatic stress disorder (PTSD), and with PTSD alone.
*Methods:* The number of participants enrolled in the study was 460. Out of them, 420 were determined to be suffering from depression, and 40 (20 males and 20 females) comprised the control group. The subjects were divided into groups, each consisting of 60 participants (30 males and 30 females) with: mild depression (MD), moderate depression (MOD), severe depression (SeD), MD and PTSD (MD + PTSD), MOD and PTSD (MOD + PTSD), SeD and PTSD (Sed + PTSD), and PTSD alone. At 7:00 a.m. all patients had blood samples collected to assess serum concentrations of the studied parameters using the Elisa method.
*Results:* Depression became more severe as the concentration levels of MIP-1α, PMN elastase, MDA, and IL-12 changed.
*Conclusion:* Studied parameters can be used as markers of chronic stress in both depression and PTSD, either comorbid or alone, to make an early diagnosis and evaluate disease severity. Revealed changes confirm the presence of a biological response in depression.


## Introduction

Both depression and posttraumatic stress disorder (PTSD) are considered multifactorial mental conditions. Major depression (MD) has been associated with various neurobiological changes such as neurotransmitter deficit, endocrine disturbance as well as impaired neural adaptation and plasticity. In many studies, neuroinflammation is described as a major factor responsible for these alterations. Both the development and progression of depressive disorder seem to be associated with chronic stress [1]. PTSD, in turn, can be linked to chronic low-grade inflammation through stress-related endocrine pathways as well as accompanying central autonomic processes. These stress-related pathways include the hypothalamus-pituitary-adrenal (HPA) axis and the sympathetic nervous system (SNS). Prolonged psychological stress has been shown to be linked to negative health outcomes, including those resulting from elevated circulating levels of inflammatory biomarkers [2]. In both depression and PTSD, corticotrophin-releasing hormone (CRH) and adrenocorticotropic hormone (ACTH) are stimulated by cytokines and chemokines, and the hypothalamic–pituitary–adrenal (HPA) axis becomes activated. Chemokine signaling plays a significant role in a variety of central nervous system (CNS) neuropathological processes in addition to its relevant role in physiological processes of neuronal migration and modulation of synaptic transmission [3–5].

The CC- and b-chemokine receptors belong to a large group of low molecular weight inducible proteins that show a wide range of proinflammatory effects *in vitro* including leukocyte chemotaxis. They contain four conserved cysteines, with the first two being connected. Macrophage inflammatory protein-1α (MIP-1α) belongs to Chemokine (C-C motif) ligand 3 (CCL-3) or ß-chemokines and has been shown to be involved in the pathogenesis of both depression and PTSD. Key functions of MIP-1α include both

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recruiting and activating mononuclear phagocytes in the CNS, as they are all sources of this chemokine. CCL-3/MIP-1α plays a key role in both depression and PTSD by recruiting T-cells, monocytes, dendritic cells, and natural killers (NK). By doing so, it participates in acute and chronic inflammatory responses [6,7].

Another marker studied was Interleukin-12 (IL-12), a heterodimeric cytokine that is formed mainly by monocytes and macrophages. It plays a major part in promoting Type 1 T helper (Th1) cells responses, and consequently, cell-mediated immunity. This is facilitated by the production of IL-12-induced interferon (IFN) from both resting and activated NK and T cells, the enhancement of NK cell cytotoxic activity, and the generation of cytotoxic T lymphocytes. Accordingly, IL-12 is considered a proinflammatory cytokine that serves an immunoregulatory function which connects intrinsic resistance with antigen-specific adaptive immunity. When IL-12 is produced at an early inflammatory stage, it allows a subsequent antigen-specific immune response that enhances Th1 T cell differentiation, while simultaneously inhibiting Th2 T cell differentiation. This shows the importance of the role of IL-12 in the cascade of cytokine regulation in depression and PTSD [1,8]. Neuroinflammation is closely related to excessive oxidative stress. The CNS is more prone to oxidative stress than other organs. The brain consumes a high amount of oxygen, lacks anti-oxidative compounds, and has a high membrane surface area-to-cytoplasmic volume ratio as well as high levels of polyunsaturated fatty acids and metal ions. Reactive oxygen species (ROS), such as superoxide, nitric oxide, and hydrogen peroxide, are highly reactive and are naturally formed as byproducts of electron transportation and energy metabolism. While ROS are responsible for enhanced cellular function, excess ROS combined with insufficient anti-oxidant activity can lead to oxidative damage [9,10].

An imbalance between the reactive oxygen and nitrogen species (ROS/RNS), production and antioxidant capacity of the body is symptomatic of oxidative stress. Alterations in the oxidative stress parameters promote neurodegenerative disorders. This is due to the targeting of different substrates in cells, which causes protein, DNA and RNA oxidation, or lipid peroxidation [11,12].

In the course of depression, neutrophils, which are part of the inflammatory and immune systems, become activated [13]. Intracellular protein breakdown in phagosomes is primarily initiated by human neutrocytes that are rich in lysosomal proteinases. Elastase is the best known of this group. Mainly found in azurophilic granules, it is actively involved in the phagocytic system of polymorphonuclear (PMN) leukocytes. PMN granulocytes utilize proteinases to decompose these agents along with tissue residues. Current research suggests that the body's response to an inflammatory stimulus is effectively mirrored by the amount of extracellular PMN elastase that is released. PMN elastase is released by activated neutrophils. Found in azurophilic granules of mature neutrophils, it is a multifunctional serine protease which can contribute to intracellular protein decay during the phagocytic process of an inflammatory response. PMN granulocytes are particularly important as they act to protect the body during an inflammatory reaction. A variety of bloodstream mediators such as cytokines, leukotrienes, and complement factors both lure and attract these cells and cause them to phagocytize and damage unnatural agents. As a result, enzymatically active PMN elastase, along with additionally generated oxidants like O2-radicals, H2O2, OH-radicals may lead to the damage of local tissue [14,15].

Malondialdehyde (MDA) is a lipid peroxidation product as well as a reliable indicator of damage to the cell membrane. As a marker of lipid peroxidation severity, it accurately reflects the degree of cellular damage resulting from depression and PTSD. High levels of MDA act as a biomarker of antioxidant defense system capability as well as oxidative damage inflicted by the ROS. Both lipid peroxidation and oxidative stress are measured by determining the concentration levels of malondialdehyde, a by-product of polyunsaturated fatty acid peroxidation and arachidonic acid. As a reactive aldehyde or reactive carbonyl compound (RCC), MDA can alter proteins to induce advanced lipoxidation end (ALE) products. Since ALE products are proinflammatory, they have detrimental effects like weakened antioxidant defense and impaired DNA repair. Increased lipid peroxidation is related to both depression and chronic stress disorders like PTSD [16,17].

There have been no studies found which assess the concentration levels of inflammatory response identifiers, namely MIP-1α, PMN elastase, MDA, or IL-12, in depressed patients of varying severity, neither with nor without comorbid PTSD. The object of this study was to assess the concentrations of inflammatory and oxidative status biomediators such as MIP-1α, PMN, MDA, and Il-12 in depressed patients with and without PTSD, and with PTSD alone. It was hypothesized that both depressed and PTSD patients would show stronger positive correlations between oxidative stress and inflammatory markers when compared to healthy subjects.

## Material and methods

The number of participants enrolled in the study was 460. Out of them, 420 were determined to be suffering from depression, and 40 (20 males and 20 females) comprised the control group. The mean age for the study group was 45.2 ± 4.5 years (range: 19–47 years). The subjects were divided into subgroups, each consisting of 60 participants (30 males and 30 females) with: mild depression (MD), moderate depression (MOD), severe depression (SeD), MD and PTSD (MD + PTSD), MOD and PTSD (MOD + PTSD), SeD and PTSD (Sed + PTSD), and PTSD alone.

All participants were enrolled between 2012 and 2016. For PTSD to be diagnosed, both the fourth and the fifth editions of the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV/DSM-V) were adopted. The DSM-IV criteria were applied to diagnose patients between 2012 and the first half of 2015. This resulted in 253 subjects comprising the study group and 20 subjects comprising the control group. Between the second half of 2015 and 2016, the DSM-V criteria were applied. In this case, 167 subjects were selected to comprise the study group and 20 subjects to comprise the control group.

Based on the DSM-V criteria, four diagnostic clusters can be used to determine the symptoms that accompany PTSD, and they include intrusion, avoidance, negative cognitions and mood, as well as alterations in arousal and reactivity. With regard to avoidance, PTSD individuals are observed to avoid trauma-related stimuli as a result of re-experiencing their traumatic events. It is also related to negative alterations in cognitions and mood. To be specific, patients are unable to recall important facts regarding traumatic events, negative beliefs and emotions, reduced affect, as well as to experience positive emotions thereafter. Trauma-related alterations in arousal and reactivity are also reported. This means that they tend to behave in an aggressive and self-destructive manner. All PTSD symptoms mentioned usually last for at least one month. In the course of personality disorders, both self and interpersonal functioning are impaired. They are also accompanied by pathological personality traits. Depression was determined using the fifth edition of the Diagnostic and Statistical Manual of Mental Disorders. Depression severity, in turn, was measured by means of the psychometric properties of the Beck Depression Inventory (BDI-II). It is comprised of twenty-one questions that relate to different depressive symptoms. In the study, the subjects were requested to answer the questions by selecting one out of

four responses that best described their emotions over the past 30 days. They could receive between 0 and 3 points for each answer. The total score that could be earned was between 0 and 63. Depression severity was classified as follows: 11 = no depression, 12–19 = mild depression, 20–25 = moderate depression, over 26 = severe depression.

Depressed patients were treated at the Department of Psychiatry between 2012 and 2016. The control group involved forty sex/age- matched healthy individuals with the mean age of 42.4 ± 4.1 years (range: 23–48 years). Patients who were excluded from the study included those with mental conditions other than studied, the CNS damage, alcohol, tobacco, or other substance dependence, as well as infectious or chronic systemic diseases. Individuals who were taking medication were also excluded from the study. With regard to females, none of them were experiencing menopause. The blood samples were collected during the follicular phase of the menstrual cycle. The blood was drawn from the median cubital vein on admission to hospital and prior to pharmacological treatment. Subjects from both study and control groups had not been on any medication for two weeks prior to blood collection.

### *Determination methods*

All study participants had blood samples collected between 7 and 9 am. Afterward, the samples were centrifuged at 3500 rpm for 10 min and pipetted into sterile 2 ml microcentrifuge tubes. The tubes were kept at −70 °C. Laboratory analysis was performed using the enzyme-linked immunosorbent assay (ELISA). The concentration levels of the following parameters were determined: MIP-1α/CCL3 chemokine (Cloud-Clone Corp., USA); PMN elastase (Immunodiagnostik, Bensheim-Germany); MDA (Cloud-Clone Corp., USA); IL-12 (Diaclone SAS, France).

The manufacturer's instructions were followed and the tests were done in duplicate. Standard solutions were added to the plate to check the results. Also, a calibration curve was prepared. The plate was coated with a monoclonal antibody specific for a particular marker (antigen). Subsequently, the plasma samples were applied to the plate and incubated. The incubation time was 60 min. After the studied antigen had bound to a specific antibody, the unbound antigens were washed away, and an enzyme-linked antibody was added. The samples were again incubated for 30 min and the plates were rinsed. After that, a substrate solution was added to produce a color reaction. Depending on the concentration levels, color intensity changed. The concentration levels were as follows: (PMN elastase), (0.011 ng/ml); (MIP-1α), (5.7 pg/ml); (MDA), (9.14 ng/ml); (IL-12), (2.2 pg/ml).

### *Ethics*

The study was approved by the Bioethics Committee. All subjects were informed about the study procedure. Afterward, they provided written informed consent. Their mental status was examined by the author of this manuscript, who is a psychiatrist.

### *Statistical analysis*

The Statistica 10.0 commercial package was used to perform a statistical analysis. First, the Shapiro-Wilk test was utilized to assess the normality of distribution. It was found that none of the analyzed parameters had a normal distribution. Afterward, a descriptive analysis was performed with a focus on the arithmetic mean, standard deviation, median, and other statistical parameters. The results are presented in Figures as the standard error of the mean (SEM).

The Mann-Whitney *U* test for the median was used to test the significance of differences in selected groups and subgroups of patients. The accepted probability level was $p < 0.05$.

The Pearson correlation coefficient was used to analyze the relationship between the parameters. A multi factor analysis of variance (ANOVA) that was performed allowed hypothesis testing. It was hypothesized that both gender and depression severity (MD, MOD, SeD) concomitant with PTSD, as well as both these factors, exert an impact on the concentration levels of studied parameters.

## Results

Based on the mean concentration levels of all four blood parameters, it was found that the differences between males and females were statistically significant ($p < 0.001$). However, it was not the case with all the groups.

For MIP-1α and PMN elastase, insignificant differences were shown between males and females from the control group. In the remaining groups, the results were reported to be statistically significant. In addition, females were observed to have higher MIP-1α concentration levels than males except for PTSD patients. Here, males were reported to have higher MIP-1α concentration levels versus females. With regard to PMN elastase, females were found to have higher concentration levels than males in all study groups except for the control group, where the differences were insignificant, (Table 1).

In the case of MDA and IL-12, significantly higher mean concentration levels were reported among females. Nevertheless, it is important to note that for MDA, the results in particular groups were not unambiguous. While in the control group as well as SeD, MOD+PTSD, SeD+PTSD, and PTSD groups, the differences were

**Table 1**
Comparative results of MIP-1α and PMN between males and females.

| Parameter | MIP-1α [pg/ml] | | | | | PMN elastase [ng/ml] | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Females (N = 230) | | Males (N = 230) | | *p*-value | Females (N = 230) | | Males (N = 230) | | *p*-value |
| Group | *M* | *SD* | *M* | *SD* | | *M* | *SD* | *M* | *SD* | |
| C | 285.55 | 9.43 | 278.08 | 12.24 | NS | 34.29 | 2.81 | 34.44 | 3.57 | NS |
| MD | 478.61 | 4.73 | 445.00 | 10.35 | $p < 0.001$ | 83.11 | 2.37 | 71.95 | 3.20 | $p < 0.001$ |
| MOD | 539.27 | 7.21 | 520.11 | 5.67 | $p < 0.001$ | 128.44 | 6.30 | 109.00 | 9.03 | $p < 0.001$ |
| SeD | 684.09 | 10.86 | 638.78 | 2.71 | $p < 0.001$ | 165.49 | 6.00 | 143.39 | 4.65 | $p < 0.001$ |
| MD + PTSD | 480.05 | 4.55 | 457.71 | 2.74 | $p < 0.001$ | 160.60 | 2.38 | 131.06 | 1.23 | $p < 0.001$ |
| MOD + PTSD | 729.28 | 40.23 | 680.86 | 4.09 | $p < 0.001$ | 114.07 | 2.07 | 105.56 | 2.45 | $p < 0.001$ |
| SeD + PTSD | 1048.66 | 40.67 | 938.26 | 3.08 | $p < 0.001$ | 242.98 | 2.71 | 195.00 | 2.48 | $p < 0.001$ |
| PTSD | 410.73 | 5.94 | 444.61 | 16.64 | $p < 0.001$ | 81.48 | 1.63 | 69.50 | 3.04 | $p < 0.001$ |

MIP-1α – macrophage inflammatory protein-1α [pg/ml]; PMN elastase - polymorphonuclear elastase [ng/ml]; C-control group, MD-mild depression; MOD-moderate depression; SD-severe depression; MD+PTSD-mild depression+posttraumatic stress disorder; MOD+PTSD-moderate depression+posttraumatic stress disorder; SeD+PTSD-severe depression+ posttraumatic stress disorder; PTSD-posttraumatic stress disorder; SD-standard deviation; M- mean.

reported to be statistically significant ($p < 0.001^{***}$), in the MD group, the probability level was at $p < 0.01^{**}$, and in the MOD group, it was at $p < 0.05^{*}$, (Table 2).

The highest MIP-1αconcentration level was observed in SeD + PTSD patients (993.43 ± 62.58 pg/ml). The lowest MIP-1α concentration level was reported in the control group (281.81 ± 11.43 pg/ml). However, slightly higher levels were found in PTSD patients (427.95 ± 21.15 pg/ml) $p\ (_{Cvs.PTSD}) < 0.001^{***}$. It it worth noting that the concentration levels in the SeD + PTSD group was 3.5 times higher when compared to the control group, (Fig. 1).

Similar findings were reported for PMN elastase. The lowest concentration level was observed in the control group (34.37 ± 3.17 ngl/ml), and the highest one in the SeD + PTSD group (218.99 ± 24.33 ngl/ml) ($p < 0.001^{***}$). In this case, however, the values failed to increase progressively in subsequent groups. PMN concentration levels dicreased in both MD + PTSD group (145.83 ± 15.01 ng/ml) and MOD group (109.82 ± 4.84 ngl/ml) $p\ (_{MODvs.MD+PTSD}) < 0.001$, (Fig. 2).

MDA concentration levels were reported to progressively increase in subsequent groups, eventually reaching the mean value of 3.71 ± 0.29 nmol/l in SeD + PTSD patients. Afterward, the mean values were reported to decrease up to 1.35 ± 0.09 nmol/l only in PTSD patients. When analyzing Fig. 3, it is necessary to look at the results by dividing depressed patients into those with comorbid PTSD and without PTSD. Here, it can be observed that the mean MDA concentrations for the subsequent severity levels increased in patients suffering from depression comorbid with PTSD, and from depression alone. This gradation of the results began at a significantly higher level among patients with depression and PTSD, (Fig. 3).

A similar observation was made for Interleukin-12. The lowest concentration level was found in the control group (6.68 ± 0.93 pg/ml). The highest IL-12 concentration levels were noted among SeD + PTSD patients (50.08 ± 4.46 pg/ml) and SeD patients (36.54 ± 4.32 pg/ml) $p\ (_{SeDvs.SeD+PTSD}) < 0.001$. The findings for the remaining groups were comparable to the other parameters. However, the gradation of the results among patients suffering from depression concomitant with PTSD began at a slightly higher level when compared to patients with depression alone, (Fig. 4).

Pearson correlation coefficient was also used to evaluate the correlations between the selected blood parameters (Table 3). The reported correlations were found statistically significant. The highest correlation was reported between MIP-1α and MDA r = 0.932 ($p < 0.05$). This means that as MIP-1α concentration levels increased, MDA levels also increased. A similar correlation was observed between MIP-1α and IL-12 (r = 0.930); ($p < 0.05$).

Based on the multi-factor analysis of variance (ANOVA) that examined depression, posttraumatic stress disorder and gender, the above findings were confirmed. The null hypothesis saying that none of the studied factors had an impact on either depression or posttraumatic stress disorder was rejected at $p < 0.001$ (F = 2415.8). It was also concluded that all levels of depression severity and posttraumatic stress disorder with and without depression had an influence on the examined blood parameters. The null hypothesis saying that gender had no impact on the disorders was also rejected at $p < 0.001$ (F = 1389.8). Therefore, a relationship between gender and the selected blood parameters was shown. Another null hypothesis saying that there is no relationship between gender and depression comorbid with PTSD was also rejected at $p < 0.001$ (F = 77.5). Thus, it is crucial to take into consideration all factors like gender, depression and/or posttraumatic stress disorder, as they all had a significant impact on the results.

## Discussion

Oxidative stress is a consequence of the body's inability to produce enough antioxidants to sufficiently neutralize the production of oxidants. This disproportion between the presence of reactive oxygen species, coupled with the failure to effectively eliminate it, leads to a number of negative consequences including lipid peroxidation as well as the oxidation of proteins and cellular components like DNA [18,19]. With regard to depression, oxidative stress occurs when neurons become vulnerable to attack by free radicals. Both ROS and cytokines are recognized as contributing factors in the onset of its etiology [20]. Pro-inflammatory cytokines include soluble proteins and peptides which are used extensively for intercellular communication. They are secreted by a variety of different immune cells and are known to participate in a number of redox processes related to depression [21,22]. As a result of faulty antioxidant defense, excess ROS exposure leads to neuron dysfunction, and ultimately, neuronal death. Furthermore, oxidative stress is reported to cause lower levels of fatty acids in the leukocyte membranes of depressed patients as well as decreased hippocampal volume in patients with major depression. Both reactive oxygen species and cytokines are perceived as significant factors responsible for the development of depression [20].

In the present study, serum concentration levels of the following parameters were measured: MIP-1α, IL-12, polymorphonuclear neutrophil (PMN) elastase – lipid peroxidation marker, and MDA. The study covered both males and females with different levels of depression severity (mild, moderate and severe), with and without PTSD.

With regard to MIP-1α, statistically significant differences between its concentration levels were reported in the groups with MD, MOD, SeD, MD + PTSD, MOD + PTSD, SeD + PTSD and PTSD

**Table 2**
Comparative results of MDA and IL-12 between males and females.

| Parameter | MDA [nmol/l] | | | | | IL-12 [pg/ml] | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Females (N = 230) | | Males (N = 230) | | *p*-value | Females (N = 230) | | Males (N = 230) | | *p*-value |
| Group | *M* | *SD* | *M* | *SD* | | *M* | *SD* | *M* | *SD* | |
| C | 0.62 | 0.10 | 0.41 | 0.10 | $p < 0.001$ | 7.34 | 0.79 | 6.02 | 0.47 | $p < 0.001$ |
| MD | 0.99 | 0.10 | 0.92 | 0.05 | $p = 0.003$ | 17.16 | 0.80 | 11.99 | 1.02 | $p < 0.001$ |
| MOD | 1.25 | 0.13 | 1.21 | 0.06 | $p = 0.042$ | 24.01 | 1.08 | 18.58 | 0.84 | $p < 0.001$ |
| SeD | 1.74 | 0.07 | 1.58 | 0.07 | $p < 0.001$ | 40.54 | 1.65 | 32.54 | 1.47 | $p < 0.001$ |
| MD + PTSD | 1.73 | 0.07 | 1.74 | 0.08 | NS | 23.79 | 1.00 | 13.28 | 1.17 | $p < 0.001$ |
| MOD + PTSD | 2.64 | 0.17 | 2.13 | 0.13 | $p < 0.001$ | 31.97 | 1.55 | 28.07 | 0.51 | $p < 0.001$ |
| SeD + PTSD | 3.97 | 0.13 | 3.46 | 0.13 | $p < 0.001$ | 54.29 | 1.99 | 46.00 | 1.03 | $p < 0.001$ |
| PTSD | 1.41 | 0.05 | 1.28 | 0.08 | $p < 0.001$ | 24.92 | 1.60 | 22.46 | 1.40 | $p < 0.001$ |

MDA – malondialdehyde [nmol/l]; IL-12 - Interleukin-12 [pg/ml]; C-control group, MD-mild depression; MOD-moderate depression; SD-severe depression; MD+PTSD-mild depression+posttraumatic stress disorder; MOD+PTSD-moderate depression+posttraumatic stress disorder; SeD+PTSD-severe depression+ posttraumatic stress disorder; PTSD-posttraumatic stress disorder; SD-standard deviation; M- mean.

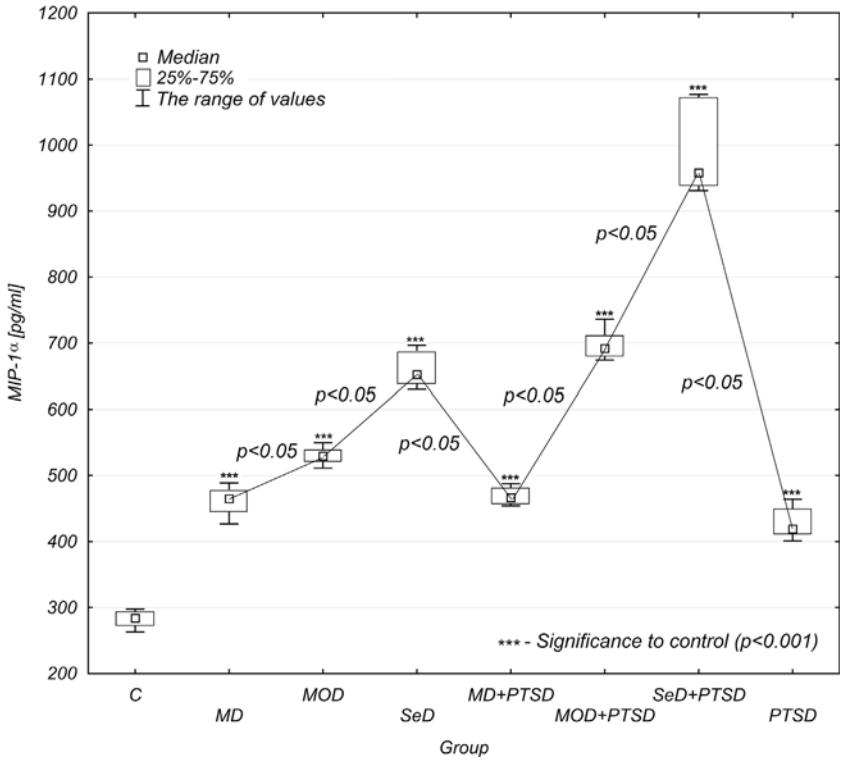


**Fig. 1.** Mean MIP-1α concentration levels in selected study groups.
MIP-1α – macrophage inflammatory protein-1α [pg/ml]; C-control group, MD-mild depression; MOD-moderate depression; SeD-severe depression; MD+PTSD-mild depression+posttraumatic stress disorder; MOD+PTSD-moderate depression+posttraumatic stress disorder; SeD+PTSD-severe depression+ posttraumatic stress disorder; PTSD-posttraumatic stress disorder.

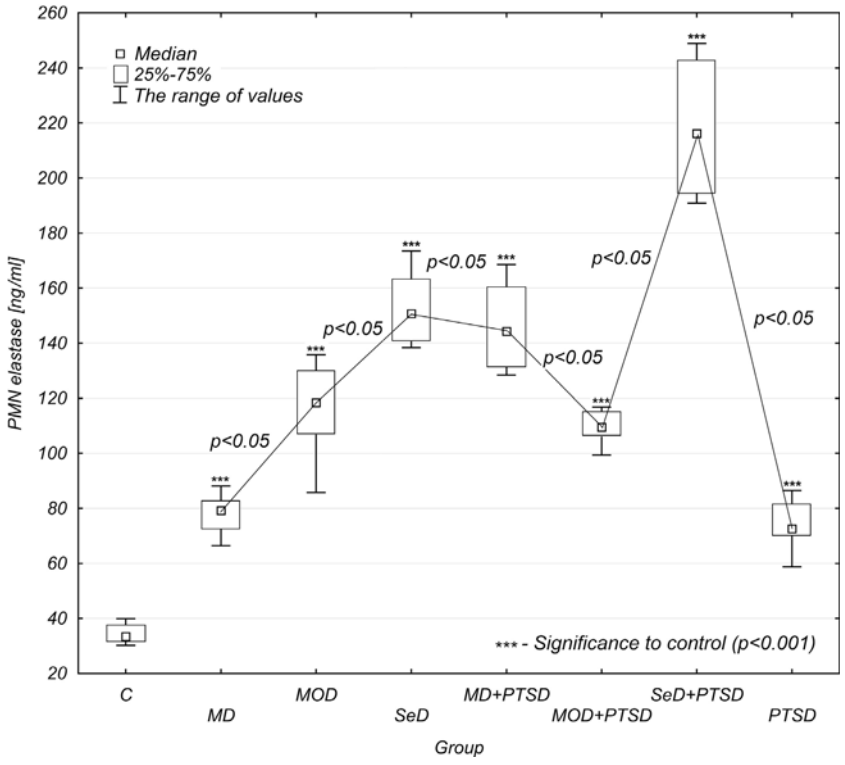


**Fig. 2.** Mean PMN concentration levels in selected study groups.
PMN elastase – polymorphonuclear elastase [ng/ml]; C-control group, MD-mild depression; MOD-moderate depression; SeD-severe depression; MD+PTSD-mild depression +posttraumatic stress disorder; MOD+PTSD-moderate depression+posttraumatic stress disorder; SeD+PTSD-severe depression+ posttraumatic stress disorder; PTSD-posttraumatic stress disorder.

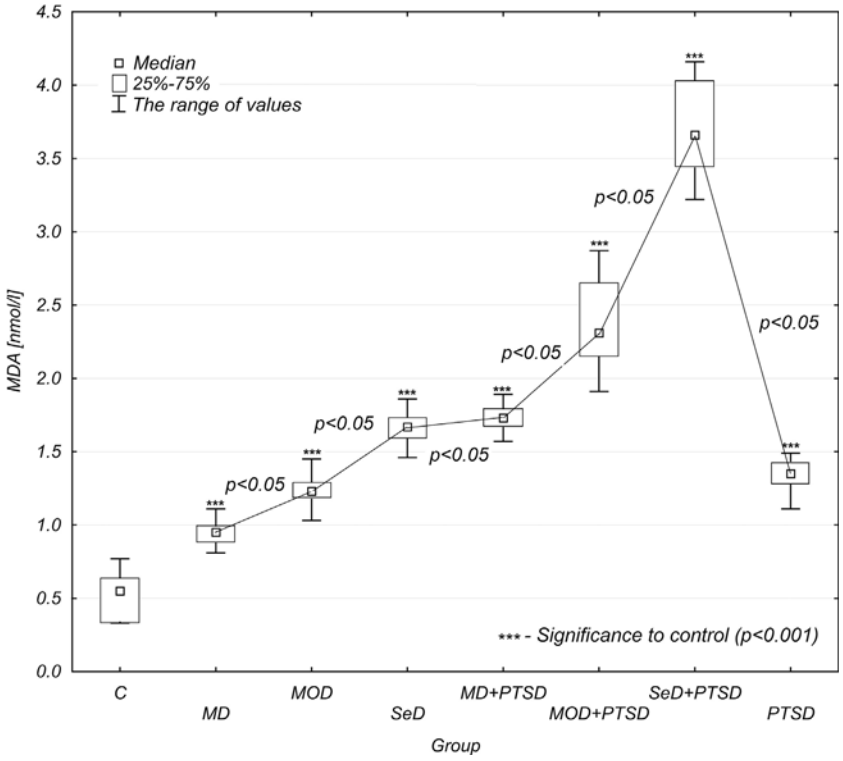


**Fig. 3.** Mean MDA concentration levels in selected study groups.
MDA – malondialdehyde [nmol/l]; C-control group, MD-mild depression; MOD-moderate depression; SeD-severe depression; MD+PTSD-mild depression+posttraumatic stress disorder; MOD+PTSD-moderate depression+posttraumatic stress disorder; SeD+PTSD-severe depression+ posttraumatic stress disorder; PTSD-posttraumatic stress disorder.

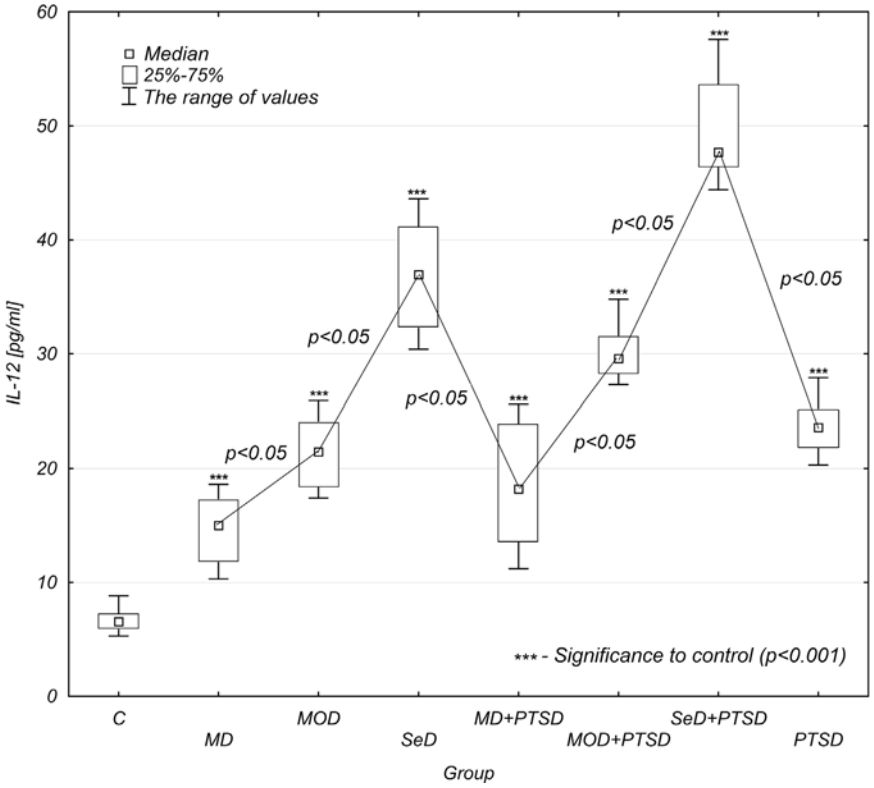


**Fig. 4.** Mean IL-12 concentration levels in selected study groups.
IL-12 – Interleukin-12 [pg/ml]; C-control group, MD-mild depression; MOD-moderate depression; SeD-severe depression; MD+PTSD-mild depression+posttraumatic stress disorder; MOD+PTSD-moderate depression+posttraumatic stress disorder; SeD+PTSD-severe depression+ posttraumatic stress disorder; PTSD-posttraumatic stress disorder.

**Table 3**
The Pearson correlation coefficient for blood parameters.

| Correlation coefficient | MIP-1α [pg/ml] | PMN elastase | MDA [nmol/l] | IL-12 [pg/ml] |
|---|---|---|---|---|
| MIP-1α [pg/ml] | 1 | | | |
| PMN elastase [ng/ml] | 0.857 | 1 | | |
| MDA [nmol/l] | 0.932 | 0.837 | 1 | |
| IL-12 [pg/ml] | 0.930 | 0.857 | 0.872 | 1 |

MIP-1α – macrophage inflammatory protein-1α [pg/ml]; PMN elastase - polymorphonuclear elastase [ng/ml]; MDA – malondialdehyde [nmol/l]; IL-12 - Interleukin-12 [pg/ml].

($p < 0.001^{***}$). As depression became more severe, the MIP-1α concentration levels increased both in depressed patients with and without PTSD. In addition, females were observed to have higher MIP-1α concentration levels than males except for PTSD patients. Here, males were reported to have higher MIP-1α concentration levels when compared to females. The highest MIP-1α concentration levels were observed in SeD + PTSD patients ($993.43 \pm 62.58$ pg/ml). The lowest MIP-1α concentration levels were reported in the control group ($281.81 \pm 11.43$ pg/ml). However, slightly higher levels were found in PTSD patients ($427.95 \pm 21.15$ pg/ml). It is worth noting that the concentration levels in the SeD + PTSD group was 3.5 times higher when compared to the control group. This was true for both males and females, (Fig. 1). It was also noted that the mean concentration levels were significantly different for all study groups ($p < 0.05$).

MIP-1α/CCL3 is traditionally considered to be a neutrophil chemoattractant. While the CNS expression of CCL3 seems to be limited to astrocytes [7], its neuronal expression is still a matter of debate [23]. This is because the CCR1/CCR5 receptors, shared with a variety of other CC chemokines, are expressed on microglia, astrocytes, oligodendrocytes and NSC/NPCs. In response to inflammation, they become upregulated, showing an increased sensitivity in response to MIP-1α/CCL3 [24]. In a study by Simon et al. [21], 49 severely depressed patients were compared alongside 49 healthy individuals, with the severely depressed patients showing higher MIP-1α concentrations versus the controls. However, the author failed to include such factors as gender, depression severity levels, or PTSD, which was examined in the present study. In a study by Geppert et al. [25], MIP-1α concentration levels were evaluated in depressed patients with comorbid Alzheimer's disease (AD). While it was shown that AD patients had lower CCL3 levels, there was a positive correlation to non-cognitive symptoms such as mood and personality changes.

With regard to PMN elastase, females were found to have higher concentration levels than males in all study groups except for the control group, where the differences were insignificant. The lowest concentration level was observed in the control group ($34.37 \pm 3.17$ ngl/ml), and the highest one in the SeD + PTSD group ($242.98 \pm 2.71$ ng/ml). Moreover, in SeD + PTSD males, the concentration levels were over 5 times higher when compared to males from the control group. SeD + PTSD females were reported to have 7 times higher levels than the female controls.

The mean value of this parameter increased for MD + PTSD patients ($145.83 \pm 15.01$ ng/ml) versus MD, SeD + PTSD and SeD patients ($218.99 \pm 24.33$ ngl/ml), and decreased for MOD + PTSD patients ($109.82 \pm 4.84$ ngl/ml) versus MOD subjects. This result is indicative of the impact that PTSD had on PMN concentration levels in the groups with MD + PTSD and SeD + PTSD, as well as the lack of (or reduced) impact in the case of MOD + PTSD patients, (Fig. 2).

A study by Değer et al. [14] analyzed the PMN elastase concentration levels in patients suffering from major depression with and without melancholia (32 subjects) and dysthymia (36 subjects). The mean PMN elastase levels were shown to be statistically higher in depressed patients without dysthymia versus healthy control subjects [14]. A study by Bekaroğlu et al. [15] also looked at PMN elastase concentration levels. It examined the differences in PMN elastase levels before and after antidepressant therapy. The study covered 40 patients with major depression and 15 patients with dysthymia. All patients had blood samples collected prior to and 3 months after the treatment. It examined the differences in PMN elastase levels before and after antidepressant therapy. After being treated with antidepressants for a 3-month period, only patients with MD showed a significant reduction in PMN elastase levels [15]. Another study by Capodicasa et al. [26] examined 39 patients (11 males, 28 females; mean age $43.2 \pm 6.02$) suffering from depression and undergoing carbonate therapy. This study assessed both polymorphonuclear leukocyte count and plasma PMN elastase levels in patients receiving chronic lithium therapy for depression.

Serum PMN granulocyte elastase concentration can reflect how severe inflammation is in depression and is a valuable indicator when monitoring therapy and evaluating disease recurrence. Leukocyte elastase is primarily localized in lysosomal granules. However, it has also been found in the nuclear membrane, the Golgi apparatus, endoplasmic reticulum and mitochondria of these cells. Granulocytes contain a large number of proteases, the role of which is to intracellularly decompose the phagocytic material. Apart from elastase, cathepsin G and many other enzymes, granulocytes are also said to produce certain amounts of gelatinases – enzymes that belong to the family of extracellular matrix metalloproteinases (MMPs). Neutrophil can be forced to release extracellular elastase by initiating an inflammatory response by pro-inflammatory cytokines and oxygen reactive species [14,16,27].

During phagocytosis of foreign substances, these enzymes are also partially excreted into the extracellular surrounding. Here, PMN elastase activity is regulated by inhibitors, such as the α1-proteinase inhibitor, α1-PI. However, α1-PI is delivered through the bloodstream and lymphatic system and ultimately forms a complex containing all extracted elastase. Thus, a correlation between the concentration of PMN elastase/α1- PI complex and released PMN elastase can be observed. This allows for measurement of granulocyte activity during an inflammatory response. However, an excessive release of PMN elastase can surpass the inhibitory potential of the α1-proteinase inhibitor [28].

With regard to MDA, no statistically significant differences between males and females were only found in the control group. However, each of the remaining groups (MD, MOD, SeD, MD + PTSD, MOD + PTSD, SeD + PTSD and PTSD) were reported to have statistically significant differences ($p < 0.05$). MDA concentration levels increased with depression severity in patients with and without comorbid PTSD (MD, MOD, SED, MD + PTSD, MOD + PTSD, SeD + PTSD), eventually reaching the mean value of $3.71 \pm 0.29$ nmol/l in SeD + PTSD patients. Subjects with PTSD alone were observed to have mean concentration levels of $1.35 \pm 0.09$ nmol/l. Moreover, severly depressed males with PTSD (SeD + PTSD) were reported to have over five times higher concentration levels than males from the control group. With regard to females with SeD + PTSD, it was seven times higher when

compared to the female controls. MDA concentration levels increased with depression severity in patients with and without comorbid PTSD (MD, MOD, SED, MD + PTSD, MOD + PTSD, SeD + PTSD), eventually reaching the mean value of $3.71 \pm 0.29$ nmol/l of SeD + PTSD patients. Increased MDA levels indicate the role of increased lipid peroxidation products, particularly with regard to severely depressed patients, (Fig. 3).

Furthermore, a number of other studies have shown increased MDA concentration levels in cases of depression. Therefore, the MDA status can effectively be used as a biomarker for oxidative stress [19]. A study by Khanzode et al. [29] found major depression to be associated with significantly increased MDA. Ozcan et al. [30] demonstrated increased concentrations of MDA in 30 patients with affective disorders when compared to 21 healthy controls. In a study by Gałecki et al. [31], the peripheral blood of patients with major depression showed elevated levels of MDA when compared to the control group. Also, it was noted that antidepressant treatment, e.g. fluoxetine reduced MDA levels. This was confirmed by Kotan et al. [32] who reported decreased MDA concentration levels after a 24-week course of antidepressant therapy. It was also observed that patients with gastric adenocarcinoma tend to have significantly increased MDA levels [33]. In a study by Sarandolet al. [34] 96 major depression patients were detected to have significantly higher MDA levels versus 54 controls.

Oxidative damage to DNA is characterized by increased levels of malondialdehyde, a byproduct of the peroxidation of polyunsaturated fatty acids and arachidonic acid. High levels of these biomarkers, coupled with significantly impaired antioxidant enzyme activity, are a feature of MDD. Lipid peroxidation is induced by elevated concentrations of proinflammatory cytokines that form free radicals and increase monoamine metabolism. Lipid peroxidation causes neuronal life span and neurofilament expression to decrease. It also results in reduced ion activity and membrane stability. Finally, it negatively influences the release of neurotransmitters, and through malondialdehyde, disrupts the connection between 5-HT and membranes. MDA is one of the most important biomarkers of oxidative stress damage to cells. In order to decrease the process of lipid peroxidation, both antidepressants and antioxidants can be administered. Because MDA inhibits the ligand-binding site of serotonin receptor cells, it affects the metabolism of serotonin [35,36].

As far as IL-12 is concerned, statistically significant differences between its mean concentration levels were reported in all study groups (C, MD, MOD, SeD, MD + PTSD, MOD + PTSD, SeD + PTSD and PTSD; $p < 0.001^{***}$). Depression severity showed a positive correlation to IL-12 concentrations in patients both with and without concomitant PTSD. The lowest mean value was noted in the control group ($6.68 \pm 0.93$ pg/ml). The highest concentration level was observed in SeD + PTSD patients ($50.08 \pm 4.46$ pg/ml), and then in SeD patients ($36.54 \pm 4.32$ pg/ml), (Fig. 4). In addition, females were found to have higher IL-12 concentration levels *versus* males.

In a study by Sutcigil et al. [8], it was found that increased IL-12 concentration levels in depressed subjects were reported to become lower after sertraline treatment. Another finding of this study, like others [37,38], was that depressed patients were observed with significantly higher plasma IL-12 levels *versus* controls and that sertraline therapy reduced these levels. The presence of elevated IL-12 levels in patients with major depression may be viewed as further evidence for the activation of a Th-1 type immune response during the course of major depression. IL-12 is a heterodimeric cytokine that is mainly formed by both monocytes and macrophages. It is essential in promoting Th1 responses, and as a result, cell-mediated immunity. This function is facilitated by the IL-12-triggered production of IFN-γ by both resting and activated NK and T cells, the enhancement of NK cell cytotoxic activity, and the generation of cytotoxic T lymphocytes. Therefore, IL-12 is a proinflammatory, immunoregulatory cytokine that connects intrinsic resistance with antigen-specific adaptive immunity [39,40].

Differences in the concentration levels of cytokines such as MIP-1α, IL-12, PMN elastase, and MDA between patients from the study groups (MD, MOD, SeD, MD + PTSD, MOD + PTSD, SeD + PTSD and PTSD) and the control group showed that as depression, both with and without comorbid PTSD, became more severe, the levels of studied parameters increased. The reason for this increase was related to the development of inflammation that occurs during both depression and PTSD. Chronic inflammation plays a significant role in the pathogenesis of depression. Cytokines in the brain are produced primarily by microglia, but can also be formed by astrocytes and, to some extent, by neurons and oligodendrocytes. Following an acute inflammatory reaction, increased CNS cytokines can protect the brain, yet under conditions of chronic immune activation, microglia can become a source of inflammatory mediators that may influence the brain neurotransmitter systems and neuronal integrity. Activated microglia can produce indoleamine-2,3-dioxygenase (IDO) and kynurenine-3-monooxygenase that catabolizes KYN, inducible NO synthase (iNOS), reactive oxygen and nitrogen species (ROS/RNS), and MCP-1/CCL2, a chemokine involved in attracting peripheral immune cells into the brain [41]. Depression is characterized by abnormalities in energetic cellular processes, which is reflected by significantly increased levels of NO. Mitochondrial hypothesis of depression is confirmed by high rates of co-morbidity with mitochondrial polymorphism, intensification of down-regulation of genes that regulate mitochondrial functions in the hippocampus and prefrontal cortex. ROS, created by the respiratory chain, are degraded by antioxidant enzymes. In situations of impaired balance of pro- and anti-inflammatory mediators and overproduction of ROS, antioxidant systems become overloaded, resulting in damage to lipids, proteins and DNA. The authors also found that there were differences in the concentrations of studied parameters in both males and females. This can indicate estrogen participation in MIP-1α, PMN elastase, MDA, and IL-12 activation processes. Estrogens inhibit neurodegenerative mechanisms that induce, among other things, the phenomenon of oxidative stress. It has been shown that the neuroprotective activity of estrogens depends to a large extent on their binding to the nuclear estrogen receptors (ER) present in the brain. Estrogen receptors belong to a large group of transcription factors [42]. Stimulated ERs attach to the corresponding DNA fragment, called the estrogen-responsive element (ERE). The ERE fragments are located in the promoter regions of many genes necessary for maintaining normal brain function, including choline acetyltransferase, proenkephalin, adrenergic receptor, serotonergic receptor, somatostatin, brain-derived neurotrophic factor (BDNF), glial fibrillary acidic protein (GFAP), and Bel-2 proteins. Estrogens, by interacting with the ERs, can selectively affect different neuronal populations. Estrogen interference in many of the metabolic pathways of these cells changes their sensitivity to degeneration-inducing factors, thereby increasing the chance of survival of these cells, and subsequently also leading to their partial regeneration [43].

## Conclusion

To sum up, based on the findings of the present study, MIP-1α, PMN elastase, MDA, and IL-12 are modified in depressed patients both with and without comorbid PTSD. This is consistent with the observation that changes in immune response are seen in both depressive and PTSD disorders. These parameters can effectively be used as sensitive biological markers to chart the course of disease activity in patients with depression.

## Conflicts of interest

The author declare that there are no conflicts of interest.

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