Research Paper
The PCSK9 Protein Is Not Necessarily a Risk Factor for Major Depressive Disorder

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A B S T R A C T

Introduction: Major depressive disorder (MDD) is one of the common psychiatric disorders that is characterized by abnormal neurobiological responses. Proprotein convertase subtilisin/kexin 9 (PCSK9) is important in cholesterol homeostasis.

Methods: This study aimed to investigate PCSK9 levels and oxidative stress with MDD disease. The study included 30 patients with MDD and 30 healthy controls. Their blood samples were collected in sterile tubes, and the serum PCSK9 concentration, superoxide dismutase (SOD), and glutathione peroxidase (GPx) activity were determined by ELISA kits. Total antioxidant capacity (TAC), total oxidant status (TOS), malondialdehyde (MDA), and copper concentration were determined manually. There was a significant increase in PCSK9 levels in the patient group (P<0.05).

Results: The receiver operating characteristic (ROC) curve with a sensitivity of 57% and specificity of 52% was 0.928 (95% CI, 0.86-0.996) for PCSK9 in the patient group (P<0.001). It was found that MDA (P=0.036) level was higher in the MDD group, but TAC (P=0.445) level, SOD (P=0.148), GPx (P=0.019) activities, and copper concentration were lower in the patient group compared with the control group.

Conclusion: The study results confirm the relationship between oxidative stress and MDD and also suggest a link between PCSK9 and MDD disease.

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Highlights

● In major depressive disorder (MDD) individuals, there is a significant increase in proprotein convertase subtilisin/kexin 9 (PCSK9) levels.

● Total antioxidant capacity, superoxide dismutase (SOD), glutathione peroxidase (GPx) activities, and copper concentration levels were lower in the MDD group.

● Total oxidant status (TOS) and malondialdehyde (MDA) were significantly higher in the MDD group.

● Oxidative stress contributes to the pathogenesis of MDD.

Plain Language Summary

MDD is one of the common psychiatric disorders in the world which alone accounts for the largest share of non-fatal diseases. These disease is caused by the complex interaction of social, psychological and biological factors, which biologically can be mentioned as an increase in oxidative stress and a disruption in the activity of inflammatory pathways. One of the factors related to oxidative stress is protein factor proprotein convertase subtilisin/kexin type 9 (PCSK9) with enzyme activity that plays an important role in regulating cholesterol homeostasis. Today, the presence of early diagnosis is very effective in reducing the progressive prevalence of these diseases. Many studies have investigated the relationship between PCSK9 levels and mental illnesses such as schizophrenia and bipolar disorder, but so far no studies have been recorded regarding the relationship between this protein factor and MDD. The present study was designed to answer the question whether MDD, like other mental disorders, is related to PCSK9 or not. 25 people with MDD and 25 healthy people participated in this research. The results of this study showed that oxidative stress has an effective role in the occurrence of MDD and it shows the relationship between PCSK9 and the disease; However, more studies are needed in this regard.

1. Introduction

Major depressive disorder (MDD) is a common psychiatric disorder worldwide. It has a prevalence rate of more than 35% in most countries' populations (Liu et al., 2021). It was reported that MDD, due to its progressive nature, commonly causes disability in subjects associated with low activity, limited social function, and high disease burden (Grases et al., 2019). MDD affects sleep patterns, appetite, perception, emotion, energy balance and mood changes, even suicidal thoughts (Michel et al., 2012). Many studies support that oxidative stress and elevated inflammation are the underlying pathophysiology of MDD and are commonly found in depressed individuals (Echeverria et al., 2016). The mechanisms by which an imbalance between oxidants/antioxidants may lead to depressive symptoms are yet to be clarified. However, it was previously reported that the brain cells are very sensitive to elevated oxygen free radicals (Liu et al., 2015). Increasing the oxidant agents to the antioxidant defense system may contribute to oxidative damage in brain cells and

necrosis (Van der Vliet et al., 1999). It was also found that elevated reactive oxygen species (ROS) generation in brain cells with a weakened antioxidant defense system causes major damage to the cell membrane lipids, proteins, and DNA, leading to cellular dysfunction and illness (Hasler et al., 2007). Oxidative stress is connected to several brain cell receptor factors, such as serotonin, gamma amino butyric acid (GABA), and glutamate (Ten Have et al., 2018). Notably, one of the proteins associated with oxidative stress is the protein factor proprotein convertase subtilisin/kexin type 9 (PCSK9). PCSK9 was expressed in various tissues, such as the central nervous system (CNS), kidney, pancreas, and intestine (Seidah et al., 2014; Seidah et al., 2003).

Accordingly, PCSK9, as an intracellular factor, plays an essential role in cholesterol metabolism by regulating low-density lipoprotein receptors (LDLR) in the membrane (Stoekenbroek et al., 2019). The LDLR is the key cell surface receptor for cholesterol trafficking and uptake, which is achieved by intaking low-density lipoprotein LDL-C and mediating its metabolism. *LDLR* gene expression is tightly regulated via cholesterol avail-

ability, which functions through a negative feedback mechanism (Schlüter et al., 2017). The PCSK9 has an enzymatic function that effectively governs the post-transcriptional function of LDLR, and possibly, over-expressed PCSK9 might induce cholesterol accumulation. Moreover, large-scale population studies revealed that PCSK9 is commonly associated with inflammatory and neurodegenerative diseases (Kurata et al., 2013). Accumulated LDL due to PCSK9 overexpression in neurons is usually linked to the generation of oxidized LDL, thereby causing oxidative damage in neurons. As a result, damage in neurons contributes to mental disorders, including MDD (Need et al., 2012). Although many studies have suggested a potential link between PCSK9 and several mental diseases, including schizophrenia and bipolar disorder, the possible link between PCSK9 and MDD has not yet been investigated (Shi et al., 2008). Therefore, the present study was designed to provide an appropriate answer to whether PCSK9 is associated with MDD disease.

2. Materials and Methods

Participants and sample collection

A total of 30 healthy individuals and 30 patients with MDD were selected after assessing the characteristics of depression by the Hamilton depression rating scale (HDRS) in Iran. Participants received written and oral information about the study and were matched in terms of age, body mass index (BMI), and sex in two groups. The inclusion criteria for participants with MDD were as follows: new case to be diagnosed by a psychiatrist, have an HDRS index above 17 in patients, ages between 18-65, non-use of selective serotonin reuptake inhibitors (SSRIs), no smoking, alcohol, and chronic physical illness such as epilepsy, autoimmune diseases, neoplasia, and infectious diseases. The exclusion criteria were taking lipids-balance medications, ethanol and drug addiction, hyperlipidemia, nephritic syndrome, infectious diseases, mental disorders, and taking antioxidant and vitamin medications from the previous month. From all control and MDD individuals in a fasting state (12 h overnight), 10 mL blood samples were obtained. Then, serum samples were separated inside sterile tubes and stored at -20 °C.

Lipid profile

The total cholesterol (TC), triglycerides (TG), and high-density lipoprotein cholesterol (HDL-C) of serum were measured by a Hitachi 7150 automated analyzer (Hitachi, Japan). Low-density lipoprotein cholesterol

(LDL-C) concentration was calculated by the formula LDL-C=TC-[HDL-C+TG/ 5], and for VLDL-C calculation, the Nobert formula was used (VLDL-C=TG/5)

Measurement of PCSK9 protein concentration

The ELISA method was used to measure the serum PCSK9 protein concentration according to the commercial kit (ZellBio GmbH, Germany) protocol and by Elisa Reader (Synergy HTX, Biotech, USA).

Measurement of total antioxidant capacity (TAC)

TAC measurement was performed using a ferric-reducing antioxidant power assay (FRAP). In this method, the antioxidant reduced ferric ions to ferrous ions. At first, FRAP reagent, including acetate buffer (25 mL, 300 mM, pH=3.6) with 16 mL acetic acid, was prepared. To obtain 2,4,6-tripyridyl-s-triazine (TPTZ) (2.5-mL), TPTZ (10 mM), HCl (40 mM), and $FeCl_3 \cdot 6H_2O$ (2.5 mL) were used. Then, 300 μL reagent was added per 10 μL of samples and incubated at 37 °C for 10 min. The maximum absorption of the complex among Fe^{2+} and TPTZ was measured by a spectrophotometer (Bell-Italy) at a wavelength of 532 nm (Mohseni et al., 2020).

Measurement of total oxidant status (TOS)

TOS measurement was assayed by ferrous oxidation in xylenol orange. After oxidation, the ferric ions can react with xylenol orange, creating a colored dye in an acidic medium. Finally, the absorption of the complex was measured at a wavelength of 560 nm (Asadi et al., 2019).

Measurement of malondialdehyde (MDA) level

This method was based on the thiobarbituric acid (TBA) (Merck, Germany) reaction with MDA as a production of lipid peroxidation. First, 1.5 mL of 20% acetic acid and 0.2 mL of 8.1% SDS were mixed gently for 50 μL of the plasma sample. After adding TBA and distilled water (4 mL), the solution was incubated in boiling water at 90 °C for 60 min. Then, 3 mL of n-butanol was added and centrifuged at 3000×g for 15 min. Finally, the absorbance of the organic layer was measured by fluorometric assay (Jasco, Japan). The fluorescence intensity was measured at the stimulus wavelength of 515 nm and the wavelength of 553 nm emission (Mazloomi et al., 2020).

Superoxide dismutase (SOD) enzyme activity

Serum SOD enzyme activity was measured by the ZellBio kit (ZellBio GmbH, Germany). This method

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converts superoxide radicals into hydrogen peroxide and oxygen under enzymatic reaction. Finally, the colored compounds from this enzymatic reaction were measured at 420 nm according to the U/mg protein unit.

Glutathione peroxidase (GPx) enzyme activity

The serum GPx enzyme level was measured using the ZellBio kit (ZellBio GmbH, Germany). In this method, the GPx enzyme, as an electron donor, reduces the selenocysteine in the active site by the glutathione. The enzyme converts glutathione to oxidized glutathione, and the remaining GSH in the reaction medium can regenerate DTNB and produce a yellow color. The yellow complex has absorption at 412 nanometers, according to the instruction. Color production is inversely related to enzyme activity. Finally, the GPx activity was reported as a U/mg protein unit.

Measurement of copper concentration

First, standard copper solutions were made and incubated for 1 hour at 20 °C. Then, 1 mL of lanthanum chloride solution (Sigma-Aldrich Co, Steinheim, Germany) was added to each 10 mL of standard solution to eliminate the interference of anions such as sulfate, nitrate, and phosphate. In the ionization process, the pH of the solutions was reduced to 2 by HCL. The regulatory specifications of the British atomic absorbing device (Thermo Fisher Scientific, Paisley, UK) were adjusted based on the cathode ray tube, acetylene flammable gas, air oxidizer, and wavelength of the emission equal to 422.7 nm, which eventually led to the reduction of copper. The samples are pH=2 before measurement and then filtered

by a 0.22-µm filter, and the absorption of the samples is measured and quantified by drawing a standard curve (Maduray et al., 2017).

Statistical analysis

Statistical analysis was performed using SPSS software, version 16 (SPSS Inc. Chicago, USA). Based on the results of the Kolmogorov-Smirnov test, the t-test was done to compare differences between study groups. The receiver operating characteristic (ROC) curve analysis was performed to investigate the PCSK9 factor. The Pearson test was used to investigate the relationship between the factors. Results were expressed as Mean±SD. The significance differences of all tests were considered at P<0.05.

3. Results

Demographic characteristics of the patients

The baseline clinical and biochemical characteristics of the patients are presented in Table 1. The MDD and control groups were similar in terms of mean age (P=0.423), as were the gender and BMI ratios between the two groups (P=0.147, P=0.085, respectively). TC and LDL-C levels increased in the MDD group (P=0.026, P=0.019, respectively). However, the two groups had no significant differences in TG and HDL-C levels (P=0.312, P=0.148, respectively).

Assessment of PCSK9 serum levels

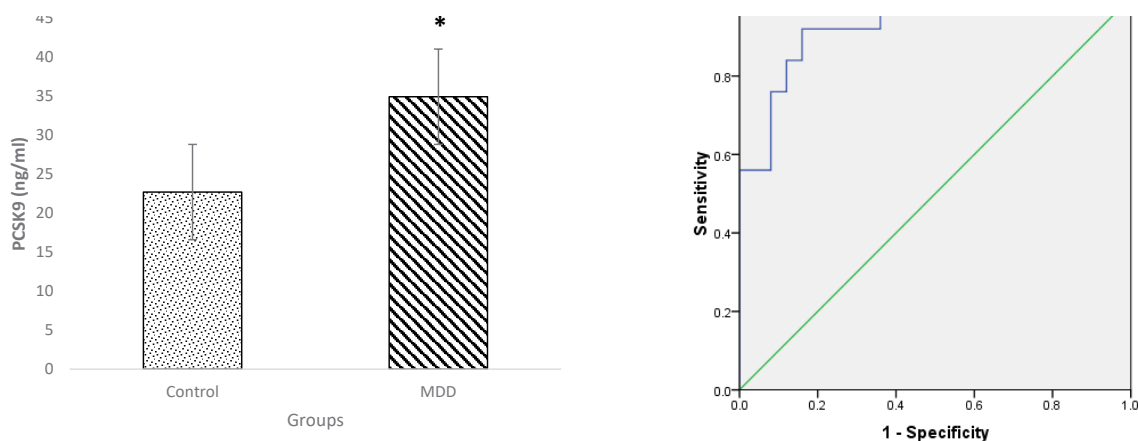According to Figure 1, the serum level of PCSK9 protein increased significantly in the patients (P<0.001).

Figure 1. A)The concentration of PCSK9 protein in the patient group compared to the control group shows a significant increase (P<0.001), *Significant differences (P<0.05)

B) The results of the analysis the ROC diagram in PCSK9 factor for MDD group [0.913 (95% CI, 0.88%, 0.975%)

Based on ROC curve analysis, the PCSK9 protein factor could be considered a diagnostic factor for MDD. The area under the curve (AUC) of PCSK9 in the MDD group and control group was obtained as 0.928 (95% CI, 0.86%, 0.996%) (Figure 2).

Evaluation of TAC, TOS, and MDA

Based on the analysis, TAC showed no differences among the study groups (P=0.445). In addition, the TOS level in the MDD group did not increase significantly compared to the control group (P=0.207) (Table 2). A significant increase in MDA as a most common indicator of oxidative stress was observed in the MDD group (P=0.036).

SOD and GPx activity

The results of SOD and GPx enzyme activity are illustrated in Table 2. As seen, the SOD activity levels between MDD and control groups were not significantly reported despite the decrease in the patient group (P=0.148). It was also found that GPx enzyme activity was significantly decreased in the MDD group (P=0.019).

Copper

The mean serum concentrations of copper ions in study groups showed that copper levels were significantly associated with the diagnosis. In fact, the serum concentrations of copper ions were significantly lower in the patient group (P=0.012) (Table 2).
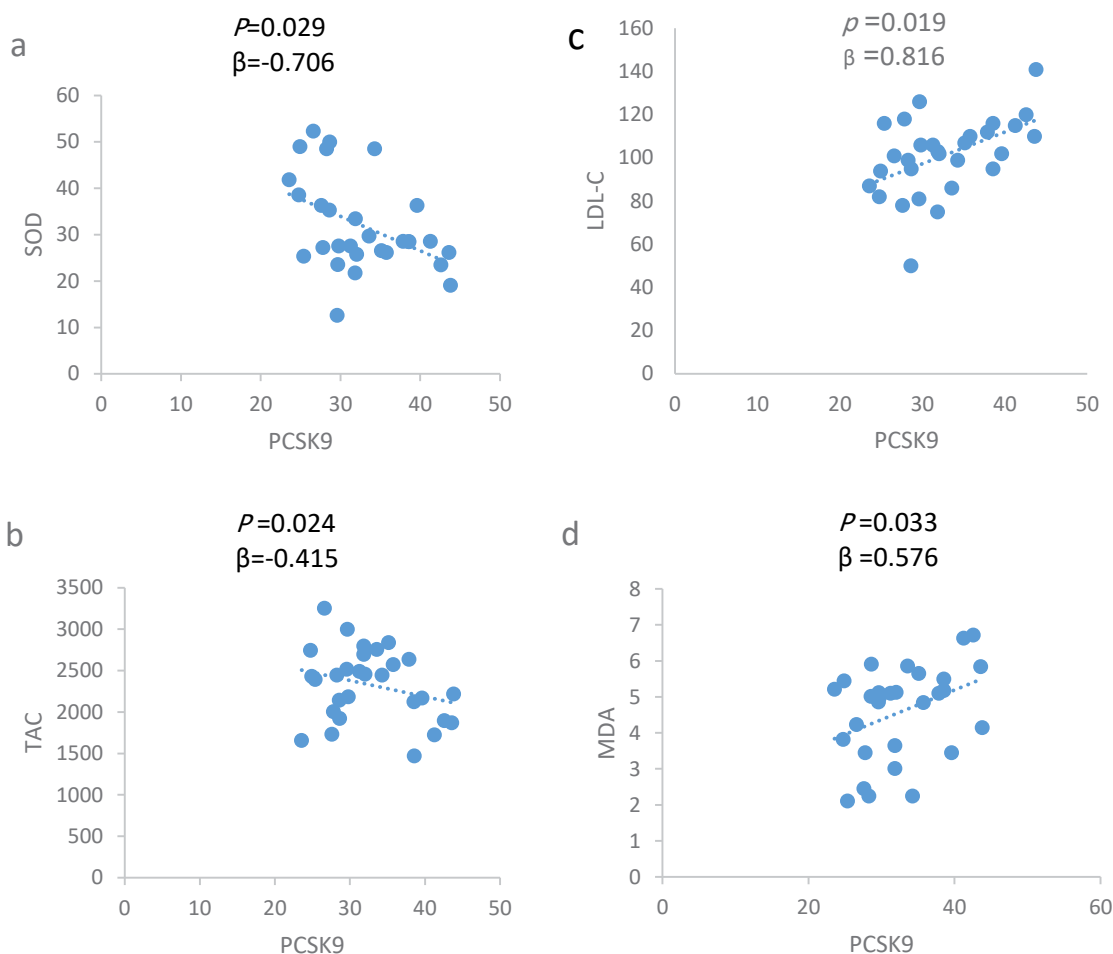

Figure 2. Association analysis of PCSK9 with LDL-C, SOD, MDA and TAC serum levels in the control group

Notes: According to association analysis, the PCSK9 variable had a negative association with SOD (a), and TAC (b), but in this group, there was a significant positive association between PCSK9 level and parameter of LDL-C (c) and MDA (d). Control group variables showing a normal distribution and are given as Mean±SD.

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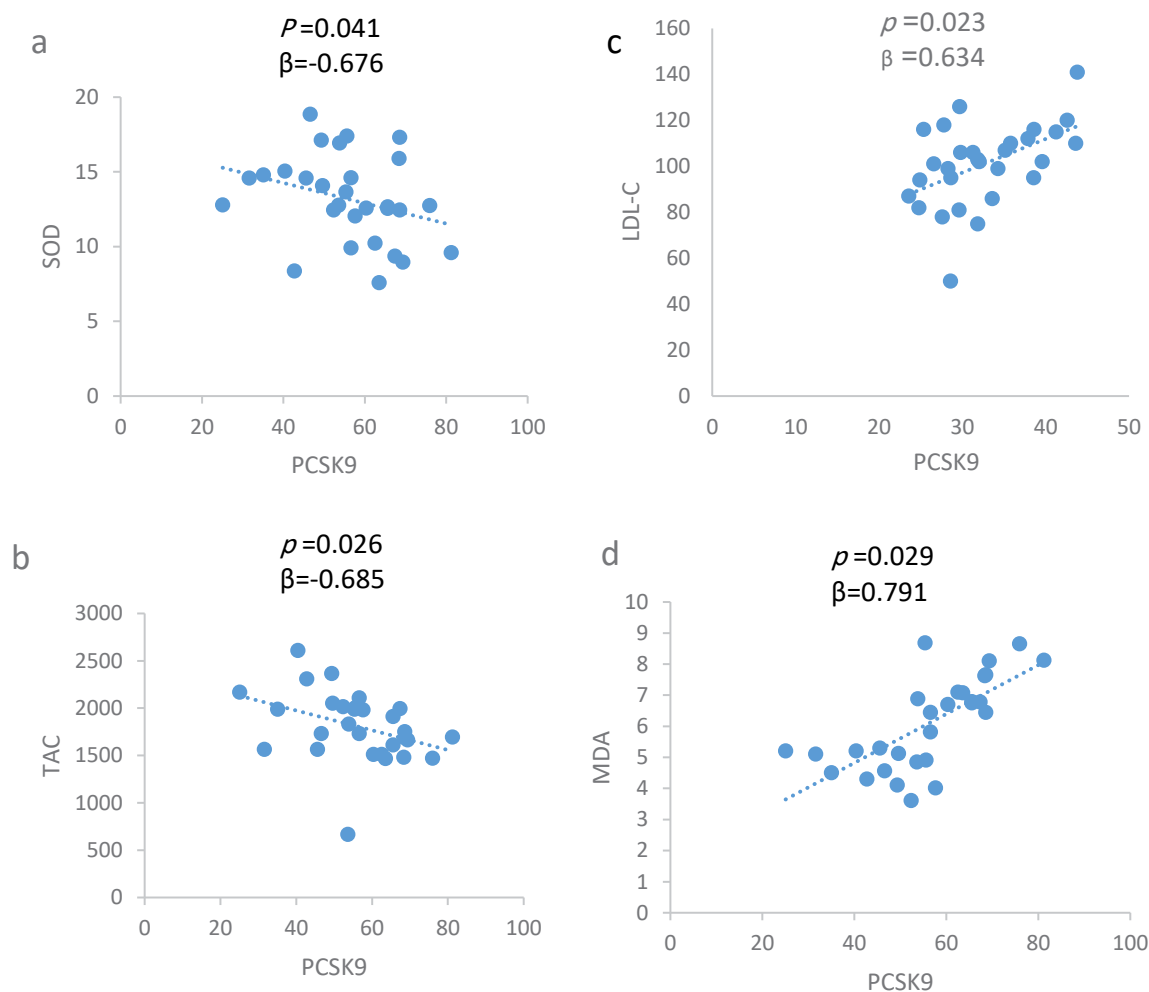
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Figure 3. Association analysis of PCSK9 with LDL-C, SOD, MDA and TAC serum levels in the MDD group

Notes: According to association analysis, the PCSK9 variable had a negative association with SOD (a), and TAC (b), but in this group, there was a significant positive association between PCSK9 level and parameter of LDL-C (c) and MDA (d). MDD group variables showing a normal distribution and are given as Mean±SD.

Association analysis of PCSK9 with LDL-C, SOD, GPx, TOS, and TAC serum levels

The association analysis showed that in the control group (as shown in Figure 2), the PCSK9 had a significant negative association with SOD (P=0.029, β=-0.706), GPx (P=0.044, β=-0.002), Copper (P=0.044, β=-0.738) and TAC (P=0.024, β=-0.615). There was a significant positive association between PCSK9 and LDL-C (P=0.019, β=0.816), MDA (P=0.033, β=0.576), and TOS (P=0.010, β=0.473). In addition, in the patient group, there was a significant negative association between PCSK9 with SOD (P=0.029, β=-0.676), GPx (P=0.037, β=-0.015), copper (P=0.034, β=-0.726), and

TAC (P=0.026, β=-0.685). But in this group, there was a significant positive association between PCSK9 and LDL-C (P=0.023, β=0.634), MDA (P=0.029, β=0.791), TOS (P=0.044, β=0.399) (Figure 3).

4. Discussion

We examined serum levels of PCSK9 as an oxidative stress indicator in individuals with MDD. In addition, oxidative stress factors, including TAC, TOS, MDA, and the activity of antioxidant enzymes SOD and GPx, as well as copper ion concentration were assessed. The association between oxidative stress and MDD has already been confirmed in several studies (Moylan et al.,

Table 1. Comparison of baseline clinical characteristics between MDD group and control group

Variables	Mean±SD		P
	Control (n=30)	MDD (n=30)	
Age (y)	7.53±34.4	9.69±32.6	0.423
Gender(male/female)	17/15	13/15	0.147
BMI (Kg/m²)	2.3±24.66	3.5±23.32	0.085
TG (mg/dL)	14.71±151.36	10.79±162.29	0.312
TC (mg/dL)	142.20±11.51	170.75±12.53	0.026[*]
HDL-C (mg/dL)	36.08±5.77	37.52±4.99	0.148
LDL-C (mg/dL)	95.73±12.65	123.87±13.70	0.019[*]

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BMI: body mass index; TG: triglyceride; TC: total cholesterol; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol.

*Significant differences (P<0.05).

2013; Gałecki et al., 2009). In the present study, TAC was reduced in the MDD group compared to the control group, but the change was not statistically different. Also, the TOS index increased in the patient group compared to the control group without significant differences. In addition, a significant increase in MDA levels was observed in the patient group compared to the controls, parallel with lipid peroxidation. Inconsistent with our finding, Sarandol et al. reported a significant decrease in TAC levels. They also indicated that TOS and MDA levels increased significantly in the serum of the MDD patients compared with the control group (Sarandol et al., 2007). On the other hand, Sofic et al. reported no significant change in serum antioxidant capacity in the patients with MDD compared with the healthy group (Sofic et al., 2002). Previous studies have demonstrated that the activities of SOD and GPx enzymes significantly decreased in the serum of MDD patients compared to the control group (Stefanescu et al., 2012; Herken et al., 2005; Srivastava et al., 2002). We found that GPx activity was significantly lower in the patient group. Moreover, SOD activity in the patient group was lower than that of the control group without significant differences.

Table 2. Results related to TAC, TOS, MDA, Cu concentrations and GPx, SOD activities in the MDD group and control group

Variables	Mean±SD		P
	Control (n=30)	MDD (n=30)	
TAC (μmol/mL)	1072.824±118.62	1048.40±93.01	0.445
TOS (μmol/mL)	9.68±3.67	11.89 ±6.17	0.207
MDA (nmol/mL)	13.506±5.35	16.83±5.86	0.036[*]
Cu (mg/L)	22.91±1.49	9.79±3.9	0.012[*]
SOD (U/mL)	6.48±76.16	60.37±4.03	0.148
GPX (U/mL)	256.93±79.38	143.18±43.06	0.019[*]

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TAC: Total Antioxidant Capacity; TOS: Total Oxidative Status; MDA: Malondialdehyde; SOD: Superoxide dismutase; GPX: Glutathione peroxidase.

*Significant differences (P<0.05).

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Copper is an essential component that plays a critical role in enzyme activity. It is the primary cofactor for some enzymes, including monoamine oxidase. Therefore, copper-related deficiencies can lead to major disorders like depression. In addition, copper as a cofactor is essential for some antioxidant enzymes, such as lysine oxidase and SOD. Its deficiency may result in the decreased activity of these enzymes (Maes et al., 2011). Some studies have also shown that copper is essential for the development and functioning of the CNS, and low copper concentrations can result in inappropriate development of CNS. At the same time, excess levels may be dangerous (Styczeń et al., 2016). Studies on brain tissue have shown that the brain is copper-rich, suggesting that copper may play an essential role in brain cell function and mood disorders (Scheiber et al., 2014). Many studies have also shown that the induction of oxidative stress pathways in major depression is associated with the activation of the inflammatory response and acute phase proteins. The changes in serum sepsis (key protein in copper storage) among the depressed patients could be considered another reason for the possible role of copper ions in depression (Etebary et al., 2010; Siwek et al., 2013; Lee et al., 2018; Russo, 2011). We found that patients with MDD had lower copper concentrations than healthy subjects. In line with our observation, some studies have also shown that copper as a trace metal may be involved in the pathophysiology of depression. Styczeń's research team found lower serum copper levels in depression than in the normal group (Styczeń et al., 2016). Furthermore, Twayej et al. (2019) observed a significant reduction in copper concentration, accompanied by upregulation of all cytokines, in depressed individuals compared to healthy ones.

Investigating the serum PCSK9 concentration in the patients with MDD showed a significant increase in this group. ROC curve analysis shows that the PCSK9 protein factor could be considered a diagnostic factor for MDD. PCSK9 protein has been identified as an important mediator in LDL-plasma cholesterol control by mediating LDL receptor degradation (Seidah et al., 2003). Moreover, this protein is a secretory protein in the liver, small intestine, kidneys, and brain tissues such as the hippocampus and cerebellum. Some studies have shown that mutations in the functional areas of this protein have led to some diseases, such as hypercholesterolemia and atherosclerosis. Studies show that mutations leading to loss of function in this protein are associated with decreased LDL-plasma cholesterol levels (Costet et al., 2006; Cohen et al., 2006; Cohen et al., 2005). As indicated by previous studies, increasing the serum level of PCSK9 can result in elevated serum ox-LDL levels

as well as overexpression of LOX1 in the vessel wall, which can lead to an increase in PCSK9 expression at later stages (Shapiro et al., 2017). Oxidized LDL causes inflammation by increasing NFkB and other inflammation factors (Bossù et al., 2009; Ogłodek., 2017). Since PCSK9 plays a functional role in raising serum oxidized (ox) LDL levels (PCSK9 acts as a factor that raises serum ox-LDL level), it can also be considered an essential factor in causing inflammation. In this regard, Tang et al. (2012) used siRNA of PCSK9 to demonstrate the effect of this factor on controlling the inflammation caused by ox-LDL through the NFkB pathway (Tang et al., 2012).

On the other hand, oxidized LDL was known as a factor that induces antigen-providing cells. Several studies have shown the importance of the MAPK pathway in depression (Kumamaru et al., 2011). A study found that changing the P38MAPK factor in the cerebral cortex (the site of production and location of serotonin receptors) may ultimately cause depression (Bruchas et al., 2011; Rasakham et al., 2012). P38MAPK activity increases the expression of HNF1α nuclear factor, subsequently leading to *PCSK9* gene overexpression (Cui et al., 2016). These results, which considered inflammation a factor in increasing PCSK9 levels, can support the present study results. According to the results, it is important that the relationship between PCSK9 and LDL-C levels, as MDA and TOS, is significantly positive in the two groups. This issue confirms our hypothesis that by increasing the PCSK9 variable, LDL-C increases as an oxidative factor, and due to the increase in MDA, oxidative stress will follow. So, PCSK9 plays an influential role in the progression of depression by creating oxidative stress and inflammation, and it can be claimed that this factor can be considered an important factor in the diagnosis or even treatment of depression. The limitations of this study are the low number of new samples, the time-consuming collection time, and the cost of performing tests related to the protein PCSK9 metabolic pathway.

5. Conclusion

For this study, the inclusion criteria for the participants comprised new cases of patients with MDD diagnosed by a psychiatrist with an HDRS index above 17. Serum samples were analyzed for related tests. In general, the results suggest that PCSK9 protein, as a factor involved in oxidative stress, was effective through specific signaling pathways in the occurrence of MDD. These findings help us gain insight into using PCSK9 as a diagnostic factor or even therapeutic goals by PCSK9 inhibition. However, our results are subject to further investigation.

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Ethical Considerations

Compliance with ethical guidelines

Funding

Authors' contributions

Conceptualization and investigation: Gholamreza Shafiee, Elahe Habibitabar, and Shima Khanverdiloo; Laboratory analysis and diagnosis: Mona Doostizadeh, Leila Jahangard, Elahe Habibitabar and Shima Khanverdiloo; Initial draft preparation: Jamshid Karimi and Gholamreza Shafiee; Final approval: All authors.

Conflict of interest

The authors declared no conflict of interest.

Acknowledgments

References

Asadi, S., Goodarzi, M. T., Karimi, J., Hashemnia, M., & Khodadadi, I. (2019). Does curcumin or metformin attenuate oxidative stress and diabetic nephropathy in rats? *Journal of Nephropathology, 8*(1), 1-8. [Link]

Bossù, P., Salani, F., Cacciari, C., Picchetto, L., Cao, M., & Bizzoni, F., et al. (2009). Disease outcome, alexithymia and depression are differently associated with serum IL-18 levels in acute stroke. *Current Neurovascular Research, 6*(3), 163-70. [DOI:10.2174/156720209788970036] [PMID]

Bruchas, M. R., Schindler, A. G., Shankar, H., Messinger, D. I., Miyatake, M., & Land, B. B., et al. (2011). Selective p38α MAPK deletion in serotonergic neurons produces stress resilience in models of depression and addiction. *Neuron, 71*(3), 498–511. [DOI:10.1016/j.neuron.2011.06.011] [PMID]

Cohen, J. C., Boerwinkle, E., Mosley, T. H., Jr, & Hobbs, H. H. (2006). Sequence variations in PCSK9, low LDL, and protection against coronary heart disease. *New England Journal of Medicine, 354*(12), 1264-1272. [DOI:10.1056/NEJMoa054013] [PMID]

Cohen, J., Pertsemlidis, A., Kotowski, I. K., Graham, R., Garcia, C. K., & Hobbs, H. H. (2005). Low LDL cholesterol in individuals of African descent resulting from frequent nonsense mutations in PCSK9. *Nature Genetics, 37*(2), 161-165. [DOI:10.1038/ng1509] [PMID]

Costet, P., Cariou, B., Lambert, G., Lalanne, F., Lardeux, B., & Jarnoux, A. L., et al. (2006). Hepatic PCSK9 expression is regulated by nutritional status via insulin and sterol regulatory element-binding protein 1c. *The Journal of Biological Chemistry, 281*(10), 6211–6218. [DOI:10.1074/jbc.M508582200] [PMID]

Sarandol, A., Sarandol, E., Eker, S. S., Erdinc, S., Vatansever, E., & Kirli, S. (2007). Major depressive disorder is accompanied with oxidative stress: short-term antidepressant treatment does not alter oxidative-antioxidative systems. Human psychopharmacology, 22(2), 67–73. [DOI: 10.1002/hup.829] [PMID]

Cui, C. J, Li, S., Zhu, C. G., Sun, J., Du, Y., & Zhang Y, et al. (2016). Enhanced proprotein convertase subtilisin/kexin type 9 expression by C-reactive protein through p38 MAPK-HNF 1α pathway in HepG2 cells. *Journal of Cellular and Molecular Medicine, 20*(12), 2374-2383 [DOI:10.1111/jcmm.12931]

Echeverria, V., Grizzell, J. A., & Barreto, G. E. (2016). Neuroinflammation: A therapeutic target of cotinine for the treatment of psychiatric disorders? *Current Pharmaceutical Design, 22*(10), 1324-1333. [DOI:10.2174/1381612822101603041125111] [PMID]

Etebary, S., Nikseresht, S., Sadeghipour, H. R., & Zarrindast, M. R. (2010). Postpartum depression and role of serum trace elements. *Iranian Journal of Psychiatry, 5*(2), 40-46. [PMID]

Gałecki, P., Szemraj, J., Bieńkiewicz, M., Zboralski, K., & Gałecka, E. (2009). Oxidative stress parameters after combined fluoxetine and acetylsalicylic acid therapy in depressive patients. Human Psychopharmacology, 24(4), 277-286. [DOI:10.1002/hup.1014] [PMID]

Grases, G., Colom, M. A., Sanchis, P., & Grases, F. (2019). Possible relation between consumption of different food groups and depression. *BMC Psychology, 7*(1), 14. [DOI:10.1186/s40359-019-0292-1] [PMID]

Hasler, G., van der Veen, J. W., Tumonis, T., Meyers, N., Shen, J., & Drevets, W. C. (2007). Reduced prefrontal glutamate/glutamine and γ-aminobutyric acid levels in major depression determined using proton magnetic resonance spectroscopy. *Archives of General Psychiatry, 64*(2), 193-200. [DOI:10.1001/archpsyc.64.2.193] [PMID]

Herken, H., Gurel, A., Selek, S., Armutcu, F., Ozen, M. E., & Bulut, M., et al. (2007). Adenosine deaminase, nitric oxide, superoxide dismutase, and xanthine oxidase in patients with major depression: impact of antidepressant treatment. *Archives of Medical Research, 38*(2), 247-252. [DOI:10.1016/j.arcmed.2006.10.005] [PMID]

Kumamaru, E., Numakawa, T., Adachi, N., & Kunugi, H. (2011). Glucocorticoid suppresses BDNF-stimulated MAPK/ERK pathway via inhibiting interaction of Shp2 with TrkB. *FEBS Letters, 585*(20), 3224–3228. [DOI:10.1016/j.febslet.2011.09.010] [PMID]

Kurata, T., Miyazaki, K., Morimoto, N., Kawai, H., Ohta, Y., & Ikeda, Y., et al. (2013). Atorvastatin and pitavastatin reduce oxidative stress and improve IR/LDL-R signals in Alzheimer's disease. *Neurological Research, 35*(2), 193-205. [DOI:10.1179/1743132812Y.0000000127] [PMID]

Habibitabar., et al. (2024). PCSK9 Protein Is Not Necessarily for MDD Disease. *BCN, 15*(4), 519-530.

527

Lee, J., Park, H., & Chey, J. (2018). Education as a protective factor moderating the effect of depression on memory impairment in elderly women. *Psychiatry Investigation, 15*(1), 70-77. [DOI:10.4306/pi.2018.15.1.70] [PMID]

LLiu, J., Fan, Y., Ling-Li Zeng, Liu, B., Ju, Y., & Wang, M., et al. (2021). The neuroprogressive nature of major depressive disorder: Evidence from an intrinsic connectome analysis. *Translational Psychiatry, 11*(1), 102. [DOI:10.1038/s41398-021-01227-8] [PMID]

Liu, Y. N., Peng, Y. L., -Liu, L., Wu, T. Y., Zhang, Y., & Lian, Y. J., et al. (2015). TNFα mediates stress-induced depression by upregulating indoleamine 2, 3-dioxygenase in a mouse model of unpredictable chronic mild stress. *European Cytokine Network, 26*(1), 15-25. [DOI:10.1684/ecn.2015.0362] [PMID]

Maduray, K., Moodley, J., Soobramoney, C., Moodley, R., & Naicker, T. (2017). Elemental analysis of serum and hair from pre-eclamptic South African women. *Journal of Trace Elements in Medicine and Biology, 43,* 180-186. [DOI:10.1016/j.jtemb.2017.03.004] [PMID]

Maes, M., Galecki, P., Chang, Y. S., & Berk, M. (2011). A review on the oxidative and nitrosative stress (O&NS) pathways in major depression and their possible contribution to the (neuro) degenerative processes in that illness. *Progress in Neuro-Psychopharmacology and Biological Psychiatry, 35*(3), 676-692. [DOI:10.1016/j.pnpbp.2010.05.004] [PMID]

Mazloomi, S., Alimohammadi, S., Khodadadi, I., Ghiasvand, T., & Shafiee, G. (2020). Evaluation of Methylene Tetrahydrofolate Reductase (MTHFR) activity and the Levels of Homocysteine and Malondialdehyde (MDA) in the Serum of Women with Preeclampsia. *Clinical and Experimental Hypertension, 42*(7), 590-594. [DOI:10.1080/10641963.2020.1739700] [PMID]

Michel, T. M., Pülschen, D., & Thome, J. (2012). The role of oxidative stress in depressive disorders. *Current Pharmaceutical Design, 18*(36), 5890-5899. [DOI:10.2174/138161212803523554] [PMID]

Mohseni, R., Karimi, J., Tavilani, H., Khodadadi, I., & Hashemnia, M. (2020). Carvacrol downregulates lysyl oxidase expression and ameliorates oxidative stress in the liver of rats with carbon tetrachloride induced liver fibrosis. *Indian Journal of Clinical Biochemistry: IJCB, 35*(4), 458–464. [DOI:10.1007/s12291-019-00845-w] [PMID]

Moylan, S., Maes, M., Wray, N. R., & Berk, M. (2013). The neuroprogressive nature of major depressive disorder: pathways to disease evolution and resistance, and therapeutic implications. *Molecular Psychiatry, 18*(5), 595-606. [DOI:10.1038/mp.2012.33] [PMID]

Need, A. C., McEvoy, J. P., Gennarelli, M., Heinzen, E. L., Ge, D., & Maia, J. M., et al. (2012). Exome sequencing followed by large-scale genotyping suggests a limited role for moderately rare risk factors of strong effect in schizophrenia. *American Journal of Human Genetics, 91*(2), 303-312. [DOI:10.1016/j.ajhg.2012.06.018] [PMID]

Ogłodek E. A. (2017). The role of PON-1, GR, IL-18, and OxLDL in depression with and without posttraumatic stress disorder. *Pharmacological Reports: PR, 69*(5), 837–845. [DOI:10.1016/j.pharep.2017.03.015] [PMID]

Rasakham, K., McGillivray, K. L., & Liu-Chen, L. Y. (2012). Sex differences in U50, 488H-induced phosphorylation of p44/42 mitogen-activated protein kinase in the guinea pig brain. *Neuroscience, 223,* 447-456. [DOI:10.1016/j.neuroscience.2012.07.040] [PMID]

Russo, A. J. (2011). Analysis of plasma zinc and copper concentration, and perceived symptoms, in individuals with depression, post zinc and antioxidant therapy. *Nutrition and Metabolic Insights, 4,* 19–27. [DOI:10.4137/NMI.S6760] [PMID]

Seidah, N. G., Awan, Z., Chrétien, M., & Mbikay, M. (2014). PCSK9: A key modulator of cardiovascular health. *Circulation Research, 114*(6), 1022-1036. [DOI:10.1161/CIRCRESAHA.114.301621] [PMID]

Schlüter, K. D., Wolf, A., Weber, M., Schreckenberg, R., & Schulz, R. (2017). Oxidized low-density lipoprotein (oxLDL) affects load-free cell shortening of cardiomyocytes in a proprotein convertase subtilisin/kexin 9 (PCSK9)-dependent way. *Basic Research in Cardiology, 112*(6), 63. [DOI:10.1007/s00395-017-0650-1] [PMID]

Shapiro, M. D., & Fazio, S. (2017). PCSK9 and atherosclerosis-lipids and beyond. J*ournal of Atherosclerosis and Thrombosis, 24*(5), 462–472. [DOI:10.5551/jat.RV17003] [PMID]

Seidah, N. G., Benjannet, S., Wickham, L., Marcinkiewicz, J., Jasmin, S. B., & Stifani, S., et al.(2003). The secretory proprotein convertase neural apoptosis-regulated convertase 1 (NARC-1): liver regeneration and neuronal differentiation. *Proceedings of the National Academy of Sciences of the United States of America, 100*(3), 928–933. [DOI:10.1073/pnas.0335507100] [PMID]

Shi, J., Badner, J. A., & Liu, C. (2008). PDLIM5 and susceptibility to bipolar disorder: A family-based association study and meta-analysis. *Psychiatric Genetics, 18*(3), 116–121. [DOI:10.1097/YPG.0b013e3282fa184b] [PMID]

Sofic, E., Rustembegovic, A., Kroyer, G., & Cao, G. (2002). Serum antioxidant capacity in neurological, psychiatric, renal diseases and cardiomyopathy. *Journal of Neural Transmission, 109*(5), 711-719. [DOI:10.1007/s007020200059] [PMID]

Scheiber, I. F., Mercer, J. F., & Dringen, R. (2014). Metabolism and functions of copper in brain. *Progress in Neurobiology, 116,* 33–57. [DOI:10.1016/j.pneurobio.2014.01.002] [PMID]

Siwek, M., Sowa-Kućma, M., Dudek, D., Styczeń, K., Szewczyk, B., & Kotarska, K., et al. (2013). Oxidative stress markers in affective disorders. *Pharmacological Reports, 65*(6), 1558-1571. [DOI:10.1016/S1734-1140(13)71517-2] [PMID]

Srivastava, N., Barthwal, M. K., Dalal, P. K., Agarwal, A. K., Nag, D., & Seth, P. K., et al. (2002). A study on nitric oxide, β-adrenergic receptors and antioxidant status in the polymorphonuclear leukocytes from the patients of depression. *Journal of Affective Disorders, 72*(1), 45-52. [DOI:10.1016/S0165-0327(01)00421-9] [PMID]

Stefanescu, C., & Ciobica, A. (2012). The relevance of oxidative stress status in first episode and recurrent depression. *Journal of Affective Disorders, 143*(1-3), 34-38. [DOI:10.1016/j.jad.2012.05.022] [PMID]

Stoekenbroek, R. M., Lambert, G., Cariou, B., & Hovingh, G. K. (2018). Inhibiting PCSK9 - biology beyond LDL control. Nature reviews. *Endocrinology, 15*(1), 52–62. [DOI:10.1038/s41574-018-0110-5] [PMID]

Styczeń, K., Sowa-Kućma, M., Siwek, M., Dudek, D., Reczyński, W., & Misztak, P., et al. (2016). Study of the Serum Copper Levels in Patients with Major Depressive Disorder. *Biological trace Element Research, 174*(2), 287-293. [DOI:10.1007/s12011-016-0720-5] [PMID]

Tang, Z., Jiang, L., Peng, J., Ren, Z., Wei, D., & Wu, C., et al. (2012). PCSK9 siRNA suppresses the inflammatory response induced by oxLDL through inhibition of NF-κB activation in THP-1-derived macrophages. International Journal of Molecular Medicine, 30(4), 931-938. [DOI:10.3892/ijmm.2012.1072] [PMID]

Ten Have, M., de Graaf, R., van Dorsselaer, S., Tuithof, M., Kleinjan, M., & Penninx, B. W. J. H. (2018). Recurrence and chronicity of major depressive disorder and their risk indicators in a population cohort. *Acta Psychiatrica Scandinavica, 137*(6), 503–515. [DOI:10.1111/acps.12874] [PMID]

Twayej, A. J., Al-Hakeim, H. K., Al-Dujaili, A. H., & Maes, M. (2019). Lowered zinc and copper levels in drug-naïve patients with major depression: Effects of antidepressants, ketoprofen and immune activation. The World Journal of Biological Psychiatry, 48(3), 425-32. [DOI: 10.1080/15622975.2019.1612090] [PMID]

Van der Vliet, A., & Bast, A. (1992). Effect of oxidative stress on receptors and signal transmission. *Chemico-Biological Interactions, 85*(2-3), 95-116. [DOI:10.1016/0009-2797(92)90055-P] [PMID]

Habibitabar., et al. (2024). PCSK9 Protein Is Not Necessarily for MDD Disease. *BCN, 15*(4), 519-530.

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