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ORIGINAL RESEARCH

Serum NOX1 and Raftlin as New Potential Biomarkers of Interest in Schizophrenia: A Preliminary Study

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Introduction: There is increasing evidence that oxidative stress (OS) and neuroinflammation play a role in the neuroprogression of schizophrenia (SCZ). Promising novel candidates which have been proposed in the search for biomarkers of psychotic illness include NADPH oxidase 1,2 (NOX1,2) and raftlin. NOX1 from the NOX family is the main source of physiological reactive oxygen species (ROS) and raftlin, the main lipid raft protein, is associated with inflammatory processes. The aim of the present study was to evaluate serum NOX1 and raftlin levels in chronic stable patients with SCZ.

Methods: We measured serum NOX1 and raftlin levels from 45 clinically stable patients with SCZ and 45 healthy controls (HCs) matched for age, sex, and body-mass index. The Positive and Negative Syndrome Scale was applied to the patient group to evaluate the severity of psychotic symptoms.

Results: NOX1 and raftlin levels in the patients were statistically significantly higher than the HCs (NOX1 $p<0.001$, raftlin $p<0.001$). Both parameters showed very good diagnostic performance (NOX1 AUC = 0.931, raftlin AUC = 0.915). We obtained positive and significant correlations between serum levels of both biomarkers and symptom severity.

Discussion: This preliminary study indicating elevations in serum NOX1 and raftlin levels in patients with SCZ supports the importance of OS and inflammatory processes in the etiopathogenesis of the illness.

Keywords: oxidative stress, oxidant, neuroinflammation, reactive oxygen species, lipid raft, diagnostic performance

Introduction

Schizophrenia (SCZ) is a heterogeneous and serious mental disorder with a worldwide lifetime prevalence of 1%.[1] Despite considerable research on the neurobiology, genetics and neurodevelopment of SCZ, its etiology remains unclear.[2] It has been suggested that oxidative stress (OS) and neuroinflammation, which are associated with neurodegenerative diseases, may also involved in the neuroprogression of SCZ.[3,4] OS, reflecting an imbalance between the reactive oxygen species (ROS) and the antioxidant defense system, is thought to play a role in the pathogenesis of SCZ by causing membrane dysfunction.[5] A number of systematic reviews and meta-analyses have been performed and generally suggest insufficiency in the antioxidant system and increase in pro-oxidant processes in SCZ patients.[6] The increase in ROS causes changes in the plasma membrane, leading to disruptions in the neurotransmission of dopamine, GABA and glutamate, which are important neurotransmitters in the pathogenesis of SCZ.[6]

Along with oxidative phosphorylation, NADPH oxidase (NOX) is also the main source of physiological ROS.[7] NOX enzymes carry an electron from NADPH to reduce molecular oxygen to superoxide anion, causing ROS production.[8] NOX1, 2 and 4 from the NOX family are mainly distributed in the central nervous system (CNS).[9] In the social isolation

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model of psychosis in animal studies, it has been reported that not only the phagocyte oxidase NOX2, but also the non-phagocyte oxidase NOX1 may have an important role.[10,11] As far as we know, there is no human study on NOX levels in patients with SCZ.

Studies related to inflammation markers and the clinical course of the disease have been conducted in the pathology of schizophrenia, and there is evidence supporting that it may play an important role in the pathology of the disease.[12] Neuroinflammation can cause disconnection along with white matter damage, associated with symptoms of schizophrenia.[13] Recent studies show that chronic neuroinflammation that can affect the CNS and the interrelationship between immune and neuroinflammatory systems play an important role in the neurobiology of schizophrenia.[14,15] Lipid rafts are micro-domains in the cell membrane, which are composed of cholesterol, sphingolipids, saturated phospholipids and various protein combinations, and are involved in cellular processes such as signal transduction.[16] Raftlin is the major lipid raft protein localized to lipid rafts by fatty acylation.[17] It has recently been reported to be an inflammatory biomarker for some inflammatory diseases.[18] Additionally, Raftlin has also been associated with oxidative and inflammatory biomarkers such as; catalase (CAT), malondialdehyde (MDA), total oxidant status (TOS), interleukin-17 (IL-17) and tumor necrosis factor-α (TNF-α).[18–21] Excessive secretion of Raftlin causes excessive production of IL-17 via Th17.[22] Th17 cells may cause deterioration in the blood-brain barrier (BBB) and may cause neuroinflammation in SCZ by infiltrating the CNS.[23] In our very recent study, we showed that patients with generalized anxiety disorder have increased serum raftlin levels compared to healthy controls (HCs), and Raftlin has a very good diagnostic performance in this disease.[24] Although there are studies on Th-17 cells, which are claimed to have an important role in inflammatory processes in patients with SCZ, to our knowledge, there is no research examining Raftlin, which is known to increase the inflammatory response associated with these cells.

Although studies on patients with SCZ mostly reported a decrease in antioxidants and an increase in pro-oxidants, the source of this abnormality is still unclear.[25,26] In this context, the present study aims to compare levels of serum NOX1, which is the main source of ROS, between patients with SCZ and HCs, and to investigate the relationship between NOX1 and symptom severity in the patient group. Secondly, the current study aims to compare levels of Raftlin, which is important in the Th17-related inflammatory response,[22] between patients with SCZ and HCs and to determine its relationship with symptom severity in the patient group.

Methods

Participants

The current study included 90 participants; 45 patients with chronic SCZ and 45 HCs. Patients with SCZ who were followed up in the outpatient service of the Necip Fazıl City Hospital psychiatry department were recruited to the patient group. A Structured Clinical Interview (SCID)[27] by an experienced psychiatrist was performed according to DSM-5 to validate the diagnosis for this group. The patients had not been hospitalized and were on stable antipsychotic medication for at least the last six months. Patients were clinically stable at the time of enrollment. 45 HCs consisted of hospital employees and their relatives. They were matched for age, sex, and body mass index (BMI) without a past history of psychiatric illness. To exclude psychiatric disorders in the control group, HC participants were also evaluated with the SCID according to DSM-5.

The following inclusion criteria were applied to the patients: (1) free from neuroinflammatory, neurodegenerative disease and major medical illness (including multiple sclerosis, Parkinson's disease, stroke, chronic obstructive pulmonary disease, inflammatory bowel disease, rheumatoid arthritis, chronic obstructive pulmonary disease, diabetes mellitus, allergies, and infections); (2) free from intellectual disability; (3) free from anti-inflammatory, immune suppressive and antioxidant drug use; (4) free from signs of acute inflammation such as elevated CRP (> 5mg/L and leukocytosis (> 10,000 mcL); (5) free from substance use disorders (including alcohol) and regular nicotine use; (6) free from comorbid psychiatric disorders (7) a score of four or less for each of the items in the The Positive and Negative Syndrome Scale (PANSS) scale for clinical stability.

The inclusion criteria for the control group included the first five criteria outlined above, and in addition they needed to be free from a current or lifetime diagnosis of psychiatric illness according to DSM-5 diagnostic criteria.

Written informed consent was obtained from the controls and patients before participating in the study. The present study was approved by the Ethics Committee of Kahramanmaras Sutcu Imam University Faculty of Medicine (approval date: 02.11.2021, number: 02). During the conduct of the study, the Declaration of Helsinki was followed.

Measurements

Clinical and sociodemographic variables were recorded. PANSS[28] scale was applied by an experienced psychiatrist to evaluate the severity of symptoms in the patient group. In this context, it was required that the items in the PANSS scale were scored four or less for clinical stability. The Calgary Depression Scale for Schizophrenia (CDSS)[29] was used to evaluate depressive symptoms in patients with SCZ.

Biochemical Analysis

Venous blood samples were taken from all participants between 08.30 and 10.30 in the morning after an overnight fast. The samples were immediately transferred to the biochemistry laboratory and centrifuged at 4000 g for ten minutes. Serum samples separated after centrifugation were stored at −30 °C until biochemical analysis. Measurement of serum levels of NOX1 and Raftlin was performed with a quantitative sandwich enzyme immunoassay technique (ELISA) using a commercial kit (MyBioSource Company, USA). During the measurement, the manufacturer's instructions were followed.

Statistical Analysis

The distributions of continuous variables were initially assessed. In presenting descriptive statistics, mean ± standard deviation for normally distributed variables and median values with interquartile ranges for non-normally distributed variables were used. Differences between patients and HC groups were examined using independent samples *t*-tests (where normally distributed) or Mann–Whitney *U*-tests (where non-normally distributed). Chi-square test was used when comparing binary variables between groups. Spearman correlation test was used to test the relationship between continuous variables. A receiver operating characteristic (ROC) curve was plotted to test the diagnostic performance of NOX1 and Raftlin in patients with SCZ.

Results

Patient and HC groups, consisting of 45 participants each, were matched in terms of age (p=0.18), gender (p=0.13), BMI (p=0.28), duration of education (p=0.23), and marital status (p=0.20). The characteristics across both participant groups, and symptom severity of patients, are shown in Table 1. There were no changes in the medical treatment of the patient group in the prior 6 months and non-drug treatments such as ECT were not applied during this period.

We found statistically significant increased serum NOX1 levels in the patient group compared to the HCs (p<0.001, 95% confidence interval: 2.02–2.17, U value: 7) (Table 2). The cut-off point for NOX1 levels was found to be 18.12ng/mL by ROC analysis (Figure 1). We found a statistically significant positive correlation between serum NOX1 levels and PANSS positive, negative, general psychopathology and total scores (respectively p<0.001, p=0.043, p<0.001, p<0.001) (Table 3).

We found statistically significant increased serum Raftlin levels in the patient group compared to the HCs (p<0.001, 95% confidence interval: 1.96–2.09, U value: 18.1) (Table 2). The cut-off point for raftlin levels was found to be 19.46ng/mL by ROC analysis (Figure 2). We found a statistically significant positive correlation between serum Raftlin levels and PANSS positive, negative, general psychopathology and total scores (respectively p=0.043, p=0.049, p<0.001, p<0.001) (Table 3).

Discussion

The present study evaluated the relationship between two new biomarkers associated with oxidative stress and inflammatory process and chronic stable patients with SCZ in a Turkish outpatient population. We report three main findings. The first of these is increased serum NOX1 and Raftlin levels in the patient group compared to HCs. Secondly, we found that both parameters have very good diagnostic performance in patients with SCZ. Third, we found statistically significant positive correlations between NOX1 and Raftlin levels and the severity of patients' symptoms.

Table 1 Demographic and Clinical Characteristics of Patients with Schizophrenia and Healthy Controls

		Schizophrenia (n=45)	Control (n=45)	*p* value
Age(years)[a]	Mean ± SD	34.86±6.74	34.35±6.46	0.18
Gender[b]	Male (n:%)	24/21	22/23	0.13
BMI (kg/m^2)[a]	Mean ± SD	24.91±2.45	24.78±3.67	0.28
Education (years)[a]	Mean ± SD	9.53±1.79	9.35±1.68	0.23
Married[b]	Single (n:%)	15/30 (33%)	16/29 (36%)	0.20
Duration of illness (years)	Mean ± SD	13.22±11.88		
Onset of illness (Age)	Mean ± SD	24.28±5.94		
Number of episodes	Mean ± SD	4.1±2.98		
Chlorpromazine equivalent (Daily)	Mean ± SD	606.6±322.31		
PANSS total	Mean ± SD	65.4±10.2		
PANSS Positive	Mean ± SD	16.88±4.29		
PANSS Negative	Mean ± SD	18.35±4.14		
PANSS General	Mean ± SD	30.17±4.61		
CDSS	Mean ± SD	2.15±1.49		

Note: [a]Independent samples *t*-test; [b]Chi-Square test; α: 0.05.
Abbreviations: PANSS, Positive and Negative Syndrome Scale; CDSS, The Calgary Depression Scale for Schizophrenia.

Table 2 Laboratory Results of Patients and Healthy Controls

		Patients (n=45)	Control (n=45)	*p* value
NOX-1	Median (Q1-Q3)	25.06 (16.3–33.82)	6.96 (1.48–12.44)	**<0.001***
Raftlin	Median (Q1-Q3)	40.63 (29.80–51.40)	18.17 (9.33–27.01)	**<0.001***

Note: Mann–Whitney *U*-test. α, 0.05. *Difference is statistically significant.
Abbreviation: NOX-1, NADPH oxidase 1.

Excessive ROS produced or insufficient antioxidant defenses due to OS are among factors thought to cause the development of SCZ.[30] It has been reported that there is a decrease in the levels of vitamin E and vitamin C, which are known to have antioxidant activity, in patients with SCZ. There are studies on the effects of taking supplements of these vitamins on psychotic symptoms.[31,32] However, vitamin E is insufficient to prevent intracellular damage of ROS, and the combined use of both vitamins may produce pro-oxidant effects.[30,33] Thus, it is important to increase understanding of NOX (the main source of ROS) and its role in psychotic illness, in order to develop more effective treatments for reducing oxidative damage in patients with SCZ. Recent animal studies on NOX enzymes support this. Firstly, in a rat model of social isolation, NOX2-related OS was reportedly of great importance in the initiation and progression of neuropathological changes[10,34] and also isolation-related HPA axis changes.[35] Another important study reported an increase in NOX1 enzyme due to increased expression of NOX in visceral fat of rats exposed to a social isolation model.[11] In the present study, we found a significant increase in serum levels of NOX1 in human SCZ patients compared to HCs, who were matched for age, sex and BMI. This is likely to result in OS-related damage due to increased production of ROS in patients with SCZ. When our results are evaluated in context along with the animal studies mentioned above, the evidence suggests that the negative consequences of deviation from redox homeostasis in SCZ patients can be regulated by targeting with the specific NOX enzyme pathway.

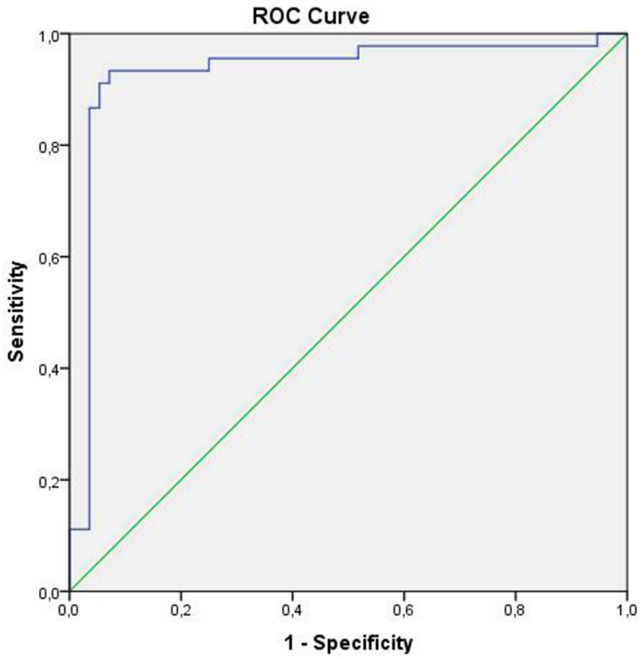

Figure 1 ROC curve analysis of NOX1. Specificity, 0.946; Sensitivity, 0.978; AUC was 0.931 for NOX1. The cut-off point was detected as 18.12 ng/mL.
Abbreviations: NOX1, NADPH Oxidase; ROC, receiver operating characteristic; AUC, area under curve.

An excessive amount of Raftlin has been shown to increase the Th17-mediated response by causing excessive IL-17 production.[22] Th17 cells contribute to progressive brain changes in SCZ by disrupting the BBB, infiltrating the CNS and causing neuroinflammation together with other cytokines and microglia.[23] Th17 cells play a role in neuroinflammation by activating microglia in the CNS and increasing the production of cytokines including IL-6, TNFα and IL-1β locally. In animal studies, an increase in NOX-related ROS production has been shown to be associated with a breakdown of the BBB.[34] The inflammatory response causes an increase in ROS production, and an increase in ROS production impairs the permeability of the BBB.[36] While increased IL-6 and TNF-α levels are generally reported in patients with SCZ,[37–39] inconsistent results are observed regarding IL-17 levels.[40–42] In the current study, we found that patients with SCZ had increased serum Raftlin levels compared to HCs, but also showed that this parameter was positively correlated with the severity of positive, negative and general psychopathological symptoms according to PANSS scores. Ding et al showed a positive correlation between plasma levels of IL-17 and Th17 cells and the severity of all clinical symptoms in patients with SCZ.[40] Interestingly, in the study showing decreased IL-17 levels in SCZ

Table 3 Correlation Analysis Between Serum Levels and Symptom Severity

	NOX1		RAFTLİN	
	r value	*p* value	*r* value	*p* value
PANSS P	0.464	**<0.001***	0.454	0.043
PANSS N	0.436	0.043	0.534	0.049
PANSS G	0.452	**<0.001***	0.498	**<0.001***
PANSS T	0.468	**<0.001***	0.499	**<0.001***
CDSS	0.222	0.552	0.238	0.0542

Note: Correlation coefficients (r) and p values were calculated via Spearman correlation analysis. *Difference is statistically significant.
Abbreviations: PANSS, The Positive and Negative Syndrome Scale; P, Positive; N, Negative; G, General psychopathology; T, Total; CDSS, The Calgary Depression Scale for Schizophrenia.

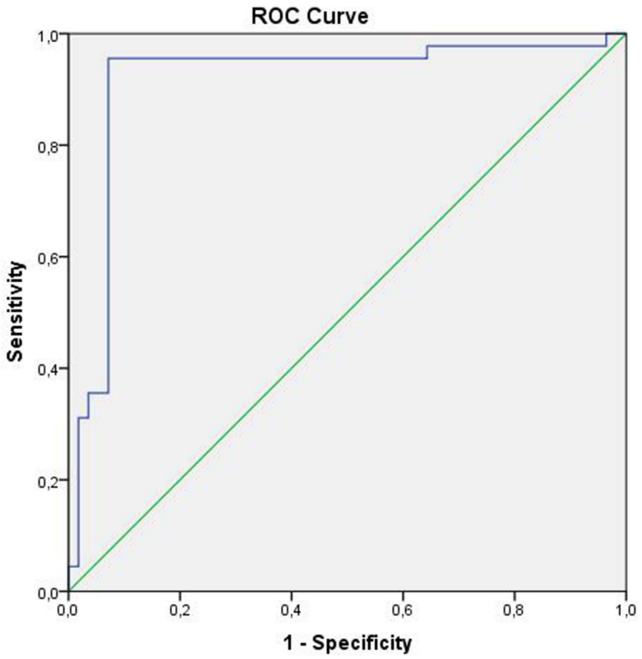

Figure 2 ROC curve analysis of raftlin. Specificity, 0.925; Sensitivity, 0.978; AUC was 0.915 for raftlin. The cut-off point was detected as 19.46 ng/mL.
Abbreviations: ROC, receiver operating characteristic; AUC, area under curve.

patients, a positive correlation was reported between PANSS scores and cytokines representing the IL-17 pathway.[43] We believe that the result we obtained regarding the excessively increased Raftlin, which is known to cause excessive IL-17 secretion, supports the increasing evidence regarding the importance of Th-17 cells and the IL-17 pathway in SCZ.

Currently, there is no blood test or imaging modality to diagnose SCZ. Since both biomarkers are being investigated for the first time in patients with SCZ, it is important to investigate the diagnostic performance of these markers. Diagnostic power according to AUC values; <0.6=fail, 0.6–0.7=poor, 0.7–0.8=fair, and 0.9–1=very good. In the present study, we found 0.931 AUC for NOX1 and 0.915 AUC for Raftlin, according to the ROC curve. These suggest that the increase in NOX1 and Raftlin levels in patients with SCZ has a very good diagnostic performance. Recently, Gunes et al reported that increased prolidase in patients with chronic SCZ had a very good diagnostic performance with an AUC value of 1.0.[44] In this context, in one of our previous publications, we showed that increased malondialdehyde levels, a lipid peroxidation marker that plays an important role in OS, have a very good diagnostic value with an AUC value of 0.979 in patients with medicated SCZ.[45] Moreover, we showed that increased GPER levels, which are known to mediate the neurological functions of 17β-estradiol,[46] have a good diagnostic value in patients with medicated SCZ with an AUC of 0.857.[47] Recent evidence suggests that GPER also increases NOX1 levels, resulting in ROS production, and that GPER inhibitors can be used as NOX-reducing regulators.[48] We believe that the increased NOX1 levels in medicated SCZ patients and the support of this result with a very good diagnostic performance are consistent with the recent results we obtained regarding GPER in the same group of patients. Despite the very good diagnostic performance we have achieved for both parameters, we do not claim that these are biomarkers to be used for diagnostic purposes, but we consider that further studies should be done in larger groups where confounding factors can be more substantively controlled for. The further studies should also examine depressive/negative symptoms closely, as in our study we did not observe an association with CDSS although the PANSS also evaluates negative symptoms and ROS systems are known to be involved in depressive disorders.

The following limitations should be considered when evaluating the results of the present study. Firstly, as a preliminary study, the sample size was small, resulting in limited statistical power. Secondly, the patient group

consisted of medicated schizophrenic patients on stable phase, an important confounding factor that could affect these results. Antipsychotics may have some effects on OS by leading to changes in the oxidation-reduction balance. However, we attempted to minimise the influence of medication by ensuring that no changes had been made recently, but we acknowledge that this and other potential confounding factors should be considered in future studies (eg, diet, exercise). Third, our study has a cross-sectional design, which prevents clarifying the roles of both biomarkers and their relationship to symptom severity in different phases of the disease. Fourth, since both biomarkers are measured in the blood, the results may not accurately reflect their levels in the brain as they are likely to be affected by BBB permeability.

Despite these limitations, the strengths of our current study are the strict inclusion and exclusion criteria applied and the matching in terms of age, gender, BMI, marital status, education level to ensure adequate homogeneity.

Conclusion

In summary, in the present study, we indicated that patients with chronic SCZ have increased serum NOX1 and Raftlin levels compared to healthy controls. To the best of our knowledge, this is the first study in which both biomarkers were evaluated in humans. In addition, we found a statistically significant positive correlation between NOX1 and Raftlin levels across clinical symptoms of patients according to PANSS. Finally, we found that both biomarkers have very good diagnostic performance in patients with SCZ. These results should be considered as preliminary, and longitudinal studies should be conducted in various phases of the disease, including drug-naive first-episode psychosis in a larger sample.

Disclosure

Dr Rebecca Strawbridge reports personal fees from Janssen, outside the submitted work. The authors report no other conflicts of interest in this work.

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