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CLINICAL STUDY

Elevated nucleosome level and oxidative stress in schizophrenia patients

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ABSTRACT

AIM: The aim of this study was to investigate the effect of oxidative stress on nucleosome levels and its relation with the clinical features in schizophrenia patients.

MATERIAL AND METHOD: Thirty schizophrenia patients and 30 healthy controls were enrolled in this study. Patients were diagnosed with schizophrenia according to the 4th edition of the Diagnostic and Statistical Manual for Mental Disorders (DSM-IV). The control group consisted of 30 healthy subjects matched to the patients with regard to age and gender and who had no history of any psychiatric disorder. The severity of schizophrenia symptoms in the patients was evaluated using the Positive and Negative Syndrome Scale (PANSS) and the Clinical Global Impression Severity Scale (CGI-S). Physical and neurological examinations were performed in each of the patients and controls.

RESULTS: Nucleosome, total oxidant levels and OSI values were higher in schizophrenia patients than in controls ($p < 0.05$). There was no significant difference in the total antioxidant levels. There was a positive correlation between the nucleosome level and PANSS positive subscale ($p = 0.028$, $r = 0.402$). There was a positive correlation between TAS and age ($p = 0.025$, $r = 0.289$), PANSS total ($p < 0.001$, $r = 0.604$). There was a negative correlation between OSI and PANSS total ($p = 0.019$, $r = –0.427$), PANSS positive subscale ($p = 0.043$, $r = –0.372$). There was a negative correlation between TOS and PANS total ($p = 0.028$, $r = –0.402$).

CONCLUSION: In this study we found a correlation between nucleosome level and PANSS positive subscale. To our knowledge, this is the first study that evaluates oxidative stress and nucleosomes released from apoptotic cells together *(Tab. 2, Ref. 50)*. Text in PDF *www.elis.sk.*

KEY WORDS: nuclesome, schizophrenia, oxidative stress.

Introduction

Although the aetiology of schizophrenia is unknown for certain, some deficits like decreased gray matter volume, synaptic indicators and neurophil are reported. These changes show that an interruption of synaptic circuitry is central in schizophrenia patients(1). Generally, patients with schizophrenia have smaller temporal, parietal, and occipital gray matter. In addition, they have ventriculomegaly, and cortical and subcortical volume loss (2). Brain matter loss and neurodegeneration are involved in the pathophysiology of schizophrenia.

Therefore apoptosis is more prominent in schizophrenia patients than in others (3). Cortical Bcl-2 level is diminished in schizophrenia patients. Lower Bcl-2 levels might cause proapoptotic effects and neuronal atrophy (4). The ratio of Bax to Bcl-2is is increased in schizophrenia, which is indicative of apoptotic tendency (5). Apoptosis increases in cases of synaptic and neuronal loss (1). Therefore, apoptosis may have a role in developing gray matter loss seen in psychoses. Apoptosis is a physiological process of genetically programmed cell death, first described by Kerr et al. (6). Neuronal cell death is observed in neurodegenerative diseases (7). Injured or diseased neurons are eradicated by apoptosis in neurodegenerative disorders (1). Nucleosome, which is composed of double-stranded DNA and histones, is the basic packing unit of DNA (8). The main role of the nucleosome is the packing of DNA, which controls genetic information by regulating protein access. This control mechanism of nucleosome is regulated by post-translational modifications like methylation, acetylation and phosphorylation of histones (8, 9). During apoptosis, nucleosomes are released into the circulation by nuclear endonucleases activation (8). Elevated levels of nucleosomes in the plasma have been found in malignant diseases, sepsis, septic shock, cerebral stroke, and systemic lupus erythematous(9).

Oxidative stress is considered to be involved in the pathophysiology of schizophrenia(6). Dopamine is one of the possible reactive products (6). Excessive oxidant production and/or insufficient enzymatic and non-enzymatic antioxidant defense leads to oxidative stress (4). Pathophysiological involvement of

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oxidative stress in some diseases is due to interference with the metabolism of carbohydrates, proteins, lipids and DNA (4). One of the effects of oxidative stress on DNA is its role in apoptosis. There is an evidence that oxidative stress leads to apoptosis in some cell types(10-12).

The aim of this study was to investigate the effect of oxidative stress on nucleosome levels and its relation with the clinical features in schizophrenia patients.

Materials and method

Patients and controls

Thirty schizophrenic patients (18 female and 12 male) and 30 healthy controls (19 female and 11 male) were enrolled in this study, which was approved by the local ethics committee. Patients were diagnosed with schizophrenia according to the 4th edition of the Diagnostic and Statistical Manual for Mental Disorders (DSM-IV) (13). The control group consisted of 30 healthy subjects matched to the patients with regard to age and gender and who had no history of any psychiatric disorder. Patients were recruited from the Psychiatry Outpatients Clinic of the Department of Psychiatry, School Medicine, RecepTayyip Erdogan University. All subjects were advised about the procedure and signed the informed consent to participate in the study. The severity of schizophrenia symptoms in the patients was evaluated using the Positive and Negative Syndrome Scale (PANSS) (14) and the Clinical Global Impression Severity Scale (CGI-S) (15). Physical and neurological examinations were performed in each of the patients and controls.

Blood sampling

Venous blood samples were collected from the left forearm vein into heparinized tubes. The blood samples were centrifuged at 3.000 rpm for 10 min at 4 °C in order to remove plasma. The buffy coat on the erythrocyte sediment was separated carefully. Plasma samples were stored at –80 °C until analysis.

Measurement of TAS, TOS and OSI levels

The total antioxidant status (TAS) was determined by the method of Erel, which allows fully automated measurement of the total antioxidant capacity of the body against strong free radicals. Similarly, the total oxidant status (TOS) was determined in an automated system developed by Erel. Oxidative stress index (OSI) was calculated from the TOS/TAS ratio (16).

Measurement of nucleosome level

The serum samples for nucleosome determination were centrifuged at 3,000g for 15 min and treated with 10 mmol/l EDTA (ethylene diamine tetraacetic acid) immediately after centrifugation and stored at –70 °C until further analysis. The commercially available Cell Death Detection ELISAPLUS Kit (Roche Diagnostics Mannheim, Germany) was used according to the manufacturer's instructions for quantification of nucleosome concentrations in serum. Absorbance (OD) of each well was determined with a microtiter plate reader (Multiskan GO, Thermo Scientific) within 5 minutes. Standard curves were fitted using Titri ELISA software, then the fitted curve was used to convert sample absorbance readings to nucleosome concentration.

Statistical analyses

The data were evaluated by the SPSS version 18.0 (SPSS Inc., Chicago, IL, USA). Both descriptive and analytical statistics were used. Chi-square / Fisher's Exact test was used for comparisons between categorical variables. Normal distribution of continues variables were tested with the one way Kolmogorov–Smirnov test. Between-group comparisons with normal distribution values were made by the Student'st-test. The Mann–Whitney U test was employed to compare the not normal distribution values. The 2-tailed significance level was set at 0.05. For correlation evaluations, Pearson's and Spearmen's correlation (2-tailed) was used.

Results

30 patients with schizophrenia and 30 age and gender-matched control subjects were included in the study. The socio-demographic characteristics of the study participants are shown in the Table 1.

Nucleosome, total oxidant levels and OSI values were higher in schizophrenia patients than in controls ($p < 0.05$). There was no significant difference in the total antioxidant levels. Nucleosome, TAS and TOS levels and OSI values are shown in the Table 2.

There was a positive correlation between the nucleosome level and PANSS positive subscale ($p = 0.028$, $r = 0.402$). There was a positive correlation between TAS and age ($p = 0.025$, $r = 0.289$), PANSS total ($p<0.001$, $r = 0.604$). There was a negative correlation between OSI and PANSS total ($p = 0.019$, $r = –0.427$), PANSS

Tab. 1. Socio-demographic characteristics of the study participants.

	Schizophrenia patients (n=30)	Control (n=30)	p
Age (mean±SD)	27.8±3.4	29.1± (6.1)	0.327
Gender n (%)			0.791
Woman	18 % (48.6)	19 % (51.4)	
Man	12 % (52.2)	11 % (47.8)	
Marital status n (%)			0.018
Married	7 % (23.3)	17 % (56.7)	
Single	21 % (70.0)	13 % (43.3)	
Widowed/Divorced	2 % (6.7)	0	
Education n (%)			0.063
Elementary school	13 % (43.3)	10 % (33.3)	
Secondary school	17 % (56.7)	15 % (50.0)	
High schol	0	5 % (16.7)	
Working status			0.108
Working	8 % (26.7)	14 % (46.7)	
Not working	22 % (73.3)	16 % (53.3)	

Tab. 2. Nucleosome, TAS and TOS levels and OSI value of cases.

	Schizophrenia patients (n=30)	Control (n=30)	p
Nucleosome (mean±sd)	2.9±0.7	2.6±0.4	0.014
TAS (median. min–max)	1.4. 0.8–4.0	1.2. 1–1.8	0.096
TOS (mean±sd)	17.9±10.6	10.3±4.8	0.001
OSI (median. min–max)	9.7. 2.7–55.7	7.2. 4–22.6	0.005

TAS – Total antioxidant status, TOS – Total oxidant status, OSI – Oxidative Stress Index

positive subscale (p = 0.043, r = –0.372). There was a negative correlation between TOS and PANS total (p = 0.028, r = –0.402).

Discussion

TOS level and OSI value were higher in schizophrenia patients compared to controls, but there was no difference in TAS levels. Studies examining the oxidative metabolism in patients with schizophrenia established that TOS level and OSI values and other oxidant parameters were significantly higher in patients with schizophrenia than those in controls (17–20). Although there are studies generally demonstrating increase of oxidants in patients with schizophrenia, a few have shown that there was no change (21, 22). Therefore, studies examining antioxidant parameters in patients with schizophrenia are conflicting. Some studies have shown increased antioxidant levels in patients with schizophrenia (23–26), while some have shown a decrease (22, 27, 28), and still some have found no change (29–31). Our results showed an increase in oxidant levels and no change in antioxidants in patients with schizophrenia.

Nucleosome level was higher in schizophrenia patients compared to controls. Nucleosome is considered to be a marker of apoptosis. Even though apoptosis is frequently determined in schizophrenia and other neurodegenerative diseases, nucleosome measurements have not been widely used. In a recent study, a susceptibility to apoptosis in antipsychotic-naive schizophrenia patients has been found (32). Another study showed an increased apoptosis in euthymic bipolar disorder patients (33). Apoptotic neurons have been shown in some neurodegenerative disorders like Alzheimer's and Huntington's diseases (4, 34, 35).

Oxidative products can cross the membranes and diffuse into the nucleus. They activate the endonucleases by increasing intracellular calcium and cause DNA fragmentation and apoptosis in nucleus (36). Dopamine's neurotoxic and neurodegenerative effects have been shown (6–9, 37). These effects are associated with oxidative metabolism (10–12, 37). Dopamine and 6-OH-DA might induce apoptosis in the presence of oxidants in various neural and non-neural cells (37).

Apoptosis can be activated by a variety of causes in addition to intracellular oxidants and oxidative stress (1, 10, 38). Fatty acid oxidation products are postulated to play a role in apoptosis in a review article (39). Dysregulation in oxidant metabolism such as reduced antioxidants and increased oxidants are observed in schizophrenia (22). Oxidative stress is implicated in the pathophysiology of schizophrenia. Lack of antioxidants might have a role in the aetiology by increasing the apoptosis and causing neuron loss. Several studies support this hypothesis showing a link between decreased GSH (which is an antioxidant enzyme) and increased apoptosis (40). Because of the role of oxidative stress in apoptosis, some antioxidants have been used for preventing apoptosis (41, 42)

We found a positive correlation between nucleosome level and PANSS positive subscale. To our knowledge, there is no data about nucleosome level and disease severity in psychiatric or neurological disorders. However, there is a correlation between disease severity and nucleosome level in some other diseases such as SLE, psoriasis and severe sepsis and septic shock (43–46).

The effect of antipsychotics in apoptosis is controversial. Haloperidol has been found to cause apoptotic neuronal death in a study (47). Atypical antipsychotics up-regulated bcl-2 mRNA which is a potent inhibitor of apoptosis in rat brain (4, 48). This means that typical antipsychotics induce and atypical antipsychotics inhibit apoptosis. However, in an experimental study antipsychotics including haloperidol, clozapine, and quetiapine were shown to activate caspase-3, which is a mediator of apoptosis without an increased DNA fragmentation. Also, it has been found that there was no association between increased Bcl-2 activity and these antipsychotics. This data suggests that antipsychotics may have non-lethal apoptotic effects (49). Another study shows that antipsychotics like haloperidol, eticlopride, raclopride, chlorpromazine and risperidone protect neuronal cells against cell death (50). Caspase-3 activity has been found to be higher in antipsychotic-naïve first episode schizophrenia patients (32). Antipsychotics can be a confounding factor in this study. Therefore, further studies are needed in patients with first-episode and drug naive schizophrenia patients.

In conclusion, in this study we found a correlation between nucleosome level and PANSS positive subscale. To our knowledge, this was the first study that evaluated oxidative stress and nucleosomes released from apoptotic cells together.

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