Article

Serum Levels of Oxidants and Protein S100B were Associated in First-episode Drug-naïve Patients with Schizophrenia

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ABSTRACT

Background: Patients with schizophrenia have been noted with an elevation of serum S100B protein concentration, but the pathological process is not known. This study was to investigate the relationship between levels of S100B protein and oxidative stress.

Methods: General information and blood sample were collected from the first-episode drug naïve or drug-free acute stage of patients who met the Diagnostic and Statistical Manual of Mental Disorders-IV (DSM-IV) criteria for schizophrenia and healthy controls. The serum levels of S100B, total oxidants (TOS) and malonaldehyde (MDA) were used to measure the level of oxidative stress in both patients and healthy controls. General linear regression analysis was performed to examine the association of S100B protein with the levels of oxidative stress.

Results: The levels of serum protein S100B were associated with the concentration of both TOS (Beta=15.77; *p*=0.0038) and MDA (Beta=7.90; *p*=0.0068) in the first-episode drug-naive patients (n=29).While both associations were no longer significant in the drug-free acute phase patients (n=29) (*p*>0.05), the levels of serum S100B was still consistently associated with TOS (Beta=12.42;*p*=0.0026) and MDA(Beta=4.11;*p*=0.0480) in the combined group of patients group(n=58). Simultaneous analysis of both oxidative markers, we still found that both TOS (Beta=12.88; *p*=0.0103) and MDA (Beta=6.46; *p*=0.0167) were associated with the serum level of protein S100B in the first-episode drug-naive patients, but not drug-free acute phase patients.

Conclusion: Our results suggest that astrocyte activity, serum levels of oxidants, and their cross-talking might be involved in the pathogenesis of schizophrenia. This warrants a further study for understanding the underlying mechanism.

KEYWORDS

Oxidative stress, S100B protein, schizophrenia, MDA, total oxidants

INTRODUCTION

Extensive evidence suggests that astrocytes play a crucial role in the central nervous system functions, which have been implicated in the dynamic regulation of neuron energy metabolism and production, synaptic network formation, neuron electrical activity, neurotransmitter release, and immune responses. S100B is a protein of the calcium-binding protein family that is localized in the cytoplasm and nucleus of cells and involved in the regulation of multiple cellular processes including cell cycle progression and differentiation. The protein S100B is glial-specific and secreted by astrocytes; it may function in neurite extension, the proliferation of melanoma cells, stimulation of Ca^{2+} fluxes, inhibition of protein kinase C (PKC)-mediated phosphorylation, astrocytosis, and axonal proliferation, and inhibition of microtubule assembly. S100B acts as a neurotrophic factor and neuronal survival protein in the developing central nervous system(CNS)(1); whereas it can spill from the injured cells and enter the extracellular space or bloodstream, and then cause an increase in the serum levels of protein S100B during the acute phase of brain damage.

While the protein S100B is considered a potential clinical marker for blood-brain barrier (BBB) permeability, CNS injury or presence of neurological conditions (2), evidence has also supported that protein S100B may play a role of immune pathology in the etiopathogenesis of schizophrenia(3), which has been believed involving brain-derived neurotrophic factor (BDNF) (4, 5), the immune and inflammatory changes (6, 7), the oxidative stress (8-11). The protein S100B has gained attention as a potential peripheral biomarker of schizophrenia (3, 12-17) and displays neuroprotective or neurodegenerative abilities that depend on its concentrations. At pico- and nanomolar concentrations, S100B promotes cell promotion and differentiation, but induces cellular death at micromolar concentrations (18-20). Elevated levels of S100B in blood have been linked to brain damage (21-24) and a group of psychiatric disorders including schizophrenia, major depressive disorder, bipolar and mood disorder (25-27). Most of these studies were based on the sample of serum, plasma, or cerebrospinal fluid in comparison with healthy controls (16, 28-30). Studies have indicated that dysfunction of glial cells, a decreased brain density of glial cells, and alterations in genes associated with astrocytes and oligodendrocytes might present a pathogenic factor in schizophrenia (15, 31). However, there is still a lack of consistent association of the levels of S100B with schizophrenia(32) or no association(33). Our recent study has shown that levels of S100B were elevated in the patients of first-episode drug-naive or acute-stage drug-free with schizophrenia in comparison with healthy controls (34).

The elevation of S100B may involve the inflammatory hypothesis of schizophrenia pathogenesis (13, 35, 36). Activated glial cells release a variety of pro-inflammatory mediators (e.g., cytokines) which can potentially contribute to neuronal dysfunction and result in the progression of the CNS pathology (37). In addition, S100B, expressed by subsets of CD3+ CD8+ T cells and natural killer (NK) cells, is suspected associated with the immune hypothesis of schizophrenia(13). Evidence has indicated that the level of S100B secretion is increased by pro-inflammatory cytokines in C6 glioma of cells and hippocampal slices of rats through the mitogen-activated protein kinase (MAPK) pathways; and the antipsychotic drugs such as haloperidol and risperidone can inhibit the secretion of S100B that is induced by interleukin (IL)-6 stimulation, in which oxidative stress is believed as a component (38).

Free radicals leading to elevated oxidative stress have similar property compared with S100B. Free radicals are species usually produced during cellular metabolism in aerobic cells. In the conditions of increase in production or decrease in scavenging of free radicals, an excessive free radical production can injure neurons in schizophrenia and affect their functions such as membrane transport, impairment of energy production in mitochondria, changes in membrane phospholipid composition, alteration of receptors and transporters as well as neurotransmission (39), which may be involved in the pathophysiology of patients with schizophrenia.

A few studies have investigated the correlation between the protein S100B and oxidative stress. Protein S100B is shown to suppress oxidative cell damage; whereas oxidative stress impairs the ability of S100 proteins to bind and activate protein phosphatase 5 (PP5), a serine/threonine phosphatase involved in oxidative stress responses (40). Studies have shown that a long-term exposure to high blood glucose concentrations leads to an increase in the TOS in the patients with diabetic ketoacidosis and that the neurotransmitter changes in response to this exposure lead to an increase in the levels of S100B (41). It is also found that copy number variations on chromosome 21 disrupted gene human serum albumin (HSA) 21 associated S100B contributes to oxidative stress and apoptosis in Down syndrome human neural progenitors (42) and the role of S100B in the development of cerebral vasospasm and brain damage may result from the induction of oxidative stress and neuroinflammation after subarachnoid hemorrhage (43). In investigating the serum levels of multiple oxidative stress biomarkers and S100B in patients with major depressive disorder (MDD) in an acute phase, only serum superoxide dismutase (SOD) and catalase (CAT) activities were found significantly higher compared with healthy subjects; the Hamilton Depression Scale (HAMD) scores have been positively associated with the levels of S100B (44).

In this study, we aimed to investigate the relationship between the level of protein S100B and oxidative stress status in first-episode naive drug patients with schizophrenia, which allows us to minimize the potential impact of confounding factors such as illness duration, medication effects, and the psychiatric and medical co-morbidities. We used malondialdehyde (MDA) and total oxidant status (TOS) in serum to measure the levels of oxidants. MDA is one of the end products of this self-perpetuating lipid peroxidation reaction, which is considered a specific and sensitive measure of lipid auto-oxidation(45); the level of TOS reflects the total effect of all oxidants existing in plasma or serum and body fluids(46).

METHHODS

Participants

Participants were recruited from Beijing Huilongguan Hospital, a Beijing-city owned and one of the largest psychiatric hospitals in China. Inpatients, who met the Diagnostic and Statistical Manual of Mental Disorders-IV (DSM-IV) criteria for schizophrenia (American Psychiatric Association, 1994) with an agreement of two senior psychiatrists using the Structured Clinical Interview for DSM-IV (SCID), were recruited. It has also been suggested that some antipsychotics, primarily typical antipsychotics may have pro-oxidant effects and increase the oxidative stress and oxidative cell injury(47). Kropp et al. reported that three weeks of treatment with typical antipsychotics might increase the plasma MDA levels compared with the atypical antipsychotics (48). To avoid potential confounding, two groups of patients were recruited. One group of patients were first-episode and had never taken antipsychotics (first-episode drug-naive); the other group of patients were acute stage and had not taken antipsychotics for

at least three weeks prior to the study (drug-free acute stage). Healthy subjects without current or past psychiatric disorders and matched on age and gender were selected from the local community, and Axis I psychiatric disorders was ruled out through psychiatric review evaluation by a psychiatrist.

A complete medical history and physical examination, laboratory tests including a urine and blood screen, and electrocardiogram were obtained from all participants, in order to rule out any neurological or other medical diseases. Neither the patients nor the control subjects had a diagnosis of alcohol or illicit drug abuse/dependence. Excluded criteria: 1) subjects who had another psychiatric disorder or drug and alcohol abuse or dependence, 2) subjects who was incapable of completing the investigation for any reason such as non-cooperation, and mental retardation, and 3) subjects who have a family history of diabetes or eating disorders either.

The ethics committee of Beijing Huilongguan Hospital approved the study (Number: 2015-012). After a complete description to all participants, the written informed consent was obtained.

Clinical evaluation

Information on general socio-demographics and clinical and psychological conditions were collected for each patient or subject with structured forms. Additional information was collected from available medical records or with assistance from a family member or relative and treating clinician. The patient's psychopathological symptoms were assessed using the Positive and Negative Syndrome Scale (PANSS) on the day of the blood draw, which was conducted independently by two psychiatrists. To ensure consistency and reliability of ratings across the study, two psychiatrists had simultaneously attended a training session in the use of the PANSS before this study started. After necessary training, an intra-rater correlation coefficient higher than 0.8 was required for the evaluation of PANSS total score. Brief psychiatric rating scale (BPRS) and the measurement and treatment research to improve cognition in schizophrenia (MATRICS) and Matrics Consensus Cognitive Battery (MCCB) were also assessed and collected for each patient at baseline as part of other studies. The concentration of MDA was measured in terms of nmol/gmHb. The levels of TOS was determined using a colorimetric method developed by Erel(46). The assay is based on the oxidation of ferrous ion to ferric ion in the presence of various oxidant species in acidic medium and the measurement of the ferric ion by xylenol orange. The assay is calibrated with hydrogen peroxide, and the results are measured in term of μmol H2O2 Equiv. /L.

Blood samples and Assays

Serum samples from all subjects were collected at 7 AM following overnight fasting for laboratory tests. 5 ml of the venous blood sample was taken from the antecubital vein of each participant. The blood sample in lithium heparin tube was centrifuged (4^{0}C, 3500rpm, 10min), aliquoted, and then stored at −70^{0}C until assayed. The same technician, who was blind to the clinical data, assayed all samples. The identity of all subjects was coded and main-

tained by the investigator until when all biochemical analyses were completed. Inter- and intra-assay variation coefficients were 7% and 5%, respectively.

Serum levels of S100B were measured within one week by the sandwich enzyme-linked immune sorbent assay (ELISA) using a commercially available kit (Ad Litteram Diagnostic Laboratories, Inc., San Diego, CA, USA). The detailed information has been described elsewhere (34). The levels of MDA were measured using monitoring thiobarbituric acid-reactive substances (TBARs) assay kit (Cayman Chemical Company, Ann Arbor, MI). The principle of the assay, based on the procedure by Dawn-Linsley, et al (49).

Statistical analysis

SPSS, Statistical Package for Social Sciences, for Windows 17.0 and R 3.2.0 Package (https://www.r-project.org/) were used for the statistical analysis. General linear regression analysis was performed to examine the association of S100B with the levels of oxidative stress as measured by TOS and MDA. The analysis was first performed using all participants together, with schizophrenia patients with the first episode drug-naive and acute phase drug-free patients with schizophrenia, and healthy controls, by fitting a group by levels of S100B interaction term while adjusting for age and sex, followed by a stratification analysis by the group. We then performed an analysis of patients only to maximize the statistical power and adjusting for age of onset (years), duration of illness, smoker status, and years of education. A threshold level of 0.05 was used for declaring statistically significant.

RESULTS

Table 1 presents the demographics and descriptive statistics of all measurement and patient characteristics including psychopathological symptom and cognitive measures. In total, the study included 29 schizophrenia patients with the first-episode drug-naive (Mean age=27.62; SD=7.18 years), 29 patients with the drug-free acute stage (Mean age=27.03, SD=7.68 years), and 50 healthy controls (Mean age=25.92, SD=6.69 years). There was no significant difference in mean age and distribution of sex among three groups (p>0.05). We noted significant differences in the levels of S100B, TOS, and cognitive functions as measured by MCCB composite score (p<0.01), but no significant difference in the levels of MDA $(p$>0.05) were found among the three study groups.

While no significant difference in the levels of protein S100B, TOS and MCCB composite score between the two groups of patients (i.e., drug-naive and drug-free patients), there were significant differences in these measures between patients and healthy control (Table 1). We noted a difference in psycho-pathological symptoms of PANSS Positive score and general psychopathology (p<0.05) between two patient groups. The first-episode drug-naive patients appear more severe in symptoms. Specifically, the schizophrenia patients of first-episode drug-naive had a higher PANSS positive score (Mean, 25.10; SD, 5.07) than the drug-free acute stage patients (Mean, 22.12; SD, 6.79), and a higher PANSS total score (Mean, 89.36; SD, 15.11) than drug-free group (Mean, 82.35; SD, 14.97).

Table 1. Demographics and descriptive statistics of serum biological markers and characteristics of the participants

	First episode drug-naive				Drug-free acute stage					Healthy control				
	N	Mean	Median	SD	N	Mean	Median	SD	Sig*	N	Mean	Median	SD	Sig**
Age (year)	39	27.62	27.00	7.18	34	27.03	26.00	7.68	n.s.	50	25.92	24.00	6.69	n.s.
Sex	39	0.59			34	0.59			n.s.	50	0.48	0.00	0.50	n.s.
S100B1(μg/L)	29	234.91	239.06	79.33	29	210.15	202.97	72.53	n.s.	50	117.72	108.50	46.91	**
Total oxidant capacity(μmol/L)	29	8.83	7.69	2.89	29	10.08	9.94	2.02	n.s.	50	15.66	15.36	4.26	**
Malondialdehyde (nmol/gmHb)	29	8.35	5.74	5.69	29	8.06	7.04	4.48	n.s.	50	6.41	4.34	4.11	n.s.
PANSS score														
Positive	39	25.10	24.00	5.07	34	22.12	21.50	6.79	*					
Negative	39	21.95	22.00	6.39	34	23.38	21.50	6.54	n.s.					
General	39	42.31	40.00	8.02	34	36.85	35.50	7.41	*					
PANSS total score	39	89.36	87.00	15.11	34	82.35	81.50	14.97	n.s					
Brief psychiatric rating score	29	47.69	46.00	7.12	29	42.76	43.00	7.64	*					
Smoker (%)	29	6.90			29	37.93			*					
Education (year)	29	12.65	12.00	2.92	29	12.48	12.00	2.31	n.s.					
Age at onset (year)	39	24.94	24.00	6.54	34	23.42	23.12	5.89	n.s.					
Total duration of illness(month)	39	31.15	20.00	33.83	34	46.29	34.00	47.14	n.s.					
MCCB composite score	35	44.74	45.00	11.19	27	45.07	44.00	11.08	n.s.	50	54.90	56.50	8.35	**

*, p<0.05, a significant difference between first episode drug-naive and drug-free acute stage of patients with schizophrenia;
**, p<0.01, a significant difference among three groups; n.s., non-significance (p>0.05);
Sig*, significance between first-episode drug naive and Drug-free acute stage;
Sig**, significance between all cases and healthy controls

Table 2. Regression analysis of oxidative stress with levels of S100B in the serum of patients with Schizophrenia

	Group	N	Beta	SE	t Value	P	R^2 (%)	P_{int}
TOS	Control	50	-0.524	1.606	-0.33	0.7458	0.22	0.0014
	Drug-naive	29	15.445	5.015	3.08	0.0050	25.28	
	Drug-free	29	11.256	6.143	1.83	0.0789	9.75	
MDA	Control	50	0.766	1.682	0.46	0.6510	0.43	0.0159
	Drug-naive	29	7.330	2.476	2.96	0.0066	23.86	
	Drug-free	29	-2.819	3.009	-0.94	0.3578	2.79	

P_{int}, the p value for the interaction between the group and oxidative stress; Analysis was performed by stratifying group while adjusting for age, sex and participant group.

In analysis of all groups together adjusting for age and sex, we found that levels of protein S100B were associated with the concentration of both TOS (Beta= 15.45; p=0.0050) and MDA (Beta=7.33; p=0.0066) in the first episode drug-naive patients with schizophrenia (Table 2). However, there was no association of TOS (Beta=11.256; p=0.0789) nor MDA (Beta=-2.819; p=0.3578) with protein S100B in the drug-free acute phase patients with schizophrenia; no signify-

cant association of either TOS (p=0.7458) or MDA (p=0.651) with S100B in the healthy controls. We noted a significant interaction between the participant group and TOS (p=0.0014) and between participant group and MDA (p=0.0159) on the levels of S100B in serum, suggesting that the association of serum oxidants and S100B was different between patients and healthy controls.
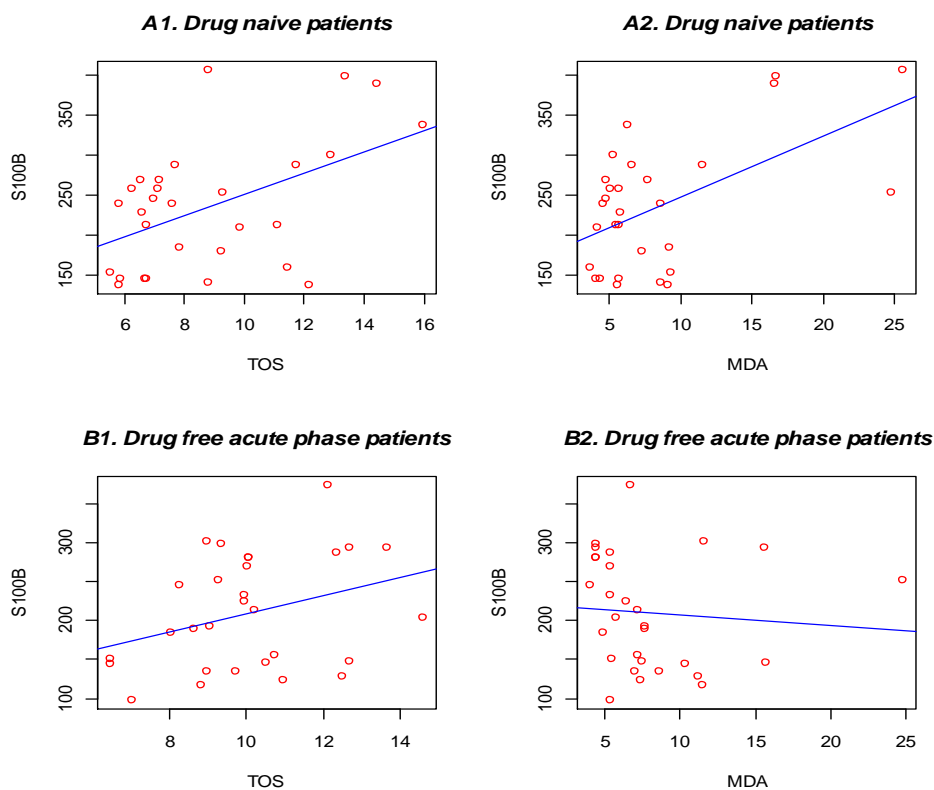

Figure 1. The best-fitted lines from the simple linear regression analysis of oxidants and levels of S100B in patients with schizophrenia (**A1**. TOS in the first-episode drug-naive; **A2**. MDA in the drug-free acute stage; B**1.** TOS in the first-episode drug-naive; **B2**. MDA in the drug-free acute stage).

In the patient specific-analysis, we found that the levels of S100B were associated with TOS (R^2=25.28%; Figure 1, A1)

and MDA (R^2=23.86%; Figure 1, A2) in the first-episode drug-naive patients. While the TOS was not associated

with the levels of serum protein S100B in the drug-free acute phase patients with schizophrenia (p=0.0789; R^2= 0.0975; Figure 1, B1), we noted a clear linear trend in the small size of sample (n=29). However, we find no trend of association of S100B with MDA (Figure 1, B2).

To control for potential confounding factors in patients characteristics such as smoke status, duration of illness and educations, we further confirmed the association of oxidants and levels of S100B in the patient-only analysis (Table 3). The associations of TOS with S100B became slightly more significant in both groups of the first-episode drug-naive (p= 0.0038) and drug-free acute phase (p=0.0670). In the combined patient groups, TOS was more significantly associated with S100B (p=0.0026), and there was no interaction between TOS and patient group (p=0.4390). However, we did not find any change in the association of MDA in either of patient groups. It is interesting to note that in the patient combined, MDA was

still associated with S100B (p=0.0480), but we noted a significant interaction between the patient group and MDA on the levels of S100B, which had been noted in the scatter plot (**Figure 1, A2 vs. B2**)

To further determine which oxidant measures were associated with S100B, we performed multiple regression analysis of two markers in the same regression model (**Table 3**). In the first episode drug-naive patients, both TOS (Beta=12.88; p=0.0103) and MDA (Beta =6.46; p=0.0167) were significantly associated with S100B. However, two markers were no longer significantly associated with S100B in the drug-free acute phase patients, although TOS showed a strong effect size (Beta=10.95; p=0.1116) on the levels of S100B. Combined together two groups of patients, only TOS was associated with S100B (Beta=12.83; p=0.0015), but not MDA (Beta= 2.52; p=0.1827).

Table 3. Multiple regression analysis of oxidants (TOS and MDA) and levels of serum S100B in patients with schizophrenia after further adjusting for potential confounding such as smoke status, duration of illness and years of educations.

	Group	Parameter	Beta	SE	t-Value	p
Simple regression						
	First-episode	TOS	15.77	4.87	3.24	0.0038
	Drug-free	TOS	12.71	6.59	1.93	0.0670
	Combined	TOS	12.42	3.91	3.17	0.0026
	Interaction	TOS*Patient group	6.33	8.11	0.78	0.4390
Simple regression						
	First-episode	MDA	7.90	2.65	2.99	0.0068
	Drug-free	MDA	-2.98	3.37	-0.89	0.3857
	Combined	MDA	4.11	2.03	2.03	0.0480
	Interaction	MDA*Patient group	8.86	4.20	2.11	0.0400
Multiple regression						
	First-episode	MDA	6.46	2.47	2.61	0.0167
		TOS	12.88	4.55	2.83	0.0103
	Drug-free	MDA	-2.29	3.16	-0.72	0.4778
		TOS	10.95	6.58	1.66	0.1116
	Combined	MDA	2.52	1.86	1.35	0.1827
		TOS	12.83	3.82	3.36	0.0015

DISCUSSION

In the present study, we found that the levels of oxidants measured by TOS and MDA in serum were associated with the elevated levels of serum protein S100B in patients with schizophrenia. The associations of S100B with both TOS and MDA were consistent in the first-episode drug-naive patients with schizophrenia, but no significant association in drug-free acute stage patients. We did not find that any significant association of oxidants with protein S100B in healthy controls.

Our findings that association of oxidants with the levels of protein S100B is consistent with the previous study (50). One speculation is that a higher level of oxidants causes an increase in the levels of serum protein S100B. The reactive

oxygen species (ROS) overproduction in myoblasts has been shown to cause an upregulation of protein S100B via nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kappaB) activation (51). In the high concentrations of oxidants, excessive reactive oxygen species (ROS) cause the damage on the components of the cells, including the proteins (enzymes, receptors), the lipids and deoxyribonucleic acid (DNA), which may consequently affect the apoptosis and the cell death(52-55). Oxidative stress is not only responsible for the neuron apoptosis but also can provoke the astroglial cell death. In the meantime, pituitary adenylate cyclase-activating poly-peptide (PACAP), a substance that can promote neuron survival, exerts a potent protective effect against the oxidative stress-induced astrocyte death. The anti-apoptotic activity of PACAP on the

astrocytes is mediated through the protein kinase A, PKC and MAPK transduction pathways, by the inhibition of the ROS-induced mitochondrial dysfunctions and caspase 3 activations. Therefore, the oxidative stress has toxic effects on the astroglial cells and then affects the synthesis and the secretion of S100B (56).

Conversely, the elevated level of protein S100B leads to an increase in oxidant levels. In the Down syndrome neural progenitor cells, constitutively over-expression of S100B leads to an increase in reactive oxygen species (ROS) formation and activation of stress response kinases(57). S100B stimulates inducible nitric oxide synthase in rat primary cortical astrocytes through a signal transduction pathway that involves activation of the transcription factor NF-kappaB (58). Furthermore, S100B-induced inducible nitric oxide synthase promoter activation was inhibited upon the mutation of the NF-kappaB response element in the promoter, whereas transfection of cells with an NF-kappaB inhibitor blocked the S100B-induced inducible nitric oxide synthase promoter activation and nitric oxide production. These studies define a signal transduction pathway by which S100B activation of glia could participate in the generation of oxidative stress in the brain. The astroglial cells are, the most important source of the free radicals in the CNS (59) and S100B was primarily expressed in astrocytes. High glucose environments enhanced the pentose phosphate pathway (PPP) in the astroglia, reducing the ROS production and thereby play a neuroprotective role (60). Activated astroglial cells produce large amounts of nitric oxide (NO) which, through the binding to the soluble guanylyl cyclase, rapidly increases the cyclic guanosinc monophosphate (GMP) concentrations. The stimulation of these glucose-metabolizing pathways by NO would represent a transient attempt by the glial cells to compensate for the energy impairment and the oxidative stress, and thus to emerge from an otherwise pathological outcome (61). These point out that the increased S100B may be a source of the oxidative stress or a compensatory response to the oxidative stress.

It is also likely that there is no direct interaction between the oxidative stress and the S100B and both may be associated with the neurodegenerative process (62). Studies have indicated a neurodegenerative component in schizophrenia (63, 64); and the accelerated aging in schizophrenia was supported by the structural and the functional brain abnormalities, the higher incidence of the aging-associated metabolic profiles and disease(65), the early cognitive decline, and the altered telomere dynamics(66). In addition, the oxidative stress and the mitochondrial dysfunction play essential roles in the pathogenesis of the neurodegenerative disorders(67) may also support the "accelerated aging" in schizophrenia. On the other hand, in an S100B over-expressing mouse model of pathological aging, the antioxidant vitamin E is shown to increase S100B-mediated microglial activation (68). There is inconsistent evidence that dietary compounds (such as trans-resveratrol) can inhibit or reverse oxidative stress, and the reduction of S100B secretion induced by H_2O_2 was not changed by resveratrol (69). In cirrhosis repre-sents, a negative correlation between S100B and oxidative stress

measured by the thiobarbituric acid method in the case group was found (70). The inconsistent results were interpreted by the difference in the assay methods on oxidative stress.

In summary, we observed that the levels of the serum protein S100B was associated with the concentration of both TOS and MDA in the first episode drug-naive group and the association of protein S100B with TOS was weaker and no association with MDA in the drug-free group.

Limitations

Our results should be read with care. This was only based on the baseline measures of S100B and TOS or MDA. We only analyzed two measures of oxidant levels: TOS and MDA but did not measure the antioxidant levels, the imbalance of antioxidants and oxidants determine the level of oxidative stress. In addition, our sample size is still limited in both inpatient groups and healthy controls, and we did not collect detailed information on smoking history except for the current smoke status, other medications, etc. To gain statistical power and test the possible causal relationship, a therapeutic study would help validate these findings.

CONCLUSION

Our study indicates that the high level of oxidants is associated with an elevated level of protein S100B. This warrants a further study for validation of this association and examines the potential causal relationship.

CONFILICT OF INTEREST

We declare that there is no conflict of interest regarding the publication of this paper.

ACKNOWLWDGEMENTS

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