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Clinical implications of oxidative stress in schizophrenia: Acute relapse and chronic stable phase

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ABSTRACT

Several studies have suggested a higher oxidative stress in schizophrenia. However, the implications of oxidative stress on clinical symptoms remain unclear. This study aimed to investigate the platelet oxidative stress in different stages of schizophrenia (i.e., chronic stable and acute relapse) in order to clarify the clinical implications of oxidative stress and the treatment effects. We recruited 43 chronic stable patients with schizophrenia and 48 non-psychiatric controls. Platelets were collected for measuring the levels of nitric oxide (NO), lipid peroxidation (LPO), and glutathione (GSH) and the activity of GSH peroxidase (GPx) and superoxide dismutase (SOD). The levels and activity were compared between patients and controls and were examined for their relationship with clinical severity. Further, we evaluated the changes of levels and activity before and after treatment in an independent sample with acute relapse ($N = 19$). Patients with chronic stable schizophrenia had lower SOD activity compared to non-psychiatric controls. In chronic stable patients, NO level was positively correlated with positive and disorganized symptoms, while the GPx activity were negatively correlated with excitement. In patients with acute relapse, the levels and activity were not different before and after four weeks of antipsychotic treatment, but LPO level was negatively correlated with pretreatment disorganized symptoms. The change of LPO can also predict the change of disorganized symptoms and negative symptoms. Our findings suggest that platelet SOD was lower in chronic stable schizophrenia. Platelet LPO may be associated with less disorganized symptoms in acute relapse patients and better treatment response.

1. Introduction

Oxidative stress is a hallmark of neurodegenerative diseases including schizophrenia (Sawa and Sedlak, 2016). The pathological consequences of oxidative stress, such as oxidative damage of cell lipids, proteins, enzymes, carbohydrates and DNA (Gutteridge and Halliwell, 2000), may play important roles in the pathophysiology of schizophrenia (Padurariu et al., 2010).

Recent studies revealed that patients with schizophrenia were in a lower antioxidant status with impaired antioxidant-enzyme activities (Miljevic et al., 2010; Nucifora et al., 2017). However, previous studies were inconsistent on specific antioxidant activities, particularly in the case of superoxide dismutase (SOD) that both decreased (Raffa et al., 2009) and increased activities (Yao et al., 1998) were reported in untreated patients; also, both decreased (Dietrich-Muszalska et al., 2005) and increased (Miljevic et al., 2010; Padurariu et al., 2010) activities were reported in schizophrenia patients who were under treatment. In contrast, glutathione peroxidase (GPx) activity and glutathione (GSH) level (Dietrich-Muszalska and Olas, 2009; Nucifora et al., 2017) were consistently decreased in treated (Raffa et al., 2009; Miljevic et al., 2010; Padurariu et al., 2010; Nucifora et al., 2017) or untreated patients (Raffa et al., 2009). A meta-analysis of 44 studies (Flatow et al., 2013) addressed the importance of clinical status and sample sources, showing that plasma SOD activity was significantly increased in first-episode psychosis (FEP) and decreased in stable outpatients, but decreased in red blood cell (RBC) across the clinical status. On the other

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hand, nitric oxide (NO) and lipid peroxidation (LPO) are frequently chosen as indicators for oxidative stress. A meta-analysis of 27 studies (Zhang et al., 2010) indicated that NO and LPO levels were significantly increased in schizophrenia. Taken together, the enhanced oxidative stress might be attributed by impaired antioxidant activity accompanied with an excess of oxidative products. Therefore, oxidative stress parameters have been suggested as biomarkers of schizophrenia risk (Yao et al., 2000). Whether the oxidative/anti-oxidative status relates to clinical status of schizophrenia is of particular interest.

Total anti-oxidative status has been shown to be negatively correlated with negative symptoms (Li et al., 2011). However, the implications of oxidative stress on other symptom dimensions and severity are still far from clear. To our knowledge, only few studies focused on the relationship between oxidative stress and specific clinical symptoms, and replicated findings were even scarce (Flatow et al., 2013). Among these, SOD and GPx activities have been reported to be lower in chronic patients who were stable for 12 months (Zhang et al., 2006), while serum SOD, GPx activity and GSH levels were shown to correlate with overall severity in acute phase (Tsai et al., 2013). Increased peripheral SOD activity has been shown to correlate with positive symptoms (i.e., hallucinations, delusions, and disorganized behavior), and is reduced upon administration of antipsychotic medications (Zhang et al., 2003; Zhang et al., 2006). The SOD activity in the CSF of recent-onset psychosis was 26.5% lower than controls and was associated with cognitive deficits (Coughlin et al., 2017). Beyond SOD and GPx, the influence of the other oxidative parameters such as NO and LPO on clinical severity is still unclear.

Another important issue is the potential confounding factors that may influence the oxidative/anti-oxidative status, including smoking, body mass index (BMI) and regular consumption of antioxidants such as vitamin E and C, green tea, etc. (Hayat et al., 2015; Mitra et al., 2017). Clinical interventions have been attempted to mitigate the effects of lipid peroxidation, such as administration of eicosapentaenoic acid (Fusar-Poli and Berger, 2012) and long-chain omega-3 fatty acid (Amminger et al., 2010). In a systematic review, psychotic symptoms rated on the Positive and Negative Syndrome Scale (PANSS) or the Brief Psychiatric Rating Scale (BPRS) were lower in those taking adjunctive antioxidants (Magalhaes et al., 2016). These studies suggest the relevance of controlling antioxidant supplement in study design. However, only few previous studies addressed these concerns in investigating oxidative stress of schizophrenia (Flatow et al., 2013), that might partly explain the inconsistent results among the studies.

In this study, we aimed to explore the altered oxidative level and antioxidant activity in the patient blood platelets. Platelet has been proposed as a possible window into brain biological processes in schizophrenia (Berk et al., 1999; Berk et al., 2000; Asor and Ben-Shachar, 2012), and a surrogate disease model of several neuropsychiatric and neurodevelopmental disorders including Alzheimer's disease (Borroni et al., 2001; Borroni et al., 2003; Casoli et al., 2010; Evin and Li, 2012; Talib et al., 2012; Veitinger et al., 2014), depression (Berk et al., 2001; Ehrlich and Humpel, 2012), and autism (Pellerin et al., 2018). Platelets appear to be one of the most promising cell models for neurobiological studies owing to their close biochemical similarities with neurons in terms of possessing monoamine oxidase, serotonin reuptake, and neurotransmitter receptors (Goubau et al., 2013), their neuronal embryological origin (Dreux and Launay, 1985), as well as mechanisms of exocytosis (Dreux and Launay, 1985; Berk et al., 2000; Plein and Berk, 2001; Dietrich-Muszalska and Kwiatkowska, 2014). Blood platelets, like other circulating blood cells, can generate reactive oxygen species (Forde and Fitzgerald, 1997). Although blood platelets are considered to be a peripheral biomarker of psychiatric illnesses (Berk et al., 2000; Plein and Berk, 2001) and may serve as an appropriate model for investigating oxidative stress (Dietrich-Muszalska and Kwiatkowska, 2014), studies using this sample source are far less than using RBC, plasma or serum. Limited studies revealed a decrease of GPx (67%) and SOD activity in blood platelets of schizophrenia patients and a correlation between SOD activity and lipid peroxidation (Dietrich-Muszalska and Kwiatkowska, 2014). As a peripheral cell model for neurons in the brain, how the altered platelet oxidative/anti-oxidative status correlate with clinical severity remained to be explored.

This study investigated the platelet oxidant levels and antioxidant activities in different stages of schizophrenia (i.e., chronic stable and acute relapse) in order to clarify the clinical implications of oxidative stress. We collected clinical data and measured the oxidant levels and antioxidant activities for patients who are chronic stable or with acute exacerbation. Smoking status, BMI, dietary habit, and antioxidant consumption were included for considerations to ensure these potential confounding factors were comparable between the two groups. We also examined whether the consequence of antipsychotic treatment was correlated with the altered enzyme activities. Our hypothesis is that patients with schizophrenia, after controlling for the potential confounding factors, showed impaired antioxidant activity in blood platelets. The alterations of oxidative/anti-oxidative system are correlated with clinical symptom severity. Antipsychotic treatment may change both clinical severity as well as oxidative/anti-oxidative status in acute phase patients.

2. Methods

2.1. Participants

The protocol of this study was approved by the Research Ethics Committee of National Taiwan University Hospital. The study was carried out in accordance with the latest version of the Declaration of Helsinki. Written informed consents were obtained from all participants after the procedures had been fully explained. The inclusion criteria included (1) a diagnosis of schizophrenia, and (2) age from 18 to 65. Schizophrenia was diagnosed based on the Diagnostic and Statistical Manual of Mental Disorders-IV criteria by two senior psychiatrists. Chronic stable patients were patients who had a diagnosis of schizophrenia for > 5 years; their symptom severity were not changed for at least 6 months and the medications were not changed during the same period. Chronic stable patients were enrolled from outpatient clinics and day care unit in the Psychiatric Department of National Taiwan University Hospital. The non-psychiatric controls, recruited by advertisement, were within the same age range and without any psychiatric diagnosis. Controls having any major systemic illness, neurological conditions, substance abuse, and recent infection or allergy were excluded. This study recruited 43 chronic stable patients with schizophrenia (37.7 ± 10.6 years; males n = 23, 53.5%) and 48 non-psychiatric controls (37.1 ± 11.2 years; males n = 23, 47.9%). Chronic stable patients had an age of onset at 22.5 ± 6.5 years, with illness duration 15.3 ± 8.8 years.

To examine the relationship between oxidative stress and symptom change after antipsychotic treatment, we recruited 19 patients within an acute schizphrenic episode (37.9 ± 10.7 years; males n = 10, 52.6%). The acute patients were recruited within 2 weeks of symptom relapse who required admission for intensive treatment. The age of onset of acute patients was 25.3 ± 6.5 years, with illness duration 12.6 ± 9.8 years.

Chronic stable patients were rated on PANSS by a psychiatrist at the same time upon blood withdrawal. Patients with acute relapse were rated on PANSS on the day of recruitment and after 4 weeks of treatment, at the same time point for blood withdrawal. Smoking, BMI, diet pattern, and antioxidant consumption were collected by face-to-face interview. Cigarette smoking was defined by frequency; patients who smoked more than three times a week in recent 3 months were categoried into current smoking. Diet content was obtained by asking the daily meals in recent 3 months. Diet patterns were categorized into vegetarian (100% vegetables), mostly vegetables (> 75%), balanced (around 50%), and mostly meat (vegetables < 25% meals). As for antioxidant consumption, patients were requested to bring all the samples

and directions of the nutrition supplements they regularly took (more than three times a week). Here we recorded the most frequently taken items in Taiwan, such as vitamin C & E, green tea, and other tea (i.e. black tea and oolong tea). There was no participants taking omega-3 fatty acid or *N*-acetyl cysteine. None of the subjects or patients received aspirin or any other antiplatelet drugs, either.

2.2. Measurement

Platelets were collected (Berk et al., 1999; Dietrich-Muszalska and Olas, 2009) for each participant by sampling peripheral blood from an antecubital vein. Platelet-rich plasma (PRP) was prepared by centrifugation of fresh human blood at 250*g* for 10 min at room temperature. Platelets were then sedimented by centrifugation at 500*g* for 10 min at room temperature. The collected platelets were frozen at −80 °C until used. The activities of SOD and GPx and levels of LPO, NO and GSH were estimated by measuring the amount of products of the enzyme reaction by the enzyme-linked immunosorbent assay. We used BCA kit to measure the amount of total protein at the same time to standardize the measurement in per mg protein. Calibration curves were built for each oxidant and each antioxidant.

The SOD activity (unit/mg protein) was measured by using RANSOD kit (RANDOX) that contained xanthine, xanthine oxidase, and 2-(4-iodophenyl)-3-(4-nitrophenol-5-phenyltetrazolium) chloride (INPT) (Raffa et al., 2009). The reaction generated $O_2^{\cdot}$, that could be reduced by SOD. The color change of INPT measured at OD_{510nm} was then translated into SOD activity (unit/mg protein).

The LPO activity was measured by the method described by Scheffer et al. (Scheffer et al., 1995), in that malonyldialdehyde (μM/mg protein) was quantified by using BCA kit that contained *N*-methyl-2-phenylindole in acetonitrile and HCl. The amount of product was measured at OD_{590nm}.

The NO level was quantified by the method previously reported (Chuu et al., 2001). In short, we added $HClO_4$ to the sample to precipicate proteins. After centrifuge at 10,000 *g*, the supernatant was applied to NO analyzer (NOA-280). NO_2^- and NO_3^- were reduced to NO^- by VCl_3, and then oxidized to $NO_2^{\cdot}$ by $O_3^{\cdot}$ The level of $NO_2^{\cdot}$ was measured and compared to the standard curve of $NaNO_2$ (2-20 μM) to estimate NO amount in the unit of μM/mg protein.

Total GSH was measured by using Bioxytech GSH-420 assay kit (Terpstra et al., 2003) that contained 2-nitrobenzoic acid, glutathione reductase and NADPH, then the product 2-nitro-5-thiobenzoic acid was quantified at OD_{414nm} (nmol GSH/mg protein).

The GPx activity was measured by the method that has been described (Miljevic et al., 2010). The GPx kit containted xanthine and xanthine oxidase that generated $O_2^{\cdot}$; $O_2^{\cdot}$ then reduced by GPx and GSH into H_2O_2 and GSSG with consumption of NADPH. OD_{340nm} was detected and the activity of GPx was presented by nmols/mg protein. The techniques used in this study were validated in several studies on platelets, e.g., (Dietrich-Muszalska and Kwiatkowska, 2014).

2.3. Clinical assessment

All patient were interviewed to assess their clinical symptoms using the PANSS (Kay et al., 1987). The chronic stable patients were assessed for one time when their blood was sampled, while the patients with acute relapse were assessed for twice, at baseline when they were recruited and at 4 weeks after the treatment. The symptoms rated by PANSS were analyzed on a four-factor structure proposed in our previous study (Hwu et al., 2002). The four symptom subscores include positive symptoms (P3 + G9 + P1 + P6), negative symptoms (G7 + N6 + G13 + N1 + N4 + N2 + N3 + N5 + G10), disorganized symptoms (P2 + G11 + N7 + G15), and excitement (G4 + G16 + G8 + P7 + S2 + G14 + S1 + S3 + P4).

2.4. Statistical analysis

The age, body weight, and BMI were compared between chronic stable patients and controls by the Student's *t*-test. The sex, smoking status, dietary habit, consumption of vitamin C or E, green tea and black tea were compared between chronic stable patients and controls by the Chi-square or Fisher exact test (if any cell was under 5). The activity of SOD and GPx and the levels of LPO, NO, GSH were compared between chronic stable patients and controls using general linear model, with adjustment of sex, age, smoking, and BMI. For acute relapse patients, the oxidative/antioxidative activity were compared between baseline and at 4 weeks by non-parametric Wilcoxon signed rank test (n = 19). The relationship between oxidative/antioxidative activity and PANSS subscores was tested by Spearman's test, partial out age, gender, and smoking. The relationships between the changes of symptoms severity (before − after) and the changes of oxidative levels and antioxidant activity (before − after) were examined by general linear regression model.

3. Results

In chronic stable patients, age and sex were comparable with controls. Patients had significantly higher body weight and BMI in average, higher rate of cigarette smoking, yet fewer education years compared to their counterparts. The pattern of diet habit and antioxidant consumption were not statistically different from controls (Table 1).

3.1. Chronic stable patients vs. controls

Chronic stable patients showed a significantly lower activity of SOD compared to controls, but had similar levels of LPO, NO, GSH, and GPx activity after adjustment for sex, age, smoking status and BMI (Table 2). There were significant correlations between SOD, LPO, NO and GSH in controls except for that between GSH and NO, whearas, only NO and LPO were significantly correlated with each other in chronic stable patients (Supplementary Table 1). The patients treated with atypical

Table 1
Demographic data patients with schizophrenia and non-psychiatric controls.

	Chronic schizophrenia (N = 43)		Non-psychiatric controls (N = 48)		Acute relapsed schizophrenia (N = 19)	
	Mean/N	SD/%	Mean/N	SD/%	Mean/N	SD/%
Age	37.7	10.6	37.1	11.2	37.9	10.7
Sex (male%)	23	53.5	23	47.9	10	52.6
Body weight*	70.4	13.0	65.2	11.8	68.3	18.5
Body mass index**	26.0	4.4	23.6	2.9	24.8	5.3
Education***	13.0	2.7	15.4	2.3	14.3	1.6
Smoking(%) [a]**	9	20.9	1	2.1	2	10.5
Diet						
Vegetarian[a]	2	4.7	2	4.2	0	0.0
Mostly vegetables[a]	4	9.3	0	0	0	0
Balanced	25	58.1	36	75.0	16	84.2
Mostly meat	12	27.9	10	20.8	2	10.5
Vitamin C or E	7	16.3	6	12.5	1	5.3
Green Tea	6	14.0	8	16.7	2	10.5
Tea	8	18.6	16	33.3	0	0.0
Age onset	22.5	6.50	–	–	25.3	6.5
Atypical antipsychotics	25	59.5	–	–	15	78.9

Note. SOD, superoxide dismutase; LPO, lipid peroxidation; NO, nitric oxide; GSH, glutathione; GPx, GSH peroxidase.
[a] Fisher exact test.
* : $p < .05$;
** : $p < .01$;
*** : $p < .001$.

Table 2
Comparisons of oxidants/ antioxidants levels/ activities between patients with schizophrenia and non-psychiatric controls.

	Schizophrenia (N = 43)		Non-psychiatric controls (N = 48)	
	Mean	SD	Mean	SD
SOD*	1.01	0.67	1.32	0.50
LPO	250.4	44.6	252.1	60.0
NO	37.3	9.6	33.7	11.3
GSH	6379	3470	6529	2932
GPx	74.8	42.5	64.5	33.8

Note. SOD, superoxide dismutase; LPO, lipid peroxidation; NO, nitric oxide; GSH, glutathione; GPx, GSH peroxidase.
* $F(4, 84) = 5.81$, $p = .018$ after adjustment for sex, age, smoking and BMI.

Table 3
Spearman's correlation coefficients between oxidants/ antioxidants levels/ activities and PANSS subscores in chronic schizophrenia.

Symptom subscores (Mean ± SD)	Positive (9.7 ± 4.1)	Negative (20.1 ± 6.5)	Disorganized (9.1 ± 3.2)	Excitement (12.8 ± 4.4)
SOD	0.00	−0.03	0.16	−0.14
LPO	0.19	0.01	0.18	0.00
NO	0.36*	0.18	0.32*	0.11
GSH	0.03	−0.00	0.10	−0.24
GPx	0.09	0.00	0.01	−0.33*

Note. SOD, superoxide dismutase; LPO, lipid peroxidation; NO, nitric oxide; GSH, glutathione; GPx, GSH peroxidase.
*: $p < .05$.

antipsychotics were not different from those treated with typical antipsychotics in the oxidative/anti-oxidative levels (data not shown).

In chronic stable patients with schizophrenia, the NO level was significantly positively correlated with the severity of positive symptoms and disorganized symptoms; while GPx activity were significantly negatively correlated with excitement symptoms (Table 3).

3.2. Acute relapse patients: pre-treatment vs. post-treatment

In patients with acute relapse, the subscores of four symptom dimensions all significantly decreased after 4-week antipsychotics treatment, while oxidant levels and antioxidant activities were not significantly different between before and after four weeks of antipsychotics treatments (Table 4). Compared to chronic stable patients, acute relapse patients were not different on oxidant levels and antioxidant activities, but had a significantly lower NO level (Supplementary Table 2). At baseline, there were significant correlation between SOD, LPO, NO and GSH except for that between GSH and NO, but after 4-week treatment, the correlation pattern changed (Supplementary Table 3).

Table 4
Comparisons of symptoms and oxidants/ antioxidants levels/ activities change between before treatment (day 0) and after antipsychotic treatment (day 28) in patients with acute relapse.

	Day 0		Day 28		Signed rank test	
	Mean	SD	Mean	SD	S	p
PANSS						
Positive	16.11	3.49	11.13	3.05	66.5	< 0.0001
Negative	25.00	8.56	19.75	5.43	39.0	0.001
Disorganized	11.79	5.41	9.06	3.43	36.5	0.002
Excitement	20.89	8.69	13.75	4.67	60.0	< 0.0001
Platelet levels/activity						
SOD	0.91	0.44	0.62	0.32	16.5	0.273
LPO	245	50	238	55	−10.5	0.497
NO	28.7	10.2	29.9	11.4	−7.5	0.670
GSH	6429	1545	6206	1217	6.0	0.677
GPx	48.2	28.9	36.0	24.6	3.0	0.831

Note. SOD, superoxide dismutase; LPO, lipid peroxidation; NO, nitric oxide; GSH, glutathione; GPx, GSH peroxidase.

Table 5
Spearman's correlation coefficients between oxidants/ antioxidants levels/ activities and PANSS subscores in patients with acute relapse.

Symptom subscores (Mean ± SD)	Positive (16.1 ± 3.5)	Negative (25.0 ± 8.6)	Disorganized (11.8 ± 5.4)	Excitement (20.9 ± 8.7)
SOD	−0.25	−0.12	0.03	−0.32
LPO	−0.20	−0.45	−0.47*	−0.35
NO	−0.03	−0.43	−0.21	−0.06
GSH	0.22	−0.17	0.18	0.02
GPx	−0.03	−0.22	−0.33	−0.19

Note. SOD, superoxide dismutase; LPO, lipid peroxidation; NO, nitric oxide; GSH, glutathione; GPx, GSH peroxidase.
* $p < .05$.

3.3. Changes of symptoms vs. treatment response

The pretreatment disorganized symptoms were negatively correlated with LPO (Table 5). Percent change (before − after) of LPO level was negatively correlated with the change of disorganized symptoms ($\beta = -8.38 \pm 3.32$, $F(1,14) = 6.37$, $p = .024$, $R^2 = 0.313$) and negative symptoms ($\beta = -16.77 \pm 5.48$, $F(1,14) = 9.35$, $p = .009$, $R^2 = 0.400$) (Fig. 1). The increase of LPO level was positively correlated with the improvement of symptom subscores of disorganized and negative symptoms.

4. Discussion

As one of first studies investigating platelet oxidative stress in schizophrenia patients, this study had three major findings. First, in chronic stable patients, SOD activity was lower than that of non-psychiatric controls; NO was positively correlated with positive and disorganized symptoms, while the GSH level and GPx activity were negatively correlated with excitement symptoms. Secondly, significant correlations among the oxidant levels and antioxidant activities were noted in non-psychiatric controls, but not in chronic stable patients. Thirdly, for patients with acute relapse, although oxidants and antioxidant activities were not changed after treatment; baseline LPO level was associated with disorganized symptoms. After antipsychotic treatment, increased LPO level predicted better improvement on disorganized and negative symptoms. Collectively, the balance between oxidative stress and anti-oxidative capacity may potentially modify clinical symptoms of schizophrenia and treatment effect.

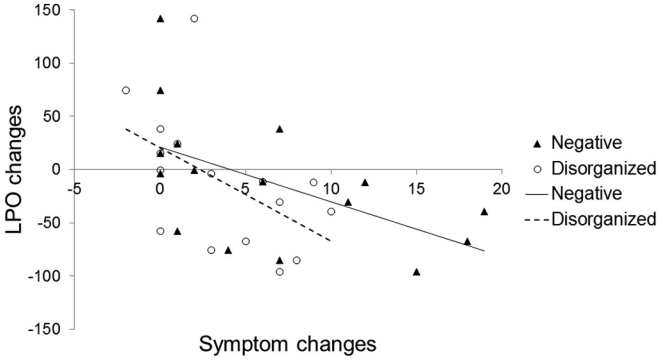

Fig. 1. Percent change of lipid peroxidation (LPO) can predict the improvement of disorganized symptoms and negative symptoms.

Our finding of lower platelet SOD activity in chronic stable patients was consistent with the previous report (Dietrich-Muszalska et al., 2005) that showed a significantly lower platelet SOD activity compared to controls. That study also found a significant correlation between the SOD activity and LPO level (Dietrich-Muszalska et al., 2005). Our data supported such correlation but only in acute phase rather than chronic stable phase, suggesting that the lower SOD activity in chronic stage may not be related to the consequence of lipid peroxidation. On the other hand, unlike a previous study showing suppressed GPx activity in the platelets (Dietrich-Muszalska and Kwiatkowska, 2014), we did not find significant difference in GPx activity, or the other oxidants (i.e., NO and LPO) or antioxidants (i.e., GSH). That study recruited samples (aged 18–36) younger than ours (mean age 37), with shorter illness duration (mean 4 years) than ours (> 10 years). Whether the lack of GPx suppression and the lack of SOD − LPO correlation can be attributable to older age, longer duration of illness, or longer exposure to antipsychotics waits to be clarified. In addition, regarding sample sources, our findings in platelets were similar to the previous meta-analysis from RBC (Flatow et al., 2013) which concluded that SOD activity was significantly decreased ($p < .01$) whilst GPx was not different from controls in stable outpatients. Taken together, the findings in platelets combined with previous research in RBC indicate an imbalance of the oxidative and anti-oxidative systems with reduced antioxidant activity in patients with schizophrenia.

Our novel approach to explore the interaction between oxidants and antioxidants revealed interesting findings. The significant correlations between levels or activity of oxidants/antioxidants found in controls were not noted in chronic stable patients. More intriguingly, such correlations still presented in patients of acute relapse, however, the correlation disappeared when symptoms improved after four weeks of treatment. This phase-specific phenomenon has not been reported yet. Whether the correlations between oxidants and antioxidants change with disease status and illness duration, or antipsychotic treatment mitigate the relationship between the oxidants/antioxidants warrants further investigations. Our findings might suggest that both oxidant level and antioxidant activity are mobilized simultaneously in acute relapse phase with a highly interactive pattern, while in chronic stage, the interactive pattern weakens, implying that the alterations in chronic phase of schizophrenia may not only occur in anti-oxidative system, but also in the dysregulation between oxidative and anti-oxidative systems. Other factors such as cytokines may also have their roles in regulating the network of oxidative and anti-oxidative processing but are out of the scope of this study.

Regarding the relationship with symptom severity in chronic stable patients, we found that GPx were negatively correlated with the severity of excitement. Previous studies have shown that plasma GSH level were inversely correlated with the overall symptom severity in patients with schizophrenia (Raffa et al., 2009; Tsai et al., 2013; Nucifora et al., 2017) but no association between plasma GPx and clinical psychopathology (Zhang et al., 2006; Tsai et al., 2013). Two studies specifically revealed an association between GSH level and negative symptoms (Matsuzawa et al., 2008; Tsai et al., 2013). Our findings support that chronic stable patients who had lower platelet GPx activity, but not GSH level, demonstrated higher excitement symptoms. Whether GSH/GPx system might be considered as a biological indicator of clinical severity of schizophrenia as previously proposed (Raffa et al., 2009; Nucifora et al., 2017) is of particular interest. On the other hand, our finding that NO was positively correlated with positive and disorganized symptoms is noteworthy. NO, a strong oxidant which is able to form hydroxyl radicals and turn into nitrogen dioxide (Wu et al., 2013), plays a pivotal role in the balance of antioxidants and free radicals by stimulating lipid peroxidation and mediates antioxidant reactions in cellular membranes at the same time (Radi et al., 1991). Such notion was supported by our finding that platelet NO level was correlated with LPO (in both acute and chronic phases) and GPx (in acute phase). The clinical correlates of platelet NO warrants further investigation.

In acute relapse patients, only the LPO level was correlated with the severity of disorganized symptoms, consistent with our clinical observation of a less disorganized manifestation in the relapse triggered by acute stress. By contrast, the disorganized/hebephrenic subtype of schizophrenia has been shown with insidious onset and poor premorbid function (Winokur, 1975; Winokur and Tsuang, 1975). Given that LPO increased under acute stress not only in the periphery (Sahin and Gumuslu, 2004; Sahin et al., 2004) but also in the striatum (Mendez-Cuesta et al., 2011) in rat models, whether the LPO level related to more abrupt relapse triggered by stress condition needs further study. Furthermore, our finding that increased LPO level after treatment was associated with greater improvement of negative symptoms and disorganized symptoms may indicate a condition characterized by acute stress but accompanied by a better resolution of negative and disorganized symptoms (Kapur and Pandurangi, 1979). LPO was one of the main consequences of oxidative reactions regulated by SOD, catalase, and GPx, like revealed by the correlations between LPO, GSH, and SOD of our data. The associations observed between the LPO level and disorganized symptoms and between LPO increase and symptom improvement suggest that oxidative stress represented by LPO may be influenced by the clinical phases of schizophrenia.

All the level/activity of oxidants/antioxidants decreased after treatment but without statistical significance. Although some studies found no change of oxidative activity after treatment (Yao et al., 1998; Sarandol et al., 2007; Virit et al., 2009), many others suggested that antipsychotics may influence oxidative status (Zhang et al., 2006; Zhang et al., 2012). Both risperidone and haloperidol reduced the elevated blood SOD activity and may normalize abnormal free radical metabolism in patients with schizophrenia (Zhang et al., 2012). Several studies have shown that the LPO level appears to be higher after treatment with classical antipsychotics (Kropp et al., 2005) while no change or even decreased when treated with atypical antipsychotics up to 90 days (Evans et al., 2003; Wei et al., 2003). Future studies may address the antipsychotic effects of different categories and various treatment duration.

Several limitations should be considered in this study. Our sample was not first episode psychosis and not drug-naive, and the sample size of patients in acute phase was relatively small. Nevertheless, we colleted dietary habit and antioxidant consumption and carefully controlled for confounding factors such as cigarette smoking and BMI in statistical analyses.

5. Conclusion

Our results provide new evidence to support dysregulated oxidant/antioxidant balance of blood platelets in chronic stable patients with schizophrenia. Their relationships with specific symptom domains, disease phases, and symptom improvement are worth clinical attention. Whether the dysregulation of the oxidants and antioxidants can be a potential biomarker for disease or relapse warrants further investigation.

Authors' contribution

Study concept and design: SYLS, HGH.
Acquisition of data: YLC, HGH, TJH, MHH, CCL, SYLN, CML.
Analysis and interpretation of data: YLC, CML, SYLS.
Statistical analysis and drafting of the manuscript: YLC.
Critical revision of the manuscript for important intellectual content: CML, SYLS.

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/

or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Declaration of Competing Interest

The authors have no conflict of interest to disclose.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pnpbp.2020.109868.

References

Amminger, G.P., Schafer, M.R., Papageorgiou, K., Klier, C.M., Cotton, S.M., Harrigan, S.M., Mackinnon, A., McGorry, P.D., Berger, G.E., 2010. Long-chain omega-3 fatty acids for indicated prevention of psychotic disorders: a randomized, placebo-controlled trial. Arch. Gen. Psychiatry 67, 146–154.

Asor, E., Ben-Shachar, D., 2012. Platelets: a possible glance into brain biological processes in schizophrenia. World J. Psychiatry 2, 124–133.

Berk, M., Plein, H., Csizmadia, T., 1999. Supersensitive platelet glutamate receptors as a possible peripheral marker in schizophrenia. Int. Clin. Psychopharmacol. 14, 119–122.

Berk, M., Plein, H., Belsham, B., 2000. The specificity of platelet glutamate receptor supersensitivity in psychotic disorders. Life Sci. 66, 2427–2432.

Berk, M., Plein, H., Ferreira, D., 2001. Platelet glutamate receptor supersensitivity in major depressive disorder. Clin. Neuropharmacol. 24, 129–132.

Borroni, B., Colciaghi, F., Pastorino, L., Pettenati, C., Cottini, E., Rozzini, L., Monastero, R., Lenzi, G.L., Cattabeni, F., Di Luca, M., Padovani, A., 2001. Amyloid precursor protein in platelets of patients with Alzheimer disease: effect of acetylcholinesterase inhibitor treatment. Arch. Neurol. 58, 442–446.

Borroni, B., Colciaghi, F., Caltagirone, C., Rozzini, L., Broglio, L., Cattabeni, F., Di Luca, M., Padovani, A., 2003. Platelet amyloid precursor protein abnormalities in mild cognitive impairment predict conversion to dementia of Alzheimer type: a 2-year follow-up study. Arch. Neurol. 60, 1740–1744.

Casoli, T., Di Stefano, G., Balietti, M., Solazzi, M., Giorgetti, B., Fattoretti, P., 2010. Peripheral inflammatory biomarkers of Alzheimer's disease: the role of platelets. Biogerontology 11, 627–633.

Chuu, J.J., Hsu, C.J., Lin-Shiau, S.Y., 2001. Abnormal auditory brainstem responses for mice treated with mercurial compounds: involvement of excessive nitric oxide. Toxicology 162, 11–22.

Coughlin, J.M., Hayes, L.N., Tanaka, T., Xiao, M., Yolken, R.H., Worley, P., Leweke, F.M., Sawa, A., 2017. Reduced superoxide dismutase-1 (SOD1) in cerebrospinal fluid of patients with early psychosis in association with clinical features. Schizophr. Res. 183, 64–69.

Dietrich-Muszalska, A., Kwiatkowska, A., 2014. Generation of superoxide anion radicals and platelet glutathione peroxidase activity in patients with schizophrenia. Neuropsychiatr. Dis. Treat. 10, 703–709.

Dietrich-Muszalska, A., Olas, B., 2009. Isoprostenes as indicators of oxidative stress in schizophrenia. World J. Biol. Psychiatry 10, 27–33.

Dietrich-Muszalska, A., Olas, B., Rabe-Jablonska, J., 2005. Oxidative stress in blood platelets from schizophrenic patients. Platelets 16, 386–391.

Dreux, C., Launay, J.M., 1985. Blood platelets: neuronal model in psychiatric disorders. Encephale 11, 57–64.

Ehrlich, D., Humpel, C., 2012. Platelets in psychiatric disorders. World J. Psychiatry 2, 91–94.

Evans, D.R., Parikh, V.V., Khan, M.M., Coussons, C., Buckley, P.F., Mahadik, S.P., 2003. Red blood cell membrane essential fatty acid metabolism in early psychotic patients following antipsychotic drug treatment. Prostaglandins Leukot. Essent. Fat. Acids 69, 393–399.

Evin, G., Li, Q.X., 2012. Platelets and Alzheimer's disease: potential of APP as a biomarker. World journal of psychiatry 2, 102–113.

Flatow, J., Buckley, P., Miller, B.J., 2013. Meta-analysis of oxidative stress in schizophrenia. Biol. Psychiatry 74, 400–409.

Forde, R.C., Fitzgerald, D.J., 1997. Reactive oxygen species and platelet activation in reperfusion injury. Circulation 95, 787–789.

Fusar-Poli, P., Berger, G., 2012. Eicosapentaenoic acid interventions in schizophrenia: meta-analysis of randomized, placebo-controlled studies. J. Clin. Psychopharmacol. 32, 179–185.

Goubau, C., Buyse, G.M., Di Michele, M., Van Geet, C., Freson, K., 2013. Regulated granule trafficking in platelets and neurons: a common molecular machinery. Eur. J. Paediatr. Neurol. 17, 117–125.

Gutteridge, J.M., Halliwell, B., 2000. Free radicals and antioxidants in the year 2000. A historical look to the future. Ann. N. Y. Acad. Sci. 899, 136–147.

Hayat, K., Iqbal, H., Malik, U., Bilal, U., Mushtaq, S., 2015. Tea and its consumption: benefits and risks. Crit. Rev. Food Sci. Nutr. 55, 939–954.

Hwu, H.G., Chen, C.H., Hwang, T.J., Liu, C.M., Cheng, J.J., Lin, S.K., Liu, S.K., Chi, Y.Y., Ou-Young, C.W., Lin, H.N., Chen, W.J., 2002. Symptom patterns and subgrouping of schizophrenic patients: significance of negative symptoms assessed on admission. Schizophr. Res. 56, 105–119.

Kapur, R.L., Pandurangi, A.K., 1979. A comparative study of reactive psychosis and acute psychosis without precipitating stress. Be. J. Psychiatry 135, 544–550.

Kay, S.R., Fiszbein, A., Opler, L.A., 1987. The positive and negative syndrome scale (PANSS) for schizophrenia. Schizophr. Bull. 13, 261–276.

Kropp, S., Kern, V., Lange, K., Degner, D., Hajak, G., Kornhuber, J., Ruther, E., Emrich, H.M., Schneider, U., Bleich, S., 2005. Oxidative stress during treatment with first- and second-generation antipsychotics. J. Neuropsychiatr. Clin. Neurosci. 17, 227–231.

Li, X.F., Zheng, Y.L., Xiu, M.H., Chen, D.C., Kosten, T.R., Zhang, X.Y., 2011. Reduced plasma total antioxidant status in first-episode drug-naive patients with schizophrenia. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 35, 1064–1067.

Magalhaes, P.V., Dean, O., Andreazza, A.C., Berk, M., Kapczinski, F., 2016. Antioxidant treatments for schizophrenia. Cochrane Database Syst. Rev. 2, CD008919.

Matsuzawa, D., Obata, T., Shirayama, Y., Nonaka, H., Kanazawa, Y., Yoshitome, E., Takanashi, J., Matsuda, T., Shimizu, E., Ikehira, H., Iyo, M., Hashimoto, K., 2008. Negative correlation between brain glutathione level and negative symptoms in schizophrenia: a 3T 1H-MRS study. PLoS One 3, e1944.

Mendez-Cuesta, L.A., Marquez-Valadez, B., Perez-De La Cruz, V., Escobar-Briones, C., Galvan-Arzate, S., Alvarez-Ruiz, Y., Maldonado, P.D., Santana, R.A., Santamaria, A., Carrillo-Mora, P., 2011. Diazepam blocks striatal lipid peroxidation and improves stereotyped activity in a rat model of acute stress. Basic Clin. Pharmacol. Toxicol. 109, 350–356.

Miljevic, C., Nikolic, M., Nikolic-Kokic, A., Jones, D.R., Niketic, V., Lecic-Tosevski, D., Spasic, M.B., 2010. Lipid status, anti-oxidant enzyme defence and haemoglobin content in the blood of long-term clozapine-treated schizophrenic patients. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 34, 303–307.

Mitra, S., Natarajan, R., Ziedonis, D., Fan, X., 2017. Antioxidant and anti-inflammatory nutrient status, supplementation, and mechanisms in patients with schizophrenia. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 78, 1–11.

Nucifora, L.G., Tanaka, T., Hayes, L.N., Kim, M., Lee, B.J., Matsuda, T., Nucifora Jr., F.C., Sedlak, T., Mojtabai, R., Eaton, W., Sawa, A., 2017. Reduction of plasma glutathione in psychosis associated with schizophrenia and bipolar disorder in translational psychiatry. Transl. Psychiatry 7, e1215.

Padurariu, M., Ciobica, A., Dobrin, I., Stefanescu, C., 2010. Evaluation of antioxidant enzymes activities and lipid peroxidation in schizophrenic patients treated with typical and atypical antipsychotics. Neurosci. Lett. 479, 317–320.

Pellerin, D., Lortie, A., Corbin, F., 2018. Platelets as a surrogate disease model of neurodevelopmental disorders: insights from fragile X syndrome. Platelets 29, 113–124.

Plein, H., Berk, M., 2001. The platelet as a peripheral marker in psychiatric illness. Hum. Psychopharmacol. 16, 229–236.

Radi, R., Beckman, J.S., Bush, K.M., Freeman, B.A., 1991. Peroxynitrite-induced membrane lipid peroxidation: the cytotoxic potential of superoxide and nitric oxide. Arch. Biochem. Biophys. 288, 481–487.

Raffa, M., Mechri, A., Othman, L.B., Fendri, C., Gaha, L., Kerkeni, A., 2009. Decreased glutathione levels and antioxidant enzyme activities in untreated and treated schizophrenic patients. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 33, 1178–1183.

Sahin, E., Gumuslu, S., 2004. Alterations in brain antioxidant status, protein oxidation and lipid peroxidation in response to different stress models. Behav. Brain Res. 155, 241–248.

Sahin, E., Gumuslu, S., Ozturk, O., Abidin, I., Yargicoglu, P., Agar, A., 2004. Marked changes in erythrocyte antioxidants and lipid peroxidation levels of rats exposed to acute, repeated and chronic restraint stress. Die Pharmazie 59, 961–964.

Sarandol, A., Kirli, S., Akkaya, C., Altin, A., Demirci, M., Sarandol, E., 2007. Oxidative-antioxidative systems and their relation with serum S100 B levels in patients with schizophrenia: effects of short term antipsychotic treatment. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 31, 1164–1169.

Sawa, A., Sedlak, T.W., 2016. Oxidative stress and inflammation in schizophrenia. Schizophr. Res. 176, 1–2.

Scheffer, R., Diamond, B.I., Correnti, E.E., Borison, R.L., Mahadik, S.P., Mukherjee, S., 1995. Plasma lipid peroxidation and HVA in first episode psychosis. Biol. Psychiatry 37, 681.

Talib, L.L., Joaquim, H.P., Forlenza, O.V., 2012. Platelet biomarkers in Alzheimer's disease. World J. Psychiatry 2, 95–101.

Terpstra, M., Henry, P.G., Gruetter, R., 2003. Measurement of reduced glutathione (GSH) in human brain using LCModel analysis of difference-edited spectra. Magn. Reson. Med. 50, 19–23.

Tsai, M.C., Liou, C.W., Lin, T.K., Lin, I.M., Huang, T.L., 2013. Changes in oxidative stress markers in patients with schizophrenia: the effect of antipsychotic drugs. Psychiatry Res. 209, 284–290.

Veitinger, M., Varga, B., Guterres, S.B., Zellner, M., 2014. Platelets, a reliable source for peripheral Alzheimer's disease biomarkers? Acta Neuropathologica Commun. 2, 65.

Virit, O., Altindag, A., Yumru, M., Dalkilic, A., Savas, H.A., Selek, S., Erel, O., Herken, H., 2009. A defect in the antioxidant defense system in schizophrenia. Neuropsychobiology 60, 87–93.

Wei, Z., Bai, O., Richardson, J.S., Mousseau, D.D., Li, X.M., 2003. Olanzapine protects PC12 cells from oxidative stress induced by hydrogen peroxide. J. Neurosci. Res. 73, 364–368.
Winokur, G., 1975. Paranoid vs hebephrenic schizophrenia: clinical and familial (genetic) heterogeneity. Psychopharmacol. Commun. 1, 567–577.
Winokur, G., Tsuang, M.T., 1975. A clinical and family history comparison of good outcome and poor outcome schizophrenia. Neuropsychobiology 1, 59–64.
Wu, J.Q., Kosten, T.R., Zhang, X.Y., 2013. Free radicals, antioxidant defense systems, and schizophrenia. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 46, 200–206.
Yao, J.K., Reddy, R., McElhinny, L.G., van Kammen, D.P., 1998. Effects of haloperidol on antioxidant defense system enzymes in schizophrenia. J. Psychiatr. Res. 32, 385–391.
Yao, J.K., Leonard, S., Reddy, R.D., 2000. Membrane phospholipid abnormalities in postmortem brains from schizophrenic patients. Schizophr. Res. 42, 7–17.
Zhang, X.Y., Zhou, D.F., Cao, L.Y., Zhang, P.Y., Wu, G.Y., 2003. Elevated blood superoxide dismutase in neuroleptic-free schizophrenia: association with positive symptoms. Psychiatry Res. 117, 85–88.
Zhang, X.Y., Tan, Y.L., Cao, L.Y., Wu, G.Y., Xu, Q., Shen, Y., Zhou, D.F., 2006. Antioxidant enzymes and lipid peroxidation in different forms of schizophrenia treated with typical and atypical antipsychotics. Schizophr. Res. 81, 291–300.
Zhang, M., Zhao, Z., He, L., Wan, C., 2010. A meta-analysis of oxidative stress markers in schizophrenia. Sci. China Life Sci. 53, 112–124.
Zhang, X.Y., Zhou, D.F., Shen, Y.C., Zhang, P.Y., Zhang, W.F., Liang, J., Chen da, C., Xiu, M.H., Kosten, T.A., Kosten, T.R., 2012. Effects of risperidone and haloperidol on superoxide dismutase and nitric oxide in schizophrenia. Neuropharmacology 62, 1928–1934.