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ORIGINAL RESEARCH

Peripheral levels of superoxide dismutase and glutathione peroxidase in youths in ultra-high risk for psychosis: a pilot study

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Introduction. Oxidative stress has been documented in chronic schizophrenia and in the first episode of psychosis, but there are very little data on oxidative stress prior to the disease onset.

Objective. This work aimed to compare serum levels of superoxide dismutase (SOD) and glutathione peroxidase (GPx) in young individuals at ultra-high risk (UHR) of developing psychosis with a comparison healthy control group (HC).

Methods. Thirteen UHR subjects and 29 age- and sex-matched healthy controls (HC) were enrolled in this study. Clinical assessment included the Comprehensive Assessment of At-Risk Mental States (CAARMS), the Semi-Structured Clinical Interview for DSM-IV Axis-I (SCID-I) or the Kiddie-SADS-Present and Lifetime Version (K-SADS-PL), and the Global Assessment of Functioning (GAF) scale. Activities of SOD and GPx were measured in serum by the spectrophotometric method using enzyme-linked immunosorbent assay kits.

Results. After adjusting for age and years of education, there was a significant lower activity of SOD and lower GPX activity in the UHR group compared to the healthy control group (rate ratio [RR] = 0.330, 95% CI 0.187; 0.584, $p < 0.001$ and RR = 0.509, 95% CI 0.323; 0.803, $p = 0.004$, respectively). There were also positive correlations between GAF functioning scores and GPx and SOD activities.

Conclusion. Our results suggest that oxidative imbalances could be present prior to the onset of full-blown psychosis, including in at-risk stages. Future studies should replicate and expand these results.

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Key words: Ultra-high risk, prodromal, oxidative stress, antioxidant enzymes, schizophrenia, psychosis.

Introduction

The neurobiology of preclinical or ultra-high risk (UHR) psychosis states has been increasingly studied using neuroimaging and neurocognition techniques (Bartholomeusz *et al.*, 2016; Chung and Cannon, 2015). Nonetheless, other lines of investigation, such as peripheral biomarkers, remain underexplored.[1–4] Replicated evidence has indicated that oxidative stress imbalances are involved in the pathophysiology of psychosis.[5–7] However, mixed results regarding two of the primary enzymatic antioxidants, superoxide dismutase (SOD) and glutathione peroxidase (GPx), have been reported in schizophrenia populations.[8–10] Clinical

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studies focusing on the early stages of psychosis, wherein the outcomes are less affected by confounders, especially medications effects, have more consistently reported abnormalities in oxidative stress pathways.[11–13]

The accumulation of reactive oxygen species (ROS), either through increased production or due to a relative lack of antioxidant defenses, leads to damage of biomolecules (ie, lipids, proteins, DNA),[14] which, in turn, has been implicated in disturbances in signal transduction and synaptic plasticity, among other processes.[13,15] Overall, the regulation of oxidative stress has been proposed as an effector system of interest in UHR states.[16] Notwithstanding, antioxidant enzymatic defenses have not yet been studied in populations that are UHR for psychosis.

The aim of this study was to compare SOD and GPx activity in a sample of subjects at UHR for psychosis with a healthy control group. Secondary aims were to verify the impact of possible associated factors (ie, mood symptoms, psychosocial functioning, body mass index, and history of childhood maltreatment) in the activity level of these enzymes.

Methods

This study was approved by the local ethics committee from Universidade Federal de São Paulo (Unifesp), and all the subjects above 18 years of age provided written informed consent prior their inclusion. For participants under 18 years old, an assent term was obtained and the written informed consent was provided by the legal responder.

Sample

Thirteen UHR subjects within ages 14–26 years were selected from those attending the PRISMA Early Intervention Program, a specialized outpatient unit for assistance and research in UHR for psychosis in São Paulo, Brazil.[17,18] All the subjects recruited for this study were seeking help for any kind of mental or emotional suffering and/or were referred to PRISMA by health professionals, including psychiatrists, psychotherapists, occupational therapists, counselors, and social workers. Approximately 1:10 patients referred to PRISMA fulfilled criteria to be considered UHR for psychosis, and all the potentially eligible subjects accepted to participate in this study. The composition and the recruitment method adopted at PRISMA were similar to those from other well-established cohorts.[19] We used the criteria for UHR for psychosis proposed and validated by Yung *et al*, according to the classification of the Comprehensive Assessment of At-Risk Mental State (CAARMS) Scale.[19] The criteria consist of three possible situations:

1. Attenuated positive symptoms: Presence of positive symptoms in moderate severity, but not fulfilling criteria for a psychotic episode; present more than 1 time per month for more than 1 hour per week, in the past year
2. Brief intermittent psychotic symptoms: Presence of brief episodes of a full psychotic illness, which might involve all of the symptoms of a psychosis (particularly delusions and hallucinations)
3. Trait and state risk factors: Vulnerable family history of psychosis in a first-degree relative or a diagnosis of schizotypal personality disorder associated with a decline in social and occupational functioning

For the control group, only healthy individuals were selected from the community, with the same age range and sex as the UHR group. Healthy volunteers had no current or lifetime history of any mental disorders according to the Structured Clinical Interview for *Diagnostic and Statistical Manual of Mental Disorders*, Fourth Edition (DSM-IV) Axis 1 (SCID-1). In addition, we included only subjects with no history of use of any psychotropic medication and negative family history of a major psychiatric disorder [defined as unipolar depression, bipolar disorder (BD), suicide, or psychosis] in a first-degree relative. The exclusion criteria for both groups were suicide risk, presence of acute or chronic general medical conditions associated with imbalances in oxidative pathways (ie, infections, cancer), or pregnancy postpartum period.

Clinical status was assessed by the following scales: Montgomery–Åsberg Depression Rating Scale (MADRS), Young Mania Rating Scale (YMRS), Global Assessment of Functioning scale (GAF), and Global Assessment of Symptoms Scale (GAS).

Blood collection

All blood samples were taken in the morning (between 8 am and 10 am) in the fasting state. After collection, blood samples were immediately placed on ice, allowed to clot for at least 30 min at room temperature, and centrifuged at 3200 rpm for 10 min. The obtained serum was aliquoted and then stored at –80 °C for a maximum of 2 years, until the assay.

Superoxide dismutase and glutathione peroxidase assessment

The activity of SOD and GPx in serum was evaluated by the spectrophotometric method using enzyme-linked immunosorbent assay kits (Cloud-Clone Corp., Houston, TX, USA.). The samples were diluted (1:50 for SOD and 1:500 for GPx) and run in duplicate or triplicate. If the coefficient of variation between the replicate was higher than 10%, the samples were repeated. Measurements were recorded using a micro-plate reader at 450 nm.

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Statistics

Statistical Package for Social Sciences (SPSS) Version 20.0 (IBM Corporation, Armonk, NY, USA) was used for the statistical analysis of data. The Shapiro–Wilk test was performed in all variables to assess normal distributions. The chi-square test was used for categorical variables. Correlations between continuous variables were made by Spearman's rank correlation test. For unadjusted comparisons, we used the Mann–Whitney U test. For adjusted analysis, we used generalized linear models. Due to the positively skewed distribution of GPx and SOD activities, models with gamma distribution were used. Due to the nonlinearity of the models, the estimated β coefficients created by the model were transformed into rate ratio (RR) estimates. Differences were accepted as significant when $\alpha < 0.05$ (2-tailed).

Results

Clinical and demographic characteristics of the sample are described in Table 1. There were no statistically significant differences in sex, age, or ethnical background between the UHR and healthy control groups.

GPx and SOD activities were not correlated with age ($r = 0.236$, $p = 0.236$ and $r = 0.354$, $p = 0.051$, respectively); GPx was positively correlated with years of education ($r = 0.454$, $p = 0.013$), whereas SOD was not ($r = 0.261$, $p = 0.208$). GPx and SOD activity were not affected by sex ($z = 1.088$, $p = 0.277$ and $z = 0.080$, $p = 0.936$, respectively) or ethnicity ($z = 0.392$, $p = 0.695$ and $z = 0.029$, $p = 0.977$, respectively).

Unadjusted comparisons showed a lower level of activity of GPx in the UHR group, whereas there was no statistically significant difference in SOD. After adjusting for age and years of education, there was a significant lower activity of SOD in the UHR group compared to the healthy control group (RR = 0.330, 95% CI 0.187; 0.584, $p < 0.001$); the lower GPx activity in the UHR group remained significant (RR = 0.509, 95% CI 0.323; 0.803, $p = 0.004$).

There were also positive correlations between GAF functioning score and GPx and SOD activities ($r = 0.399$, $p = 0.029$ and $r = 0.427$, $p = 0.030$, respectively). There were no correlations between GPx and SOD activities and total MADRS score ($r = -0.241$, $p = 0.200$ and $r = -0.103$, $p = 0.617$, respectively) and total YMRS score ($r = 0.016$, $p = 0.939$ and $r = -0.237$, $p = 0.225$, respectively).

Discussion

The results of this preliminary study indicate that, after adjusting for age and years of education, individuals at UHR for psychosis had lower activities of both SOD and GPx activity compared to the healthy control group. In addition, lower activity of GPx and SOD was associated with worse psychosocial functioning. This was the first study to assess GPx and SOD activities in an UHR for psychosis population, and provides potentially useful data for the study of oxidative imbalances in the preclinical stages of psychosis.

Indeed, although oxidative stress has a well-recognized role in the pathophysiology of schizophrenia and psychotic affective disorders, there is a scarcity of studies examining the effects of oxidative stress on at-risk populations. Recently, Coughlin *et al*[5] described a reduction in SOD activity in the cerebrospinal fluid. Only one study to date has assessed the cellular oxidative damage to nucleic acids in this population; the authors were not able to demonstrate differences between UHR and healthy controls.[20]

Studies assessing SOD and GPx in mental illnesses have provided heterogeneous results. The findings seem to be influenced by ethnicity, gender, stage of illness,

TABLE 1. Clinical and demographic characteristics of the sample

	UHR (n = 13)	HC (n = 19)	Test-value	P-value
Sex (female), n (%)	4 (30.8)	6 (31.6)	0.002[a]	0.961
Age (years), mean (SD)	17.77 (3.60)	20.37 (4.60)	1.704[b]	0.099
Education (years), mean (SD)	10.27 (3.74)	12.74 (3.28)	1.884[b]	0.070
Ethnicity (Caucasian), n (%)	6 (54.5)	9 (50.0)	0.056[a]	0.812
MADRS, total score, median (IQR)	19.50 (0.25–25.25)	0 (0–2)	2.816[c]	0.005
YMRS total score, median (IQR)	1.50 (0–4)	0 (0–2)	1.350[c]	0.177
GAF functioning, median (IQR)	55.50 (47.50–68.00)	95 (90–100)	3.983[c]	<0.001
SOD, median (IQR)	2.43 (1.81–5.98)	8.00 (4.18–13.27)	1.952[c]	0.051
GPx, median (IQR)	4.35 (2.54–10.35)	14.35 (9.78–19.64)	3.243[c]	0.001

[a]Chi-square test; [b]T-test; [c]Mann-Whitney test.

UHR: ultra high risk; HC: healthy controls; SD: standard deviations; MADRS: Montgomery–Åsberg Depression Rating Scale; IQR: interquartile range; YMRS: Young Mania Rating Scale; GAF: General Assessment of Functioning; SOD: superoxide dismutase; GPx: glutathione peroxidase.

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medication, comorbidities, obesity, and smoking.[21–25] Interestingly, this heterogeneity also appears in studies assessing the activity of these enzymes in individuals experiencing their first episode of psychosis.[22,23]

Possible differences between chronic patients with schizophrenia and individuals transitioning to psychosis have not been directly evaluated. Nevertheless, indirect evidence could be found in one study that assessed travel-induced psychosis, in which the patients had higher SOD compared to controls.[26] In addition, studies assessing the effect of antipsychotics suggest that reduction in SOD commonly found in chronic patients could be due to medication.[27,28]

This study has important limitations. We evaluated a relatively rare group of individuals, in one observation, with small sample size and therefore more susceptible to sampling error and type II error. The cross-sectional characteristics of this study do not allow the identification of causality effects.

Conclusion

The importance of early recognition and early intervention in the outcomes of individuals with schizophrenia is now well established.[29,30] It has been hypothesized that preventive interventions during putatively prodromal phases could delay, reduce severity, or primarily prevent the development of a psychotic illness.[31–33] There is, however, insufficient data to reliably identify who will and who will not transition to psychosis based solely on clinical characteristics.[34,35] Therefore, oxidative stress imbalances emerge as a promising candidate to be incorporated in future investigations of neurobiology of at-risk mental states.

Disclosures

Maiara Zeni-Graiff, Adiel Rios, Pawan Maurya, Lucas Rizzo, Sumit Sethi, Ana Yamagata, Rodrigo Mansur, Pedro Pan, Elson Asevedo, Graccielle Cunha, Ary Gadelha, and Elisa Brietzke have nothing to disclose. André Zugman reports grants from CAPES, outside the submitted work. Rodrigo Bressan reports grants, personal fees, and nonfinancial support from Janssen, personal fees from Lundbeck, and personal fees from Ache, outside the submitted work; and the governmental funding research agencies: CAPES, CNPq, and FAPESP. Elisa Brietzke reports grants from CAPES, CNPq and FAPESP and honorarium as speaker-advisory board from DAiichi-Sankyo, outside the submitted work.

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