



Systemic oxidative DNA and RNA damage are not increased during early phases of psychosis: A case control study



Dorte Nordholm^{a,b,*}, Henrik Enghusen Poulsen^c, Carsten Hjorthøj^a, Lasse Randers^{a,b}, Mette Ø. Nielsen^{b,d}, Sanne Wulff^{b,d}, Kristine Krakauer^{a,b}, Henrik Nørbaek-Emig^{b,d}, Trine Henriksen^c, Birte Glenthøj^{b,d}, Merete Nordentoft^a

^a Mental Health Center Copenhagen, Copenhagen University Hospital, Denmark

^b Lundbeck Foundation Center for Clinical Intervention and Neuropsychiatric Schizophrenia Research (CINS), Mental Health Center Glostrup, Copenhagen University Hospital, Denmark

^c Laboratory of Clinical Pharmacology, Copenhagen University Hospital, Bispebjerg, Copenhagen, Denmark

^d Center for Neuropsychiatric Schizophrenia Research (CNSR), Mental Health Center Glostrup, Copenhagen University Hospital, Denmark

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ABSTRACT

It has been suggested that patients with schizophrenia develop higher levels of oxidative stress, which may contribute to deteriorating mental illness. In order to examine oxidative stress in the early stages of severe mental illness, we examined the levels of systemic Deoxyribonucleic Acid (DNA) and Ribonucleic Acid (RNA) oxidation, 8-oxo-7,8-dihydro-2'-deoxyguanosine and 8-oxo-7,8-dihydroguanosine, perceived stress and recent life events in patients at ultra high-risk (UHR) of developing psychosis, in antipsychotic naïve patients with first-episode schizophrenia (FES), and in healthy controls. We included 41 UHR patients, 35 FES patients, and 29 healthy controls. There was no difference in the level of DNA/RNA oxidative damage between UHR patients and FES patients compared with healthy controls. We found no association between levels of DNA/RNA oxidative damage and perceived stress/life events. Based on the results, we suggest that DNA and RNA oxidative markers are not increased during the early stages of illness, but further longitudinal studies in first-episode psychosis should be carried out to examine whether DNA and RNA oxidative damage are potential markers of severe illness.

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1. Introduction

Widely used in research on psychotic disorders, the stress-vulnerability model of psychosis suggests that a combination of genetics and previous life stressors creates a vulnerability to psychosis (Zubin and Spring, 1977). When vulnerable individuals are exposed to stressors and/or experience increased levels of stress, they might develop psychosis and thus most likely expose body and brain cells to increased levels of stress (Walker and Diforio, 1997). Researchers have explored possible disease mechanisms and one hypothesis is that schizophrenia is associated with higher levels of oxidative stress, which may contribute to deteriorating mental illness (Poulsen et al., 2012; Yao and Keshavan, 2011; Flattow et al., 2013; Morris and Berk, 2015). DNA/RNA oxidative damage also seems to contribute to the severity of some physical diseases, such as diabetes and cardiovascular disease (Broedbaek

et al., 2011b; Poulsen et al., 2012). The literature suggests that patients with schizophrenia are at greater risk of developing somatic illness (Laursen et al., 2011, 2013; Heila et al., 2005), a discussion ensuing as to whether this is due to lifestyle or directly related to mental illness.

One of the components of oxidative stress is the oxidation of cellular macromolecules by reactive oxygen species (ROS). ROS are endogenous agents that, when they exceed the body's defence mechanisms, result in oxidative stress (Wu et al., 2013). The targets of the oxidation are DNA, RNA, proteins, and lipids in the cell membrane, lysozymes and mitochondria. During these processes, it is possible that the signalling processes in the cells are impaired, leading to accelerated cellular aging (Finkel and Holbrook, 2000; Yao and Keshavan, 2011).

Oxidative stress can be determined by measuring the involved components of oxidative stress mechanisms, e.g. ROS, anti-oxidants, enzymes, or the oxidised products. The method of measurement chosen depends on the macromolecule of present interest, e.g. nucleic acids. Oxidative stress involving DNA and RNA is also called genotoxic stress or DNA/RNA oxidative damage. Urinary 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG) and 8-oxo-

* Correspondence to: Present address: Psykiatrisk Centre Nordsjælland, Dyrhavsvej 48, 3400 Hillerød, Denmark.

E-mail address: dorte.nordholm@regionh.dk (D. Nordholm).

Table 1
Inclusion and exclusion criteria for healthy controls, patients with first-episode schizophrenia, and patients at ultra high-risk of developing psychosis.

Healthy controls	First-episode psychosis	Ultra high risk of psychosis
Inclusion criteria		
18–45 years old	18–45 years old diagnosis of schizophrenia or schizoaffective psychosis (ICD-10) ^{a)}	18–40 years old Fulfil CAARMS ^{b)} criteria for at least one at-risk group: Attenuated positive symptoms group Brief limited intermittent psychotic symptoms group Trait and state risk factor group Also a decline in functioning (at least a 30% drop in SOFAS score, sustained for at least one month) or sustained low functioning (SOFAS score \leq 50, for at least one year).
Exclusion criteria		
Any previous or current psychiatric illness or drug abuse	Treatment with antipsychotics (lifetime)	Past history of a treated or untreated psychotic episode of \geq one week's duration
Any first-degree relatives with psychiatric diagnosis	Use of antidepressants within the last month	Organic brain disease, e.g. epilepsy, inflammatory brain disease
	Current drug dependency (ICD-10)	Any physical illness with psychotropic effect, if not stabilised
	Treatment with methylphenidate (lifetime)	Current treatment with mood stabilisers or methylphenidate
	History of major head injury	Past antipsychotic exposure equivalent to a total lifetime haloperidol dose of > 50 mg. Diagnosis of a serious developmental disorder, e.g. Asperger's syndrome IQ < 70 and a documented history of developmental delay or intellectual disability Current pregnancy Symptoms entirely explained by use of drugs.

^{a)} ICD-10: International Classification of Diseases, Tenth Revision.

^{b)} CAARMS: Comprehensive Assessment of At-Risk Mental States.

7,8-dihydroguanosine (8-oxoGuo) are products of the enzymatic repair mechanism of the DNA and RNA after 8-hydroxylation, the majority of which is produced in the brain, liver, and bones (Poulsen et al., 2013). Urinary 8-oxodG has been validated as a marker of oxidatively generated DNA damage through a number of studies (Deng et al., 1998; Evans et al., 2010b).

In a 2012 review (Poulsen et al., 2012) the authors proposed a novel disease mechanism for degenerative brain diseases (e.g. Alzheimer disease): RNA oxidation occurring early in the degenerative processes. The authors also emphasised that RNA markers could be potential biomarkers of various degenerative brain diseases. In a 2015 review the authors likewise propose that mitochondrial dysfunction and defects in oxidative metabolism are characteristic feature of many chronic illnesses (e.g. bipolar disorder, multiple sclerosis, Parkinson's disease, schizophrenia, autism, and chronic fatigue syndrome). The oxidation also occurs in age-related conditions such as type 2 diabetes and cardiovascular disease (Broedbaek et al., 2011b; Harrison et al., 2003; Bonomini et al., 2015).

Reductions of plasma antioxidant capacity are seen in patients with chronic illness as well as early in the course of schizophrenia (Yao and Keshavan, 2011; Flatow et al., 2013), the authors have reported the presence of oxidative damage to proteins, lipids and DNA, and there is evidence of ROS overproduction and reduced levels of antioxidants (Morris and Berk, 2015).

Oxidatively generated DNA/RNA damage has been examined in patients with schizophrenia and they had significantly higher levels of damage compared to healthy controls (Jorgensen et al., 2013; Sertan et al., 2015). The patients were not in their first episode of schizophrenia and they were taking antipsychotic medication.

Knowing whether increased oxidative stress appears during the early phases of illness is of great interest. If the oxidative damage is only mildly increased during early phases, it might be

possible to prevent or diminish their severe acceleration and potentially the deterioration of the illness. Another aspect of interest is to study the association of oxidative stress levels with perceived stress and recent life events, since the stress experienced could be targeted in treatment strategies.

This is the first study to examine DNA/RNA oxidative damage in UHR and FES patients. Patients at ultra high-risk (UHR) of developing psychosis have subtle psychotic symptoms, brief psychotic symptoms, or a schizotypal disorder in combination with a low level of functioning. In our study, they represented the earliest phase of severe mental illness. The second group was antipsychotic naïve patients with early-stage psychosis, all of them recently diagnosed with first-episode schizophrenia (FES).

First, we hypothesised a stepwise increase in perceived stress, recent life events, 8-oxodG and 8-oxoGuo in UHR and FES patients compared to healthy controls. Second, we hypothesised a positive association between perceived stress, recent life events and 8-oxodG and 8-oxoGuo.

The aim of the study: 1) To examine the levels of DNA/RNA oxidative damage; and 2) to examine the association between DNA/RNA oxidative damage perceived stress and recent life events in patients during early phases of psychosis and in healthy controls.

2. Methods

The study was conducted in accordance with the Declaration of Helsinki and approved by the Regional Ethics Committee of the Capital Region, Denmark (FES: H-D-2008-088 and UHR: H-D-2009-013). All participants signed an informed written consent to participate. The patients were recruited from psychiatric hospitals, psychiatric outpatient departments, and other mental health services in the Capital Region of Denmark between December 2008

and June 2013. Table 1 presents the inclusion and exclusion criteria.

2.1. UHR patients

Forty-one UHR patients were included in our study at the Mental Health Centre Copenhagen. Because Denmark does not have UHR services/clinics, the psychiatric services treat them according to the dominant UHR symptoms, e.g. anxiety, depression, or schizotypal disorder. They underwent a comprehensive psychopathology and demographic assessment at baseline. The diagnoses were based on a semi-structured interview, the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-I) and DSM-IV Axis II Disorders (SCID-II), while inclusion was based on the UHR criteria of the Comprehensive Assessment of At-Risk Mental States' (CAARMS) (Yung et al., 2005). Patients could meet the criteria for more than one of the CAARMS subgroups, as presented in previous studies and in the CAARMS criteria (Yung et al., 2005). The study had two assessors, both of whom systematically underwent SCID-I training. One assessor (DN) participated in international CAARMS training workshops and both assessors were involved in deciding whether to include a patient or not.

2.2. Patients with FES

Thirty-five patients with FES were assessed and treated at the Mental Health Centre Glostrup in Denmark. They were all a part of the large first-episode project called, Pan European Collaboration on Antipsychotic Naïve Schizophrenia (PECANS). Data on disturbances in reward and early information processing and dopamine D2/3 receptor binding from this cohort have previously been published (Nielsen et al., 2012a, 2012b; During et al., 2014; Wulff et al., 2015; Nielsen et al., 2016; Ebdrup et al., 2016; Düring et al., 2015). They underwent a comprehensive assessment battery at baseline when they were antipsychotic naïve. Their diagnoses were based on the semi-structured diagnostic interview Schedules for Clinical Assessment in Neuropsychiatry (SCAN) version 2.1 (Wing et al., 1990). Trained assessors measured the psychopathology with the Positive and Negative Syndrome Scale (PANSS) (Kay et al., 1987) and the level of functioning with the Social and Occupational Functional Assessment Scale (SOFAS) (Goldman et al., 1992).

2.3. Healthy controls

Twenty-nine healthy controls were recruited from the community using an ad on a website for healthy controls to participate in studies. At baseline, they underwent a SCAN interview (Glostrup) or a SCID-I interview (Copenhagen) in order to detect any severe mental illness. They were matched on group level for age and gender with the two patient groups.

2.4. Stress scales

We used the Perceived Stress Scale (PSS) (Cohen et al., 1983), which has previously been validated and used in Danish populations (Nielsen et al., 2008; Eskildsen et al., 2015). The purpose of the PSS was to measure the degree to which life situations were appraised as stressful by considering coping resources and feelings of control (Cohen et al., 1983). The scale consists of 10 questions that cover the two weeks prior to the PSS being administered and is scored on a scale ranging from zero to 40 (high scores = high levels of perceived stress).

Using the Brief Life Events Questionnaire (Brugha and Cragg, 1990), we collected information about stressful life events during the six months prior to the questionnaire being administered.

Comprised of 12 items covering various life events, e.g. illness or injury, death of a close friend or relative, unemployment, financial loss and loss of important relationships, individual scores could range from zero to 12 (maximum number of events).

2.5. Oxidative stress: 8-oxodG and 8-oxoGuo

Urine samples were obtained between 9 AM and 3 PM. A freshly voided spot urine sample was obtained at either Mental Health Centre Glostrup or Mental Health Centre Copenhagen using a standard sampling kit without any additives. The urinary content of the oxidised nucleosides 8-oxodG and 8-oxoGuo was quantified using a modified version of an ultra-performance liquid chromatography and tandem mass spectrometry (UPLC-MS/MS) assay, described in details in other studies (Henriksen et al., 2009). The urinary creatinine concentration was determined by Jaffe's reaction. The 8-oxodG/8-oxoGuo excretion is defined as the urinary concentration of the nucleoside normalised to urinary creatinine concentration (Evans et al., 2010a). Circulating 8-oxodG/8-oxoGuo is completely cleared through renal excretion and the urinary excretion thereby reflects the overall whole-body oxidative stress on DNA (Evans et al., 2010b).

2.6. Statistics

Data were analysed using the Statistical Package for Social Sciences, Version 20.0 (SPSS Inc.). In the ANOVA, we compared mean values between the groups and Bonferroni correction was used for post hoc tests. Spearman rank correlation was used to test the association between two variables. We used a generalised linear model when we added the covariates to the regression analyses, and a backward model to fit the parameters we had hypothesised would have an impact on oxidative stress levels.

3. Results

There was no difference in age between the three groups of patients (Table 2) and we found no significant difference in gender distribution within the three groups (Table 2). Both the UHR and FES patients had an equally low level of functioning (Table 2) and the controls attended school or were getting an education for a significantly longer period than both UHR and FES patients (Table 2).

Nine patients received SSRI or SNRI and three patients received antipsychotics and SSRI or SNRI. Five patients received second generation antipsychotics, and less than the equivalent lifetime dose of 50 mg of haloperidol. According to the SCID-I interview (and DSM-IV Axis I diagnoses), 12 of them had a diagnosis of previous alcohol abuse (or addiction) and 11 of them had a diagnosis of previous illicit drug abuse (or dependency). None of the UHR patients had current illicit drug or alcohol abuse (or dependency).

Six of the FES patients had a previous dependency or current alcohol abuse; six patients had a previous and/or current cannabis abuse; and one had a previous dependency or current benzodiazepine abuse. None of the patients had a current diagnosis of dependency.

Sixteen healthy controls, 27 UHR patients and 18 FES patients said that they regularly smoked cigarettes. There was no additional classification of how often they smoked, but this could range from daily to monthly.

Duration of untreated illness (DUI) in the FES patients was defined as having psychotic symptoms and impaired level of functioning. DUI was 61.8 weeks (SD ± 63.0) on average and ranged from two to 300 weeks.

Table 2
Demographics, psychopathology, and stress scales in healthy controls, patients with first-episode schizophrenia, and patients at ultra high-risk of developing psychosis.

	Healthy controls mean (SD) ^{a)}	Ultra high-risk patients mean (SD)	Patients with first-episode schizophrenia mean (SD)	P-value (p < 0.05)
Age (years) (N=29/41/35)	24.7 (4.9)	23.9 (4.7)	23.3 (5.6)	p=0.735
Gender (male/total) (% males)	19/27 (58.7%)	23/18 (43.9%)	18/22 (55.0%)	p=0.364
Height (N=13/41/34)	170.8 (30.2)	173.9 (9.4)	174.1 (9.1)	p=0.760
Weight (N=25/41/34)	79.3	76.0	74.8	p=0.915
Years of schooling/education (N=28/41/30)	14.9 (2.4)	12.9 (2.9)**	11.7 (1.8)**	p < 0.001
Functioning (SOFAS score)	–	43.3 (6.5)	43.2 (11.4)	p=0.975
CAARMS group (N=41):				
Trait and state	–	25	–	–
Attenuated positive symptoms	–	38	–	–
Brief limited intermittent psychotic symptoms	–	3	–	–
PANSS (N=34):				
Positive	–	–	20.3 (4.2)	–
Negative	–	–	19.1 (6.7)	–
General	–	–	40.2 (7.4)	–
Total	–	–	79.7 (14.1)	–
Stress scales				
Perceived stress N=29/39/20	9.6 (5.2)	25.1* (6.5)	25.0* (5.6)	p < 0.001
Life events (6 months) N=30/40/20	0.8 (0.8)	1.4* (1.1)	2.1** (2.29)	p = 0.007
Oxidative stress				
8-oxodG (N=30/42/35)	1.35 (0.46)	1.41 (0.46)	1.38 (0.48)	p=0.370
8-oxoGuo (N=31/42/35)	1.69 (0.48)	1.74 (0.36)	1.84 (0.49)	p=0.857

a) SD: Standard deviation.

* p < 0.05 versus HC.

** p < 0.01 vs. HC.

There was no significant difference in levels of 8-oxodG and 8-oxo-Guo between the three groups of subjects (Table 2).

We found a 4.2% difference between the median excretion of 8-oxoGuo in FES patients vs. healthy controls and a 9.8% difference between the median excretion of 8-oxodG in UHR patients vs. healthy controls.

Both UHR and FES patients experienced significantly more perceived stress and more life events than the healthy controls (Table 2). Perceived stress and recent life events were not

Table 3
The effect of potential confounders on the 8-oxodG and 8-oxoGuo in a multi-variable analysis. We hypothesise that these measures would have an impact on oxidative stress levels.

	Beta ^{a)}	Confidence limits	p-value*
8-oxodG			
Perceived stress	0.002	–0.009 to 0.14	p=0.700
Life events	–0.020	–0.091 to 0.052	p=0.590
Age	–0.011	–0.032 to 0.011	p=0.337
Gender ^{b)}	–0.15	–0.226 to 0.019	p=0.890
Education	0.003	–0.038 to 0.044	p=0.873
Smoking	0.116	0.027 to 0.204	p=0.011
8-oxoGuo			
Perceived stress	0.002	–0.009 to 0.013	0.744
Life events	–0.044	–0.113 to 0.025	0.208
Age	0.008	–0.014 to 0.029	0.486
Gender	0.035	–0.168 to 0.238	0.736
Education	–0.009	–0.049 to 0.030	0.649
Smoking	0.019	–0.067 to 0.105	0.660

a) Beta is the regression coefficient from a generalised linear model; illustrates a certain parameter affect of the 8-oxodG and 8-oxo-Guo if it increases one level/score.

b) The difference between females and males (males have –0.15 lower 8-oxodG compared to females).

* p < 0.05.

significantly correlated. Only smoking was positively associated with 8-oxodG levels (Table 3).

4. Discussion

We found no significant difference in levels of DNA/RNA oxidative damage between healthy controls, UHR and FES patients. Likewise, we found no associations between oxidative stress and perceived stress/life events.

Patients who suffer from schizophrenia and other severe mental disorders have a shorter life expectancy compared to healthy controls (Nordentoft et al., 2013; Laursen et al., 2011). While this is probably multi-causal, it has been hypothesised to be partially attributable to inheritable increased levels of stress. To our knowledge, this is the first study of its kind to examine oxidative DNA/RNA damage in the early stages of psychosis. The oxidative stress levels show huge variability and even though 8-oxodG shows a tendency to increase (Table 2) in patients, the result is non-significant.

According to previous studies, it is still unknown at what point during the disease that the levels of oxidative stress increase. Some markers of oxidative stress are increased during FES (e.g. red blood cell catalase and plasma nitrite), but not all markers of oxidative stress are increased in FES (Flatow et al., 2013). The authors of this meta-analysis also suggest that some oxidative stress parameters could be trait markers and some could be state markers for acute exacerbations of psychosis (Flatow et al., 2013). Researchers assessed the oxidative stress and oxidative DNA damage in schizophrenia patients with and without symptomatic remission (Sertan et al., 2015) and found that the total oxidant status was higher in patients with schizophrenia compared to controls, but oxidative DNA damage was higher in only patients without remission compared to healthy controls. These studies

support our results. Oxidative stress might be increased during early phase of the illness, but it seems that DNA/RNA oxidative damage increases in severely ill patients.

The authors of a previous study of diabetes found 8-oxoGuo to be an independent predictor of long-term mortality in a large cohort of newly diagnosed type II diabetic patients (Broedbaek et al., 2011a). Patients with schizophrenia have a high comorbidity of metabolic diseases and a shorter life expectancy than people not diagnosed with schizophrenia and people who have been diagnosed with the disease early.

We expected and found higher levels of perceived stress and life events in UHR and FES patients compared to healthy controls, as described in the literature (Thompson et al., 2007; Jorgensen et al., 2013; Garner et al., 2011). Our data from healthy controls also corresponded to the finding in the general Danish population (Nielsen et al., 2008). As a result, we believe our sample is representative with respect to the stress scales. Most likely, the lack of difference of perceived stress between UHR and FES patients and the lack of association with DNA/RNA damage, reflect the fact that many factors, such as age, social status, and education, contribute to how patients perceive stress and interpret life events (Nielsen et al., 2008).

In our sample we found an association between smoking and 8-oxodG. A previous study showed an association between smoking and oxidative damage to DNA in a cohort study (Loft et al., 1992) and in a randomised smoking cessation study (Prieme et al., 1998). Other cohort studies, however, have not found any differences between smokers and non-smokers (Harman et al., 2003). Further studies are needed to confirm whether or not there is an association between smoking and oxidative DNA/RNA damage.

4.1. Study limitations

The transition rates of UHR patients vary, but a long-term follow-up study described an estimated overall rate of transition of 34.9% over a ten-year period (Nelson et al., 2013). In a previous study of FES, 14% met criteria for full recovery at the ten year follow-up and nearly a third of the cohort achieved full recovery at some time during the ten-year follow-up period (Austin et al., 2013). As a result, we have potentially presented a sample in which a minor number of patients will be likely to develop a deteriorating mental disorder.

A consequence of our small sample size is the increase of type 2 error. Another limitation is that alterations of oxidative stress parameters in the urine might not be mirrored in the central nervous system and do not necessarily relate to the same pathophysiological mechanisms.

4.2. Clinical implications and future studies

Based on the results from this study we suggest that DNA/RNA oxidative damage is not increased during early stages of illness and that they do not only depend on perceived stress or life events. According to our results, controlling for smoking is important. Longitudinal studies should be carried out in first-episode psychosis cohorts in order to examine whether 8-oxodG and 8-oxoGuo are potential markers of exacerbations of psychosis.

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Conflict of interest

All the authors declare that they have no conflicts of interest.

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