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Original article

# Elevated levels of 8-oxoGuo and 8-oxodG in individuals with severe mental illness – An autopsy-based study☆

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ABSTRACT

Elevated systemic oxidative stress levels of 8-oxoGuo and 8-oxodG have been reported in individuals with severe mental illness (SMI). As no previous studies have addressed the link between local levels of 8-oxoGuo and 8-oxodG in the central nervous system (CNS), measured in cerebrospinal fluid (CSF), and urinary systemic levels, we employed autopsy-based material to elucidate this aspect. Additionally, we investigated the impact of 8-oxoGuo and 8-oxodG levels on the prevalence of somatic co-morbidities.

Based on post mortem samples from deceased individuals with SMI ($N = 107$), we found significantly elevated urinary levels of both 8-oxoGuo and 8-oxodG compared to mentally healthy living controls. While we found an association between urinary and CSF 8-oxodG levels ($r = 0.50$, $P < 0.001$), a similar correlation was not evident for 8-oxoGuo ($r = 0.15$, $P = 0.16$). Additionally, the two $r$-values were significantly different ($P < 0.001$). Neither marker in urine or CSF was associated with obesity-related variables, metabolic syndrome or type 2 diabetes. The post mortem interval did not affect the results, but the agonal phase seemingly introduced bias.

This study provided novel insights into the cellular oxidative stress levels in individuals with SMI. We demonstrated that increased oxidative stress locally and systemically is correlated and is a clear phenomenon in SMI. Although post mortem measurements contain some weaknesses, our study indicates DNA as the main site of oxidative stress modifications in the CNS in SMI. This may provide novel opportunities for treatment modalities. Additionally, our study demonstrated the applicability of post mortem material investigating systemic and local 8-oxoGuo and 8-oxodG levels.

## 1. Introduction

Physiological oxidative stress, or oxidative eustress, describes the steady state of oxidation-reduction (redox) reactions in the organism [1]. Oxidants include reactive species such as reactive oxygen species (ROS) and reactive nitrogen species. The sources of these reactive species stem mainly from the mitochondria and cytosolic superoxide dismutases (SOD). To control potential rampant oxidation, antioxidant enzyme systems serve as counterweights and oxidant sinks [1]. Deviation in favour of the oxidant part of oxidative eustress may cause damage not only to proteins and lipids, but also to RNA and DNA.

Cellular degradation and repair systems exist to handle RNA and DNA damage, respectively, and oxidized bases and nucleosides are ultimately excreted in the urine [2]. As guanine has the lowest redox potential, it is the predominantly oxidized base. The nucleosides 8-oxo-7,8-dihydroguanosine (8-oxoGuo) and 8-oxo-7,8-dihydro-2`-

*Abbreviations:* AT, adipose tissue; BMI, body mass index; CNS, central nervous system; CSF, cerebrospinal fluid; DM, diabetes mellitus; HbA1c, glycated haemoglobin; hsCRP, high sensitive C-reactive protein; MetS, metabolic syndrome; NA, not applicable; SMI, severe mental illness; WHR, waist-to-hip ratio

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deoxyguanosine (8-oxodG) originate from RNA and DNA, respectively, and they reflect the level of cellular oxidative eustress. While the levels of 8-oxoGuo and 8-oxodG in specific organs echo the local balance of their formation and removal, the levels of the markers in urine mirror the formation rate of oxidized bases, which can be interpreted as systemic oxidative stress in the body [3]. Thus, urinary 8-oxoGuo and 8-oxodG have served as robust markers for the systemic oxidative stress state in conditions affecting multiple cell types/organs, such as diabetes mellitus (DM), metabolic syndrome (MetS), obesity and severe mental illness (SMI) [4–11].

Individuals with SMI have a diminished life expectancy of up to 20 years compared to the background population even after adjusting for accidents, suicides and homicides [12–14]. Part of this excess mortality may be explained by the fact that SMI has repeatedly been linked with an increased prevalence of comorbidities, such as DM and MetS [15–17]. A common denominator in both psychiatric and somatic conditions is elevated levels of oxidative stress [4–6,8–11].

Post mortem studies of individuals with SMI have demonstrated local elevated oxidative stress levels in brain samples [18,19]. Other studies have suggested that the major site of oxidative stress in the brains of individuals with SMI is DNA, partly due to increased levels of SOD and nitric oxide [20–22]. Moreover, a recent review has proposed several mechanisms for elevated oxidative stress in the brain, including redox signalling and RNA oxidation [23], and novel mechanisms of exosomal transfer of signalling molecules such as microRNA (miRNA) have recently emerged. Oxidation of these miRNAs has been shown to change their regulatory properties dramatically in a pathogenic direction [24].

Regarding DM, 8-oxodG has been proposed as a possible early clinical biomarker [25], and other studies have found urinary 8-oxoGuo to be an independent predictor of both all-cause and cardiovascular mortality in patients with type 2 DM [26,27]. Increased oxidative stress has also been linked to each of the criteria composing MetS (central obesity, dyslipidaemia, elevated blood glucose and hypertension), and has furthermore been suggested as a central underlying pathogenic factor for the syndrome [28,29].

A major underlying factor of both type 2 DM and MetS is obesity, and increased levels of oxidative stress have repeatedly been linked to this condition. Adipose tissue (AT) possess a large array of endocrine functions regulating energy homeostasis through secretion of hormones and adipokines. Especially visceral AT have repeatedly been shown to possess more adverse properties than subcutaneous AT [30–32]. With excessive AT accumulation, the secretion of adipokines and oxidized miRNAs increases, potentially exerting profound metabolic disturbances [33]. Excessive caloric intake can lead to mitochondrial dysfunction resulting in increased ROS production [34]. With a continuous state of excess energy, AT transitions into a state of chronic inflammation where adipocytes secrete several pro-inflammatory adipokines, stimulating production of ROS and resulting in further increased oxidative stress [7]. Consequently, as individuals with SMI are more prone to suffer from the abovementioned diseases, multiple factors, several with self-perpetual properties, may form the basis for elevated oxidative stress markers in these individuals.

Validated methods exist for quantifying levels of urinary, and recently also cerebrospinal fluid (CSF), 8-oxoGuo and 8-oxodG [35–38]. While urinary levels have been extensively investigated, the interest in CSF levels of 8-oxoGuo and 8-oxodG as markers of local oxidative stress in the central nervous system (CNS) have only recently started to gain traction.

To our knowledge, the association of systemic and local oxidative stress levels (CNS) in individuals with SMI has not previously been addressed. Additionally, no studies have elucidated the effect of obesity, MetS and DM on local and systemic oxidative stress levels of individuals with SMI.

In this study, we investigated the impact of local 8-oxoGuo and 8-oxodG levels in the CNS with systemic levels and the prevalence of somatic co-morbidities. Thus, the study addressed an emerging aspect of the characterisation of SMI. Based on the SURVIVE study [39], we employed, prospectively and systematically, sampled autopsy-based material and data to elucidate novel disease mechanisms in a group of individuals with severe health challenges.

## 2. Material and methods

### 2.1. Study population

The present study was based on the forensic autopsy-based SURVIVE study. The SURVIVE study included all deceased individuals with a known or suspected psychiatric disorder who had undergone forensic autopsy at the three Departments of Forensic Medicine in Denmark from May 2013 to May 2015. Cases were included based on health information from police reports available prior to the autopsy. Exclusion criteria included (1) advanced putrefaction with discolouration of the skin or gaseous development in the liver and/or brain detected at CT-scan prior to autopsy, (2) no CT-scan performed prior to the autopsy, and (3) cases of homicide or suspected homicide, as material sampling for the study would interfere with the legal investigation. Diagnosis codes (ICD10) from psychiatric hospitalizations from 1994 until time of death were obtained from the National Psychiatric Patient Registry (NPPR). Likewise, ICD10 codes from somatic hospitalizations from 1994 until time of death were procured from the National Patient Registry (NPR). A detailed account of the SURVIVE study is described elsewhere [39]. For the present study, we selected individuals from the SURVIVE study diagnosed with schizophrenia or a schizo-affective disorder, bipolar disorder or depression. Individuals with known cancer or DM were excluded and, finally, individuals where both urine and CSF samples where had been assayed were retained (total: $N = 107$; schizophrenia $N = 69$; bipolar: $N = 8$; depression: $N = 30$; Fig. 1).

Based on information from NPPR, we determined the duration of the psychiatric disorder of each individual from the date of the first SMI diagnosis to death. Additionally, using the number of psychiatric admission days one year prior to death as a proxy, we estimated the severity of the psychiatric disorder.

We selected living age- and gender-matched mentally healthy controls from the General Suburban Population Study (GESUS) [40], excluding individuals with psychiatric disease, cancer, and DM, at a case-control ratio of 1:3 ($N = 321$)

To validate our post mortem measurements, we identified living individuals with diagnosed SMI from the GESUS study ($N = 112$) and compared urinary 8-oxoGuo and 8-oxodG levels with the deceased individuals with SMI from the SURVIVE study.

The study complied with the Helsinki II declaration. The SURVIVE study was approved by the Danish National Committee on Research Ethics (reference number: 1305373) and the Danish Data Protection Agency (reference number: SUND-2016-16). The GESUS study was approved by the Regional Ethics Committee (reference number: SJ-114/1–01-83-0002-07) and the Danish Data Protection Agency

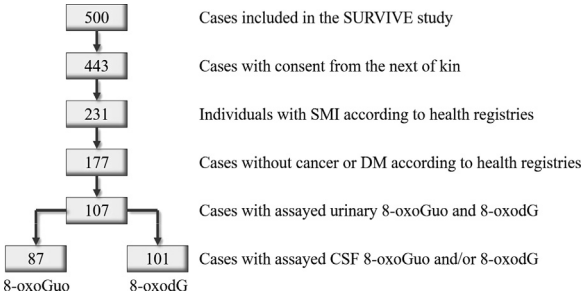


**Fig. 1.** Inclusion of deceased individuals from the SURVIVE study.

(reference number: SJ-114/REG-21–2014). In the SURVIVE study, consent from next of kin was acquired in accordance with Danish legislation concerning research on tissue from forensic autopsies; and in the GESUS study, informed consent was acquired from each participant.

### 2.2. Autopsy-related measurements

The SURVIVE study was based on an extended autopsy algorithm that was developed and implemented nationwide at the Departments of Forensic Medicine in Denmark. At autopsy, anthropometric variables were measured and included height, weight, waist and hip circumference, and omental fat weight, among others. Femoral blood or subsidiary cardiac blood from the left ventricle, urine and CSF samples were collected in vials, with no additives, and subsequently stored at −20 °C or −80 °C.

Blood samples were analysed for high sensitive C-reactive Protein (hsCRP) and glycated haemoglobin (HbA1c) using Enzyme-linked Immunosorbent Assay (ELISA) and a Siemens DCA Vantage® (Siemens, Erlangen, Germany), respectively, at the Department of Forensic Medicine in Copenhagen. Furthermore, standard forensic toxicological screening of blood samples for medication present at the time of death was performed at the respective Department of Forensic Medicine where the autopsy was performed according to the international standard ISO/IEC 17025:2005 using ultra performance liquid chromatography with tandem mass spectrometry (UPLC MS/MS).

We determined the prevalence of MetS based on a post mortem definition by the same research group [41]. An HbA1c ≥ 48 mmol/mol determined whether a deceased individual had suffered from undiagnosed DM.

Post mortem interval in the SURVIVE population was estimated in a similar fashion to Holm and Linnet [42] as one-half the time from a subject being last seen alive to identification as deceased plus the time from identification to autopsy. If the death was witnessed, only the time from death to autopsy was used. We subsequently correlated the post mortem interval with levels of 8-oxoGuo and 8-oxodG to test for possible influence.

### 2.3. Measurement of oxidative stress markers

Urinary and CSF levels of 8-oxoGuo and 8-oxodG were assayed using validated methods with UPLC MS/MS. For the urinary samples, chromatographic separation was performed on an Acquity® UPLC system (Waters, Milford, MA, USA). The column used was an Acquity UPLC BEH Shield RP18 column (2.1 × 100 mm, 1.7 μm) protected with a VanGuard precolumn (2.1 × 5 mm, 1.7 μm). A Xevo TQ-S triple quadrupole mass spectrometer from Waters using electrospray ionisation operating in the negative mode was used for MS detection. Urinary markers were normalized to concentrations of urinary creatinine determined by Jaffe's method.

For the CSF samples, chromatographic separation was likewise performed on an Acquity® UPLC system. The column used was an HSST3 column (2.1 × 100 mm, 1.8 μm) protected with an HSS T3 precolumn (2.1 × 5 mm, 1.8 μm). A Waters XEVO TQ-s tripe quadrupole mass spectrometer with electro spray ion source operating in the negative mode was used for MS detection. Details of the analyses have been described elsewhere [36,38].

SURVIVE urine samples underwent one cycle of thawing and freezing prior to analysis. All controls individuals from GESUS had urinary markers measured in spot urine using the same assay at the same laboratory [40].

### 2.4. Statistical analyses

Parametric data are presented as mean ( ± standard deviation [SD]) and were analysed using Student's *t*-test. Non-parametric data are presented as median (interquartile range [IQR]) and were analysed using Wilcoxon rank sum test or Kruskal-Wallis rank sum test, as applicable. Post hoc corrections were conducted using the Bonferroni method. A chi-square test was used for contingency tables. Cohen's *d* was used to assess effect size, and Pearson's *r* was applied for urinary excretion of 8-oxoGuo and 8-oxodG within the SMI and control group, as well as for CSF excretion in the SMI group. To select controls from the GESUS study, we used the MatchIt software package, and to test for differences in Pearson correlations, we used the cocor software package, both for the R programming language [43,44]. Linear regression was used to adjust for variables with significant differences between the SMI and control group. Non-parametric, dependent variables were transformed with the natural logarithm. Statistical analyses were performed using R version 3.4.2 [45], and a *P*-value < 0.05 was considered significant.

**Table 1**
Basic data of deceased individuals with SMI and living mentally healthy controls[a].

| | SMI (*N* = 107) | Controls (*N* = 321) |
|---|---|---|
| Age (years) | 46.8 ( ± 13.5) | 47.0 ( ± 12.6) |
| Gender (M/F) | 84/23 | 252/69 |
| BMI (kg/m$^2$) | 25.5 (21.7–29.9) | 26.1 (23.8–28.8) (NA = 7) |
| WHR[†] | 0.983 ( ± 0.079) | 0.919 ( ± 0.083) (NA = 1) |
| HbA1C (mmol/mol) [†] | 32 (29–37) (NA = 21) | 36 (34–38) |
| hsCRP (mg/L) [†] | 2.3 (0.7–8.1) (NA = 21) | 1.1 (0.6-2.0) (NA = 20) |
| Omental adipose tissue (g) | 286 (159–556) | NA |
| SMI duration (months) | 131 (42–195) | NA |
| Psychiatric admission days | 12 (0–50) | NA |

BMI, Body mass index; HbA1c, Glycated haemoglobin; hsCRP, High-sensitive C-reactive protein; IQR, interquartile range; NA, not applicable; SD, standard deviation; SMI, Severe mental illness; WHR, Waist-to-hip ratio.
[a] Continuous data presented as mean ( ± SD) or median (IQR).
[†] $P < 0.001$.

## 3. Results

### 3.1. Elevated levels of 8-oxoGuo and 8-oxodG in SMI compared to controls

Basic data of individuals with SMI and controls are presented in Table 1. While there was no difference in the BMI between the SMI and control group, the SMI group did have significantly larger WHR and an elevated hsCRP (both $P < 0.001$). Conversely, the control group had a significantly higher HbA1c level compared to the SMI group ($P < 0.001$). We found no differences in urinary or CSF levels of 8-oxoGuo or 8-oxodG across the three SMI diagnostic groups (all samples $P > 0.05$). Thus, we referred the SMI group as one entity in the subsequent analyses.

The urinary excretion of both 8-oxoGuo and 8-oxodG was significantly increased in the SMI group compared to the controls (8-oxoGuo: 3.37 [2.29–6.03] vs. 1.94 [1.61–2.35] nM/mM creatinine, $P < 0.001$; 8-oxodG: 1.61 [1.17–3.13] vs. 1.50 [1.21–1.90] nM/mM creatinine, $P < 0.05$) (Fig. 2).

Cohen's *d* on log-transformed data showed a large effect for 8-oxoGuo (1.60) and a small effect for 8-oxodG (0.41). The median excretion difference of 8-oxoGuo and 8-oxodG between the SMI group and the controls was 74% and 8%, respectively. Furthermore, the two markers were significantly correlated in both groups (SMI: Pearson's $r = 0.39$, $P < 0.001$. Controls: Pearson's $r = 0.69$, $P < 0.001$). Significant differences in the 8-oxoGuo and 8-oxodG concentrations persisted after adjusting for variables with significant differences in Table 1.

The group of living individuals with SMI from the GESUS study was

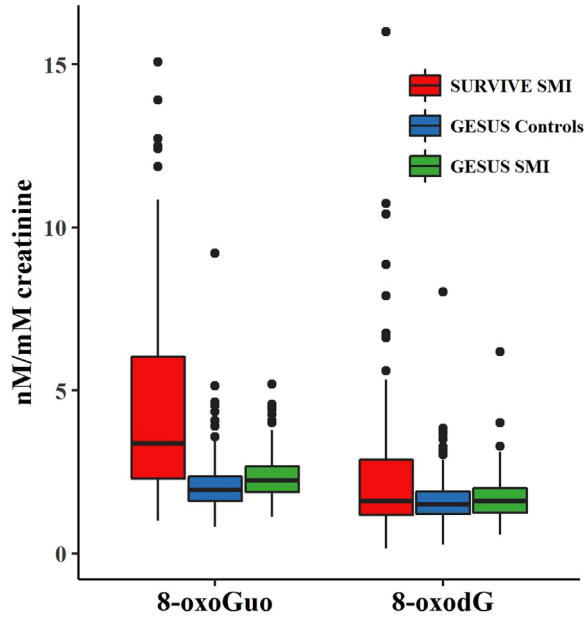


**Fig. 2.** Urinary excretion of 8-oxoGuo and 8-oxodG. Difference in urinary excretion of 8-oxoGuo and 8-oxodG in deceased individuals with SMI (SURVIVE SMI), living mentally healthy controls (GESUS controls), and living individuals with SMI (GESUS SMI). We found elevated excretion levels of both 8-oxoGuo and 8-oxodG in the SURVIVE SMI group compared to GESUS controls ($P < 0.001$ and $P < 0.05$, respectively). Comparing SURVIVE SMI with GESUS SMI showed significant difference for 8-oxoGuo ($P < 0.001$) but not for 8-oxodG ($P = 0.14$). Four outliers in the SURVIVE SMI group in 8-oxoGuo lie outside the figure (range: 17.0–28.1 nM/mM).

significantly older (mean age 52.4 [ ± 12.5], $P < 0.01$) than our SURVIVE SMI group and with significant gender differences (male [$N = 31$], female [$N = 81$], $P < 0.001$). While the median (IQR) urinary 8-oxoGuo concentration was lower in the GESUS SMI group (2.25 [1.88–2.67] nM/mM creatinine, $P < 0.001$) compared to the SURVIVE SMI group, there was no difference in the urinary 8-oxodG concentration (1.61 [1.24–2.00] nm/mM creatinine, $P = 0.14$). On a side note, comparing the GESUS SMI group with the GESUS controls yielded elevated 8-oxoGuo levels in the GESUS SMI group ($P < 0.001$) but no difference in 8-oxodG levels ($P = 0.31$).

### 3.2. Positive correlation of 8-oxodG between CSF and urine samples

For the CSF samples in the SMI group, we determined the median concentration to be 350.7 pM (236.2–705.7 pM) for 8-oxoGuo and 156.6 pM (86.8–248.7 pM) for 8-oxodG. Correlating the CSF concentration of each marker with the respective urinary marker concentration, we determined a significant correlation for 8-oxodG ($r = 0.50$, $P < 0.001$) but no correlation for 8-oxoGuo ($r = 0.15$, $P = 0.16$) (Fig. 3). The 8-oxodG correlation was significantly larger than the 8-oxoGuo correlation (difference = 0.35, 95%CI [0.16; 0.53], $P < 0.001$).

### 3.3. No associations between 8-oxoGuo or 8-oxodG and somatic co-morbidities or psychiatric disease duration and severity

Investigating the SMI group further, we found no associations between urinary and CSF 8-oxoGuo or 8-oxodG and several obesity-related clinical characteristics (BMI, WHR, weight of the greater omentum, HbA1c or hsCRP) after Bonferroni correction for multiple comparisons (Table 2).

Looking into the prevalence of MetS, undiagnosed DM discovered at autopsy, and the levels of oxidative stress markers, we expected an increase in concentrations as an individual suffered from MetS or DM. Surprisingly, we found no such association in our study population in either marker in urine or CSF. Likewise, we found no correlations with urinary or CSF 8-oxoGuo or 8-oxodG with the duration of psychiatric disease or severity (admission days).

### 3.4. Post mortem interval did not affect the results

The median (IQR) post mortem interval in the SMI group was 114 (84 – 156) hours (not applicable [NA] = 17). We found no difference when correlating the post mortem interval with either urinary marker or CSF 8-oxoGuo (urinary 8-oxoGuo: $r = 0.06$, $P = 0.57$; urinary 8-oxodG: $r = 0.13$, $P = 0.24$; CSF 8-oxoGuo: $r = 0.15$, $P = 0.20$). We did, though, find a significant positive correlation of 8-oxodG in CSF with the post mortem interval ($r = 0.21$, $P < 0.05$). Of note, we did not perform repeated post mortem measurements of the samples. However, applying the post mortem interval as a covariate in calculations of 8-oxodG in CSF did not affect the results.

## 4. Discussion

Studies investigating post mortem oxidative damage in SMI have mainly applied immunohistochemical methods to measure oxidative stress levels and have not contextualised the results with systemic levels [18,19]. To our knowledge, this is the first study to investigate both systemic and local oxidative stress levels in deceased individuals with SMI incorporating state-of-the-art methods.

We found significantly elevated levels of urinary 8-oxoGuo and 8-oxodG in deceased individuals with SMI compared to living mentally healthy controls. Even after adjustment for obesity-related variables, the difference persisted. However, especially regarding the elevated levels of 8-oxoGuo, our results could be a derived effect of increased systemic oxidative stress in the agonal phase prior to death. Other variables, such as smoking, alcohol intake and exercise, are known to have an effect on oxidative stress levels [46]. However, it was not possible to include these variables in the SMI group as police reports available at autopsy rarely include this information or only relay them in general terms. Other studies examining oxidative stress levels in SMI found no effect of, e.g., smoking, on the overall result after adjusting for this parameter [8–10].

Our results are in line with those of other studies that established elevated oxidative stress in individuals with SMI [8–11]. Jorgensen et al. demonstrated elevated urinary levels of both 8-oxoGuo and 8-oxodG in schizophrenia compared to mentally healthy controls [8]. While they found an evenly elevated excretion fraction of 8-oxoGuo and 8-oxodG between groups, our study demonstrated an increased median urinary excretion difference of 8-oxoGuo of almost a factor 10 compared to 8-oxodG (74% vs. 8%, respectively) between the SURVIVE SMI group and the GESUS controls. In another study, Jorgensen et al. furthermore demonstrated that increasing elevated urinary levels of 8-oxoGuo correlated with the severity of depression, while no difference was found in 8-oxodG levels [9]. These results, as well as ours, corroborates the notion that RNA is more exposed to systemic oxidative damage compared to DNA [5].

As urinary 8-oxoGuo and 8-oxodG are a reflection of systemic oxidative stress, the corresponding CSF markers may reflect local oxidative stress on RNA and DNA in the CNS [47–49]. As SMI, per se, originates from the brain, correlating levels of 8-oxoGuo and 8-oxodG in CSF with the corresponding levels in urine might indicate to what extent SMI contributes to the systemic oxidative stress level. The correlation coefficient of 8-oxodG in CSF and urine ($r = 0.50$) in our study indicates a moderate association; however, we were unable to demonstrate a correlation of 8-oxoGuo. As RNA is more prone to damage by oxidation, the systemic 8-oxoGuo levels seemingly eclipse the relatively low levels contributed by the CNS. Conversely, as DNA is less prone to oxidative stress damage, elevated levels of 8-oxodG in the CNS as a consequence of SMI may account for a larger fraction of the combined

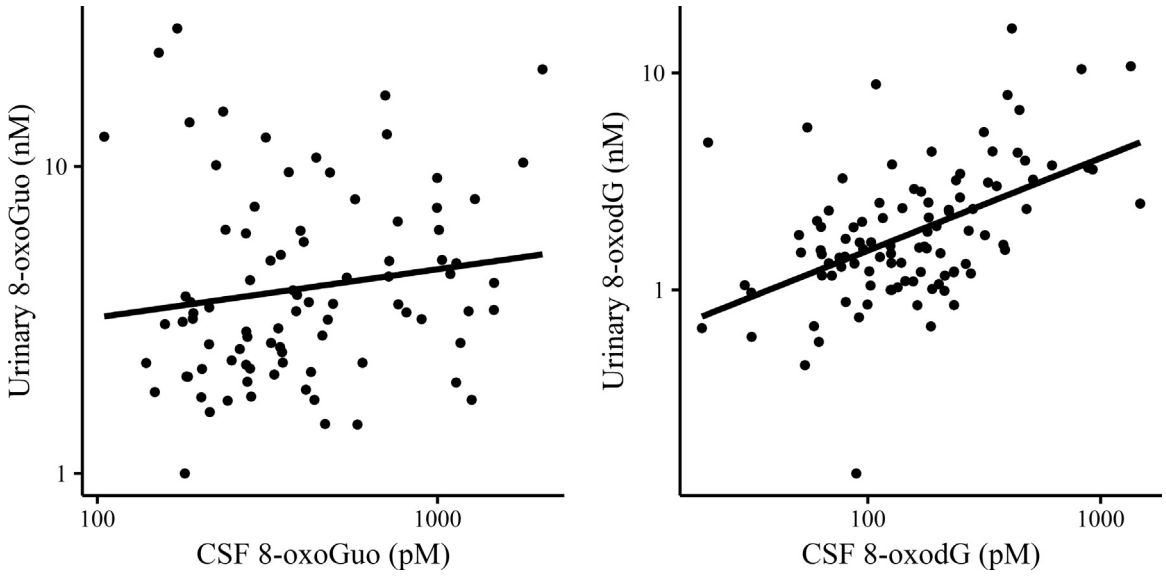


**Fig. 3.** Correlations of CSF and urinary 8-oxoGuo and 8-oxodG levels. We found a significant correlation with 8-oxodG (r = 0.50, P < 0.001) but no correlation with 8-oxoGuo (r = 0.15, P = 0.16). Furthermore, the r-values of the two markers were significantly different (P < 0.001).

**Table 2**
Correlations between obesity-related variables and 8-oxoGuo and 8-oxodG levels. Data are presented as Pearson's *r*. Linear regression analyses, adjusted for PMI and age, with post hoc Bonferroni corrections (*P*-value significant at a 0.002 level) left no significant associations.

| | Urinary markers | | CSF markers | |
|---|---|---|---|---|
| | 8-oxoGuo | 8-oxodG | 8-oxoGuo | 8-oxodG |
| **Somatic co-morbidities** | | | | |
| Omental adipose tissue | -0.01 | -0.11 | -0.11 | -0.16 |
| BMI | -0.05 | -0.20 | -0.09 | -0.26 |
| WHR | -0.08 | -0.15 | -0.08 | -0.22 |
| HbA1c (NA = 21) | 0.12 | 0.08 | 0.10 | 0.02 |
| hsCRP (NA = 21) | -0.02 | 0.05 | 0.17 | 0.02 |
| **Psychiatric disease** | | | | |
| Duration of SMI | 0.08 | 0.28 | -0.08 | 0.05 |
| Admission days | -0.10 | 0.02 | -0.10 | -0.06 |

BMI, body mass index; CSF, cerebrospinal fluid; HbA1c, glycated haemoglobin; hsCRP, high sensitive C-reactive protein; NA, not applicable; SMI, severe mental illness; WHR, waist-to-hip ratio

systemic load. Interestingly, placing our CSF measurement results in context, the median concentration of CSF 8-oxoGuo and 8-oxodG in our study was increased 4-fold and 15-fold, respectively, compared to the median concentration of healthy controls in a recent study by Jorgensen et al. [50]. Underscoring the striking difference, the mean age of our SMI group was 10 years lower than said control group. Caution should be taken when comparing concentrations of oxidative stress markers, particularly in CSF [3]. However, we must stress that the measurement of oxidative stress markers in both studies was conducted in the same laboratory, although with a slightly different assay, thus eliminating major inter-laboratory variation as a source of the difference. Our finding of a 15-fold increase in CSF 8-oxodG and 'only' a 4-fold increase in CSF 8-oxoGuo may imply that the main site of cellular oxidative stress in the CNS of individuals with SMI can be found in the DNA, which is in line with general perception [22]. In contrast, Che et al. indicated increased oxidation of RNA but not DNA in hippocampal cells of deceased individuals with SMI [18]. However, as this study employed fluorescence intensity to quantify oxidized nucleosides, a direct comparison should be made with caution. Another possible explanation for the relatively increased levels of 8-oxodG compared to 8-oxoGuo in the CNS could be the brain's exclusively aerobic and glucose-dependent metabolism. Thus, glucose-induced oxidative stress possibly contributes far more in the CNS than in other tissue [23,51].

Whether SMI is the consequence of oxidative stress or vice versa remains unanswered. Nordholm et al. demonstrated no difference in urinary oxidative stress markers in patients with ultra-high risk of developing psychosis and in patients with first episode schizophrenia compared to healthy controls [52]; further, in a prospective study, Jorgensen et al. found that increased urinary 8-oxodG levels were inversely associated with incidence of psychiatric illness in patients with type 2 DM [53]. While SMI may not exhibit increased levels of oxidative stress in the early phase [52], one might expect increased oxidative stress levels as the SMI develops. Yet, we did not find a correlation of 8-oxoGuo or 8-oxodG with the duration of the SMI in our study. However, the aforementioned markedly elevated 8-oxodG levels in CSF in our study population indicates that a causality between disease duration and oxidative stress levels could be inferred with positive correlations in CSF than in urine. In line with this, Jorgensen et al. likewise found no correlation between disease duration and urinary oxidative stress levels in schizophrenia [8]. This should prompt future studies of psychiatric disease duration and oxidative stress markers to explore CSF rather than urinary oxidative stress.

As oxidative stress levels have repeatedly been linked with obesity, MetS and DM [25,28,29,54], we expected to find increasing levels of 8-oxoGuo and 8-oxodG associated with obesity-related variables and among individuals suffering from MetS or DM. Surprisingly, this was not the case in our study. Regarding MetS and DM, relatively few individuals in our study suffered from these diseases, which could account for the lack of association. While our SMI group in general could be characterised as borderline overweight, their BMI did not differ from the mentally healthy controls in the GESUS study. As the SMI group demonstrated elevated abdominal adiposity and increased chronic inflammation but decreased HbA1c compared to controls, this could indicate that chronic inflammation precedes adverse blood glucose status independent of oxidative stress markers. This could warrant further investigation of the coupling of different levels of overweight/obesity and SMI related to oxidative stress.

Few studies have employed post mortem material to investigate oxidative stress markers. When using post mortem material, one must consider the possible effect of PMI. While Che et al. found a slight positive increase in oxidative stress levels with prolonged PMI in one of three regions of the brain (dentate gyrus), the PMI had no effect on the overall result [18]. Likewise, we demonstrated a slight increase in CSF

8-oxoGuo with PMI, which did not alter the outcome of the findings. Still, our results indicate that 8-oxoGuo and 8-oxodG in urine and 8-oxodG in CSF appear stable post mortem. Thus, while an investigation of oxidative stress in a post mortem population appears valid, direct comparisons with a living population should be regarded with some reservation. To strengthen the validity of post mortem samples in general, repeated measurements in specific time intervals after death combined with detailed information of the agonal phase may elucidate this aspect further.

There are some limitations to this study that warrant mentioning: (1) Numerous variables related to oxidative stress were not available to us as we used autopsy data. Smoking status, exercise, and the opportunity to assess the severity of an individual's SMI through scoring systems are just some factors that could have elucidated the association of SMI with oxidative stress further. (2) Regarding urine, and to some extent CSF samples, we did not know the circumstances surrounding the deaths of the subjects. Several unknown factors in the agonal phase might have introduced bias to our results. (3) Due to logistic reasons, some urine and CSF samples had been stored at -20 °C ($N = 22$) and not -80 °C. Only CSF 8-oxoGuo samples stored at -20 °C had significantly higher values compared to samples stored at -80 °C ($P < 0.001$). However, if the storage temperature would have caused a systematic bias, we would have expected differences in the urine as well as CSF 8-oxodG samples. Additionally, we found no correlation with PMI and CSF 8-oxoGuo, indicating only a discrete effect, if any, on the measurements. (4) Due to the comprehensive multicentre study design, it was not possible to include matched, mentally healthy deceased controls in the study. Thus, the control group was selected from an independent study [40]. As the oxidative stress markers in both studies were assayed at the same laboratory using the same method, the results were comparable, still taking limitation 2 into account. (5) As the SURVIVE study was based on forensic autopsy material, there was a selection bias for the inclusion of cases. Only cases for which the Danish police had requested an autopsy due to suicide, accident or uncertainty over the manner and/or cause of death at the medico-legal inquest were eligible for inclusion. Thus, the selection of deceased individuals with SMI was not necessarily representative of the general population with SMI.

In conclusion, our results corroborated earlier findings of increased levels of systemic oxidative stress in individuals with SMI compared to mentally healthy controls. Furthermore, we demonstrated markedly elevated oxidative stress levels of both 8-oxoGuo and 8-oxodG in CSF, with the latter being 15-fold higher compared to mentally healthy individuals in other studies. This indicates that DNA and not RNA is the primary site for oxidative cellular damage in the CNS in individuals with SMI. Contrary to what we expected, no associations of oxidative stress levels and obesity-related co-morbidities were found. Our study demonstrated the applicability of post mortem material and data to elucidate disease mechanisms and conditions in individuals with SMI. A further understanding of the link between oxidative stress and SMI may provide novel treatment modalities for a group of patients with complex health challenges.

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## Author disclosure statement

No competing financial interests exist.

## Authors' contributions

M.R.C. analysed the data and drafted the manuscript. T.H. performed the UPLC MS/MS analyses of urine samples. A.W. performed the UPLC MS/MS analyses of CSF samples. C.E. was the head of research of the GESUS study and provided data of the living control group. J.B. was the head of research of the SURVIVE study. H.E.P., N.L, J.R. and J.B. critically reviewed the drafts. All authors approved of the final manuscript.

## Declarations of interest

None.

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