Skin advanced glycation end products as biomarkers of photosensitivity in schizophrenia

Running title: Carbonyl stress and photosensitivity

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ABSTRACT

Objectives: Photosensitivity to ultraviolet A (UVA) radiation from sunlight is an important side effect of treatment with antipsychotic agents. However, the pathophysiology of drug-induced photosensitivity remains unclear. Recent studies demonstrated the accumulation of advanced glycation end products (AGEs), annotated as carbonyl stress, to be associated with the pathophysiology of schizophrenia. In this study, we investigated the relationship among skin AGE levels, minimal response dose (MRD) with UVA for photosensitivity and the daily dose of antipsychotic agents in patients with schizophrenia and healthy controls.

Methods: We enrolled 14 patients with schizophrenia and 14 healthy controls. Measurement of skin AGE levels was conducted with AGE scanner, a fluorometric method for assaying skin AGE levels. Measurement of MRD was conducted with UV irradiation device.

Results: Skin AGE levels and MRD at 24, 48 and 72 h in patients with schizophrenia showed a higher tendency for photosensitivity than in the controls, but the difference was statistically insignificant. Multiple linear regression analysis using skin AGE levels failed to show any influence of independent variables. MRD did not affect skin AGE levels.

Conclusions: Photosensitivity to UVA in patients with schizophrenia recieving treatment with antipsychotic agents might not be affected by skin AGE levels.

Keywords: schizophrenia, advanced glycation end products, carbonyl stress, photosensitivity

1 | INTRODUCTION

Researchers have long sought validated, reproducible, sensitive and specific biomarkers of schizophrenia. Previous studies have demonstrated significant alterations in the levels of peripheral biomarkers, such as serum amino acids and inflammation markers levels of schizophrenia (Chan et al., 2015; Nishimon et al., 2017; Ohnuma and Arai, 2011; Ohnuma et al., 2008). While the majority of previous studies have demonstrated the potential of diagnostic or prognostic biomarkers, few cross-sectional observational studies have also examined the safety or toxicity of biomarkers for treatment with antipsychotic agents (Lai et al., 2016; Tomasik et al., 2016). The accumulation of advanced glycation end products (AGEs) associated with glycation stress is known as carbonyl stress, and several recent studies have demonstrated an association between the pathophysiology of schizophrenia and carbonyl stress (Arai et al., 2010; Katsuta et al., 2014; Kouidrat et al., 2013; Miyashita et al., 2013; Takeda et al., 2015). Previously, we reported that high serum levels of pentosidine, a marker of carbonyl stress, were associated with high doses of antipsychotic agents and the duration of polypharmacy treatment with antipsychotic agents in acute-stage schizophrenia (Katsuta et al., 2014; Sannohe et al., 2017). Other researchers demonstrated possible correlation between AGE levels and the duration of antipsychotic treatment as well as antipsychotic dose

(Hagen et al., 2017). Serum pentosidine levels have not been associated with the severity of the symptoms of schizophrenia (Katsuta et al., 2014; Sannohe et al., 2017).

A photosensitive reaction, manifesting as a skin rash, is one of the side effects of antipsychotic drug-induced toxicity. Photosensitivity is believed to reflect a disturbed immune function after exposure of skin to sunlight and after the administration of not only typical antipsychotics, including phenothiazine (Wolnicka-Glubisz et al., 2005) but also current main stream of pharmacotherapy with atypical antipsychotics, including clozapine, alanapine and aripiprazole (Al-Aojan and Al-Khalifah, 2018; Gregoriou et al., 2008). In addition, some cases transferred to persistent light reaction with cross-reaction mechanism, although the causal substance (antipsychotics) has been eliminated (Amblard et al., 1982; Barbaud et al., 2001). The main mechanism of photosensitivity was considered to be immunoreaction, especially delayed-type allergy. Photohaptens are the main causative substances in first sensitization of photosensitivity and bind covalently to protein under exposure to UVA via Langerhans cells photomodified with a photohapten, which can stimulate immune T cells; then these mechanisms were elicited by UVA irradiation (Tokura, 2000). Finally, these reactions cause skin inflammation that was also caused by accumulation of AGEs, which was caused by antipsychotics dose and considered as one of the pathophysiologic mechanisms of schizophrenia (Ohnuma et al.,

2018). Thus, the accumulation of skin AGEs also might be involved in the pathophysiology underlying drug-induced photosensitivity. Indeed, the accumulation of AGEs in skin has been shown to be involved in the pathophysiology of some dermatological diseases, such as solar elastosis (Yoshinaga et al., 2012), prurigo nudularis in dialysis patients (Dyer et al., 1993; Meng et al., 2001) and perforating dermatosis (Fujimoto and Tajima, 2004; Seite et al., 1998).

In the current study, we investigated the relationship between photosensitivity and possible causative factors, including skin AGE levels, minimal response dose (MRD), daily antipsychotic doses and serum pentosidine and pyridoxal levels in patients with schizophrenia treated with antipsychotic agents and in healthy controls. We aimed to investigate whether a high glycated stress state is associated with overdose of antipsychotic drugs and the potential implication of using glycated stress as a biomarker of photosensitivity in schizophrenia.

2 | METHODS

2.1 | Subjects

The Ethics Committee of the Juntendo University School of Medicine approved the study protocol (15-052). All participants provided written informed consent prior to participation. Fourteen stable outpatients diagnosed with schizophrenia and treated with a fixed dose of antipsychotics for ≥30 days were enrolled in a cross-sectional observational study at Juntendo University Schizophrenia Project (Ohnuma et al., 2008). First-episode, drug-naïve patients were excluded from this study. All included patients met the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) criteria for schizophrenia (Association, 2013). Clinical symptoms were assessed using the Brief Psychiatric Rating Scale (BPRS), with each item rated on a seven-point scale as previously described (Katsuta et al., 2014). Three patients treated with first-generation antipsychotics, ten treated with second-generation antipsychotic and one treated with both types of antipsychotics (polypharmacy) were enrolled. Total administered antipsychotics were converted to the chlorpromazine (CP) dose (Inada and Inagaki, 2015). In addition, 14 healthy controls without current or a history of psychosis according to the Japanese latest version of Structured Clinical Interview for DSM-IV were recruited through the website of the Juntendo Hospital. The inclusion criteria in this study were as follows: 1) age between 20 and 60 years to exclude ageing-related effects; 2) no photosensitivity caused by factors such as porphyria or xeroderma

pigmentosum syndrome; 3) no polypharmacy, i.e. no prescription history of >3 antipsychotic drugs; 4) no history of diabetes, atopic dermatitis or kidney dysfunction; 5) no more than moderate obesity (i.e. BMI < 30 kg/m^2); 6) no medication use resulting in hyperesthesia optica and 7) no history of smoking or alcoholism. A schematic of the study protocol is presented in Figure 1.

2.2 | Measurement of pentosidine

All blood samples were collected prior to breakfast to control for the influence of food and exercise. The samples were immediately centrifuged at room temperature for 5 min at 3500 rpm (2400 ×g), and the supernatant was frozen at −80 °C until further use. The concentration of serum pentosidine was measured using a competitive enzyme-linked immunosorbent assay kit (FSK Pentosidine, FUSHIMI Pharmaceutical Co., Ltd., Kagawa, Japan), as previously described. Briefly, pronase was added to the serum to expose the protein-bound pentosidine molecules, and the samples were incubated at 55 °C for 1.5 h. After the stipulated reaction time, the mixtures were heated in boiling water for 15 min for enzyme inactivation. An antibody against pentosidine and the pre-treated sample or a standard pentosidine solution were added to each well and incubated at 37 °C for 1 h. After washing, a peroxidase-labelled goat anti-rabbit IgG

polyclonal antibody was added and incubated at room temperature for 1 h. Subsequently, a colour development reagent containing 3,3′,5,5′-tetramethylbenzidine (TMB) was added to each well. The reaction was stopped 10 min later by adding a TMB stop buffer. Absorbance was measured within 10 min at 450 and 630 nm (main and reference wavelength, respectively) (Katsuta et al., 2014).

2.3 | Measurement of pyridoxal

Serum pyridoxal levels were measured using high-performance liquid chromatography (HPLC) on a Hitachi L-7000 series (Hitachi, Ibaraki, Japan). Then, 200 µl aliquots of the serum were added to 200 µl citrate buffer solution and 75 µl acid phosphatase followed by incubation at 37 °C for 1 h. After completion of hydrolysis, 300 µl of 20% trichloroacetic acid was added to the mixture followed by centrifugation for 10 min at 12000 rpm (12000× g) at 4 °C. Subsequently, 400 µl of the upper aqueous layer was neutralised with 75 µl sodium acetate solution and used as HPLC reagent. The HPLC reagents were analysed with a Fluorescent Detector FP-2025 (Ex λ: 325 nm, Em λ: 395 nm; JASCO Corporation, Tokyo, Japan) and a Wakosil-II $_5C_{18}$HG (4.6φ × 250 mm) column (Wako Pure Chemical Industries, Osaka, Japan). Detailed information regarding the HPLC conditions can be accessed in a previous study by (Katsuta et al., 2014).

2.4 | Measurements of skin AGE levels

Measurement of skin AGE levels was conducted with a TrūAge Scanner™ (Morinda Worldwide Inc.), which represents a fluorometric method for assaying AGE levels in the skin. The forearm of the patients' dominant arm was placed on the scanner. A prerequisite for this assay is that the skin in the area being measured must be healthy; homogeneous; free of birthmarks, tattoos or excessive hair growth and without recent exposure to skin creams or any substance possibly having fluorescent properties. All measurements lasted approximately 15 s and was performed in triplicate after which the results were presented as an average value (Hagen et al., 2017).

2.5 | Measurement of MRD

Measurement of MRD was conducted with UV irradiation device (UV109A, Herbert Waldmann GmbH & Co., KG). We conducted a "preliminary study" with some healthy volunteers, our preliminary data (unpublished) showed that MRD range of UVA in Japanese healthy controls were 15–30 J/cm^2 with present methods. In addition, the lowest MRD is known as 320kJ/m^2 for UVA (360nm), approximately 30 J/cm^2, and appearance of erythema at 24 to 72 h with under 15 J/cm^2 UVA radiation means these

subjects assumed to have photosensitivitity (Hawk et al., 2013). Thus, the maximum irradiation time was defined as 30 J/cm^2 in this study. Eight patch holes (0% = 30J/cm^2, 20%, 30%, 40%, 50%, 60%, 70% and 80%) were selected in a V strength rate cut-off filter. First, we irradiated the skin on the patients' front arm with UVA light. After 24, 48 and 72 h, the exposed skin area was examined during a hospital visit (http://www.jove.com/video/50175) (Heckman et al., 2013). Unfortunately, six patients who underwent MRD at 24 h, one who underwent MRD at 48 h and two who underwent MRD at 72 h were unable to visit the hospital owing to personal reasons. Thus, the number of patients who underwent MRD at 24, 48 and 72 h was considered to be 8, 12 and 13, respectively.

2.6 | Statistical analysis

All statistical analyses were conducted using SPSS version 22 (IBM Corp., Armonk, NY). Chi-square tests were conducted to analyse the difference in terms of sex distribution between the groups. A p-value < 0.05 was defined as the level of statistical significance. Differences in terms of serum pentosidine, pyridoxal and skin AGE levels and MRD between the unpaired groups were examined using a two-tailed Mann–Whitney U-test for comparison between the two groups (schizophrenia vs. healthy

control). Correlation between the clinical features (e.g. age, BMI and daily CP equivalent dose) and measured biomarkers was analysed using single correlation analysis (Spearman's correlation test).

Multiple linear regression analysis included factors that potentially contributed to significantly different skin AGE levels. These factors were selected on the basis of their significant correlation with reported altered clinical characteristics such as BMI, age and daily CP equivalent dose and were set as independent variables. Finally, stepwise multiple regression analyses were performed for the potential significantly different skin AGE levels as dependent variables and for the three above-mentioned clinical variables as independent variables. The post-hoc power analysis ($1 - \beta$) were performed with G*Power (http://www.gpower.hhu.de/) with the condition; $\alpha = 0.05$, effect size = 0.5 and each sample group size = 14.

3 | RESULTS

3.1 | Comparison of clinical variables

Clinical variables including the measured biomarker levels were compared between the patients and healthy controls (Table 1). Sex ratio, age and BMI as well as serum

pentosidine did not differ between the groups (Table 1). However, the serum pyridoxal levels in the patients were significantly lower than in the healthy controls (Table 1). While skin AGE levels in the patients with schizophrenia showed a higher tendency compared with the controls, they did not reach statistical significance. Furthermore, the MRD at 24, 48 and 72 h showed higher tendencies in the patients with schizophrenia than in the controls, but the difference was also statistically insignificant. Post-hoc power analysis for this statistic showed $(1 - \beta) = 0.08$.

Single correlation analysis was performed among the carbonyl stress markers (serum pyridoxal and pentosidine and skin AGEs), MRD (24, 48 and 72 h) and the three clinical variables (age, BMI and daily CP equivalent dose) in patients with schizophrenia (Supplementary Table 1). As expected, skin AGE levels showed a significant correlation with age ($r = 0.477$, $p = 0.010$) using single correlation analysis, but not with BMI ($r = 0.114$, $p = 0.562$) and daily CP equivalent dose (patients, $r = 0.018$, $p = 0.952$). Other carbonyl stress markers did not show significant correlation with any clinical variables (all $p > 0.05$). MRDs at 48 h ($r = 0.386$, $p = 0.047$) and 72 h ($r = 0.394$, $p = 0.047$) showed a marginally significant correlation with BMI. In the nature of things, three MRDs showed significant correlations with each other

(Supplementary Table 1). Post-hoc power analysis for this statistic showed $(1 - \beta) = 0.07$.

3.2 | Multiple linear regression analysis

Stepwise multiple linear regression analysis was performed in patients with schizophrenia using skin AGE levels as a dependent variable. Age, BMI and CP equivalent dose showed a significant association with skin AGE levels in the present study (age) and previous studies (BMI and CP equivalent dose) (Sannohe et al., 2017; Takeda et al., 2015) as independent variables. In this analysis, the three independent variables were excluded in the first stage of the analysis in predicting skin AGE levels. However, forced-entry multiple linear regression analysis was performed using the same dependent and independent variables; however, no independent variables were shown to affect skin AGE levels ($F = 0.61$, $p = 0.62$). The stepwise multiple linear regression analysis was performed using MRD (24, 48 and 72 h) as a dependent variable and age, BMI, CP equivalent dose and skin AGE levels as independent variables. The results of these analyses showed that BMI was a significant variable for the prediction of MRD for 72 h in the first stage of analysis ($F = 11.1$, $p = 0.009$), whereas age, CP equivalent dose and skin AGE levels were not significant. The

predictive equation for calculating MRD at 72 h was as follows: MRD at 72 h (J/cm^2) = −6.6 + 0.98 × BMI. All independent variables were excluded in the first stage of the analysis for predicting skin AGE levels for MRD at 24 and 48 h. Post-hoc power analysis for this statistic showed $(1 – \beta) = 0.06$.

4 | DISCUSSION

In the present study, we investigated the relation between photosensitivity using MRD and variable antipsychotic doses as the potential causative pathophysiological agents underlying the accumulation of skin AGEs. The results that higher tendency of serum pentosidine levels and significantly lower levels of serum pyridoxal in the patients with schizophrenia than in the healthy controls were consistent with and confirmed previous well-established results of studies that used serum biomarkers as indicators of carbonyl stress in schizophrenia (Arai et al., 2010; Katsuta et al., 2014; Kouidrat et al., 2013; Miyashita et al., 2013; Takeda et al., 2015). These results verified that the subjects enrolled in this study possibly presented features of carbonyl stress in a population suffering from schizophrenia. While skin AGE levels in the patients with schizophrenia

showed a higher tendency than in the healthy controls, they did not reach statistical significance. Measurement of skin AGE levels with the TrūAge Scanner reflected on the levels of pentosidine, crossline (Obayashi et al., 1996) and pyrropyridine (Hayase et al., 2008) as those exerting fluorescence and crosslinkage with collagen in the skin (Meerwaldt et al., 2005). Although apparent measured AGEs were not specifically restricted (Sell et al., 2018), skin AGE levels showed a clear correlation with the concentration of skin pentosidine levels (Meerwaldt et al., 2005). The accumulation of skin AGEs related to risk for further complications in diseases such as cardiovascular disease, macular degenerative disease etc (Bos et al., 2011; Lutgers et al., 2009) could prove useful as a surrogate marker of the status of carbonyl stress in the skin (Macsai et al., 2013). Thus, the status of carbonyl stress in patients with schizophrenia might vary depending on the affected tissues, e.g. accumulation in the skin or peripheral blood.

The current study has certain limitations, such as the small number of subjects (14 patients with schizophrenia) with low statistical power (1-β; from 0.06 to 0.08) compared with a previous large serum AGE level-driven study (274 patients with schizophrenia) (Sannohe et al., 2017). Further, all patients of the present study were outpatients who showed milder symptoms and were prescribed lower daily doses antipsychotic agents compared with previous studies that included patients suffering

with severe schizophrenia (Sannohe et al., 2017). Both factors, namely, mild disease symptoms and low dosing of antipsychotics, may affect the levels of peripheral carbonyl stress markers (Arai et al., 2010; Katsuta et al., 2014; Kouidrat et al., 2013; Miyashita et al., 2013; Takeda et al., 2015). Indeed, skin AGE levels were significantly elevated in patients with severe schizophrenia (Kouidrat et al., 2015). Furthermore, in skin AGEs levels, elevated these showed relation with antipsychotics dose and its accumulative exposure periods in patients with a recent onset of psychosis (Hagen et al., 2017), as we also showed in serum study (Sannohe et al., 2017).

Unfortunately, this study failed to demonstrate any significant association between daily antipsychotic drug dose and MRD with UVA on skin AGE levels. Further, a control for the specific type of antipsychotic drugs was not accounted for in this study. Among the 14 patients with schizophrenia, only 1 patient treated with polypharmacy showed the most influenced peripheral AGE levels (Sannohe et al., 2017). Although the results presented in this study are clinically relevant, they need to be reproduced in studies that include larger sample sizes and patients with severe schizophrenia treated with high doses of antipsychotic drugs. Patients treated with antipsychotic drugs showed a minor tendency to exhibit lower photosensitivity to UVA. Additionally, higher BMI might influence the MRD at 72 h to show a higher value (i.e.

lower photosensitivity to UVA) in patients with schizophrenia. Obesity has been suggested to trigger a higher status of carbonyl stress (Miyata et al., 2005; Nangaku et al., 2005). The current results demonstrated an inverse relation between BMI and MRD. Overall, we conclude that photosensitivity to UVA in patients with schizophrenia was not clinically apparent and was unaffected by skin AGE levels and related factors such as low antipsychotic drug dose.

Further studies using non-invasive measurement of skin AGE levels on a larger subject population, including those suffering from severe schizophrenia and especially those with demonstrated history of photosensitive reaction to antipsychotic agents, might be beneficial in clinical practice for the treatment of schizophrenia.

CONFLICT OF INTEREST

None to declare

AUTHORS CONTRIBUTIONS

Eriko Tani and Tohru Ohnuma designed the study protocol, recruited subjects, collected clinical data, performed statistical analysis and wrote the manuscript. Yuto Takebayashi, Narimasa Katsuta and Shohei Nishimon designed the study, recruited subjects and

collected clinical data. Hitoki Hirose, Ken Nakayama, Wanyi Mao, Mariko Nakadaira, Narihiro Orimo, Hiroki Yamashita and Yasue Miki assisted in manuscript preparation. Toshio Hasegawa, Etsuko Komiyama, Yasushi Suga and Shigaku Ikeda contributed to study design and interpretation of analysed dermatological data. Heii Arai contributed to the interpretation of all analysed data. All authors contributed to manuscript preparation and approved the final version.

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Table 1. Comparison of clinical variables between the patients with schizophrenia and healthy controls

	Patients with Schizophrenia	**Healthy Controls**	**Statistical Test and *P*-value**	
			Mann–Whitney *U*-test	
	(n = 14)	**(n = 14)**	***t***	***P*-value**
Variables				
Sex, M/F	3/11	8/6	3.743 (x^2)	0.120
Age, mean (y)	42.6 ± 10.7	35.7 ± 9.6	−1.819	0.069
BMI (kg/m^2)	22.9 ± 4.39	21.8 ± 3.24	−0.965	0.335
DOI (y)	18.1 ± 11.7	NA	NA	NA
Total BPRS scores	24.5 ± 3.1	NA	NA	NA
CP equivalent dose (mg/day)	333.9 ± 234.9	NA	NA	NA
Serum pyridoxal (ng/ml)	10.65 ± 6.52	27.58 ± 38.51	−2.112	0.035
Serum pentosidine (ng/ml)	48.27 ± 14.65	45.94 ± 14.69	−0.291	0.771
Skin AGEs (AU)	246.45 ± 65.17	211.69 ± 31.96	−1.817	0.108
MRD 24 h (J/cm^2)	17.63 ± 8.40	13.93 ± 6.82	−0.997	0.319
MRD 48 h (J/cm^2)	14.54 ± 6.46	14.36 ± 6.98	−0.222	0.825
MRD 72 h (J/cm^2)	16.25 ± 6.45	14.79 ± 7.00	−0.316	0.752

Variable data are presented as mean ± standard deviation and range.

AGEs, advanced glycation end products; BMI, body mass index; BPRS, Brief Psychiatric Rating Scale; CP equivalent dose, chlorpromazine equivalent dose; DOI, duration of illness, MRD, minimal response dose

Figure legends

Figure 1 Inclusion criteria and measurement of minimal response dose (MRD) to UVA