High doses of antipsychotic polypharmacy are related to an increase in serum levels of pentosidine in patients with schizophrenia

High doses of antipsychotic polypharmacy are related to an increase in serum levels of pentosidine in patients with schizophrenia

Running title: Carbonyl Stress in Acute Schizophrenia

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ABSTRACT

Background: Carbonyl stress has been reported in patients with schizophrenia as an increase in peripheral pentosidine levels. This cohort study tested whether the accumulation of pentosidine was related to the disease severity or the treatment (routine administration of high antipsychotic doses).

Methods: We followed up our original investigation using a new group of 137 patients with acute schizophrenia and 45 healthy subjects, and then pooled the two cohorts to conduct the following analysis on a total of 274 patients. The impact on serum pentosidine and pyridoxal levels of duration of education, estimated duration of medication, the severity of symptoms, and daily doses of antipsychotics, antiparkinsonian drugs, and anxiolytics was evaluated by multiple linear regression analysis.

Results: The combined cohort of 274 patients exhibited abnormally high serum levels of pentosidine associated with the daily dose of antipsychotic drugs and the estimated duration of medication. This was also observed in the patients treated with antipsychotic polypharmacy, but the serum pentosidine levels of patients treated with first or second generation antipsychotic monotherapy showed no relationship with these two variables.

Conclusion: High levels of serum pentosidine were associated with high daily doses of antipsychotic drugs and the estimated duration of medication in patients treated with antipsychotic polypharmacy.

Highlights

- The large cohort of 274 schizophrenia exhibited high serum levels of pentosidine.
- Pentosidine levels were associated with the daily dose of antipsychotic

polypharmacy.

- Pentosidine levels were associated with the duration of medication of polypharmacy.
- Antipsychotic monotherapy at an optimal dose may be better for patients' health.

Keywords: antipsychotics, dose, pentosidine, polypharmacy, schizophrenia

Abbreviations: AGE, advanced glycation end-product; BMI, body mass index; BPRS, Brief Psychiatric Rating Scale; DSM-IV, Diagnostic and Statistical Manual of Mental Disorders-IV; FGA, first-generation antipsychotics; GLO1, glyoxalase I; SD, standard deviation; SGA, second-generation antipsychotics

1. Introduction

Schizophrenia is a devastating mental disease plagued with limited treatment success due to the high heterogeneity of the disease and a limited understanding of the underlying mechanisms. While positive symptoms are managed by antipsychotics, new drugs are needed to improve cognitive dysfunctions and promote recovery from negative symptoms. Thus, it is crucial to uncover the biochemical anomalies that may be responsible for these aspects of schizophrenia.

Carbonyl stress and associated advanced glycation end-products (AGEs) have recently been the focus of research into neurodegenerative diseases in aging, such as Alzheimer's disease (Takeuchi and Yamagishi, 2008; Yamagishi et al., 2005). Recent studies have also reported high serum levels of toxic AGEs and related molecules in patients suffering from schizophrenia (Arai et al., 2010; Emanuele et al., 2011; Katsuta et al., 2014; Kouidrat et al., 2013; Miyashita et al., 2013; Miyashita et al., 2014; Steiner et al., 2009; Takeda et al., 2015), compared to healthy subjects. A cross-sectional study of 45 schizophrenic patients and 61 healthy subjects performed a detailed investigation of the disease characteristics ranging from psychosocial to genetic factors (Arai et al., 2010). This showed that a raised level of AGEs was not a disease-specific feature of schizophrenia, but that a subpopulation of schizophrenic patients exhibited significantly higher serum levels of pentosidine, a well-established biomarker for AGEs, as well as significantly lower serum levels of pyridoxal, a form of vitamin B6 that detoxifies reactive carbonyl compounds, than the healthy subjects. This study was repeated with another cohort of patients with schizophrenia and healthy subjects, which confirmed the significant increase in pentosidine and decrease in pyridoxal in the peripheral blood of a subpopulation of the patients (Miyashita et al., 2014). A further study by these authors

that included 163 patients with schizophrenia revealed that, compared with patients with lower AGE levels, those under higher carbonyl stress were not characterized by any particular clinical variables but tended to be inpatients, to have a low education status, to require longer hospitalization, and to receive higher doses of antipsychotic medication (Miyashita et al., 2013).

We recently investigated the clinical significance of these potential serum markers in a cross-sectional study of 137 patients with acute schizophrenia, and we were able to confirm the decreased serum levels of pyridoxal (Katsuta et al., 2014).(Katsuta et al., 2014) On the other hand, although the overall mean pentosidine level was not significantly altered, some of the patients with schizophrenia had extremely high serum levels of pentosidine. Unfortunately, this study could not identify any of these markers as symptomatic biomarkers (i.e., for the severity of symptoms). Pentosidine and pyridoxal failed to show any correlation with clinical features of schizophrenia (e.g., duration of illness, age at onset, and family history). On the other hand, a significant positive correlation was found between serum pentosidine levels and the daily antipsychotic dose or cumulative antipsychotic exposure (calculated as the daily dose of antipsychotics × the duration of medication) (Katsuta et al., 2014). However, this study did not address the influence of different types and doses of antipsychotics, such as first-generation antipsychotics (FGA; e.g., haloperidol and chlorpromazine), second-generation antipsychotics (SGA; e.g., risperidone, olanzapine, and aripiprazole), and their combinations.

The aim of the present study was to investigate the relationship between high serum pentosidine levels and schizophrenia using correlation and stepwise multiple linear regression analyses. The largest cohort of patients with schizophrenia ever used in a

single analysis was obtained by pooling the data obtained from the patients recruited in the present study with those of our previous study (Katsuta et al., 2014). This approach allowed us to determine whether the accumulation of serum pentosidine is indicative of the patients' clinical characteristics and/or the regimen of antipsychotics. This is an important distinction. Carbonyl stress is associated with the pathogenesis of a number of diseases, including Alzheimer's disease and renal disease; thus, if the kinds and dose of antipsychotic regimen are implicated in producing carbonyl stress (by mechanisms that have yet to be elucidated), this would have implications for patients' long-term health.

2. Materials and methods

2.1. Study population

This study evaluated 137 patients admitted to the Juntendo Koshigaya Hospital (Saitama) or the Juntendo Hospital (Tokyo) due to worsening symptoms of schizophrenia. They met the criteria of the Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV) for the diagnosis of schizophrenia. The exclusion criteria were as follows: participation in the previous study (Katsuta et al., 2014), any comorbidity known to influence AGE levels (i.e., diabetes mellitus and chronic renal disease), systemic or neurological diseases, past head trauma with loss of consciousness, and a lifetime history of alcohol or substance abuse. This study also enrolled 45 healthy volunteers who did not meet current or past criteria for any Axis I disorder in DSM-IV and had no present physical disease. All participants provided their written informed consent prior to participation, and the Ethics Committee of the Juntendo University

School of Medicine approved the protocol (2015014).

2.2. Physical examination and laboratory tests

On the day of admission, all patients had a physical exam, including the measurement of body mass index (BMI). In addition, a blood sample was collected to measure the baseline levels of glucose, glycated hemoglobin (HbA1C), creatinine, and urea nitrogen. Glomerular filtration rate (normal > 60 ml/min) and urinalysis were also used to measure renal function. The healthy subjects underwent the same physical exam and blood tests as the patients, conducted on the day the serum samples for the biomarkers were collected.

2.3. Evaluation of clinical symptoms

Clinical symptoms were assessed using the Brief Psychiatric Rating Scale (BPRS), with each item rated on a 7-point scale, as previously described (Katsuta et al., 2014; Takeda et al., 2015). The overall total rating and scores related to positive and negative symptom clusters were compared (Bech et al., 1986). The age at onset of the illness was determined as the age at which any schizophrenia symptom described in the diagnostic criteria of DSM-IV first appeared, established from the medical records and/or by detailed questioning of the patients and their family members. A family history of psychiatric disease was defined as having a first- or second-degree relative who met any current or past criteria for any Axis I disorder of DSM-IV; this was ascertained by asking patients or their healthy family members about this. The Juntendo University Schizophrenia Project (JUSP) (Ohnuma et al., 2008) is a retrospective, observational study; the use of drug therapy is not controlled so that each patients' symptoms can be

controlled in the most effective manner.

2.4. Measurement of carbonyl stress markers

All blood samples were taken before breakfast to exclude the influence of food and exercise. Serum pentosidine was measured using a competitive enzyme-linked immunosorbent assay, and serum vitamin B6 (pyridoxal) as measured using high-performance liquid chromatography, as we previously described (Katsuta et al., 2014).

2.5. Statistical analysis

The potential differences in clinical characteristics of the previous and present study patient cohorts were compared using Student's t-tests or χ^2 tests, as appropriate, employing SPSS Statistics Version 21 (IBM, Chicago, IL, USA). The serum pentosidine and pyridoxal levels of unpaired groups (e.g., healthy subjects vs schizophrenia patients, or patients groups receiving different antipsychotics) were compared using two-tailed Mann–Whitney *U* tests for two-group comparison and Kruskal–Wallis tests for the comparison of three or more groups. The post hoc analysis of Kruskal–Wallis tests was conducted with two-tailed Mann–Whitney *U* tests. The significance of the *P*-value for these analyses was set based on the Bonferroni correction (the probability level of $P < 0.05$ divided by the number of comparisons in each analysis).

The multiple linear regression analysis included the following factors that potentially contribute to increased pentosidine levels, based on recent studies: 1) duration of education (Miyashita et al., 2013), 2) being an inpatient (number of admissions) (Arai et al., 2010; Miyashita et al., 2013), 3) the duration of the psychiatric

illness (Katsuta et al., 2014; Miyashita et al., 2013), 4) the severity of the symptoms of schizophrenia (the total BPRS score) (Miyashita et al., 2013), 5) the estimated duration of medication (in years, calculated as the difference between the total duration of the illness and the duration of untreated psychosis) (Katsuta et al., 2014), 6) the daily dose of antipsychotics (Katsuta et al., 2014; Miyashita et al., 2013) and also frequently accompanied, 7) the daily dose of antiparkinsonian drugs, and 8) the daily dose of anxiolytics (including sedatives and hypnotics). As would be expected, three of these variables, 2) the number of admissions, 3) the duration of the illness, and 5) the estimated duration of medication, showed significant correlations with each other (r = 0.24 to 0.93, all $p < 0.005$); from these, the estimated duration of medication was chosen as an independent variable to represent antipsychotic medication. Finally, stepwise multiple regression analyses were performed for the potential biomarkers pentosidine and pyridoxal as dependent variables and the following six independent variables: 1) the duration of education, 2) the severity of the symptoms (the total BPRS score), 3) the estimated duration of medication, 4) the daily dose of antipsychotics, 5) the daily dose of antiparkinsonian drugs, and 6) the daily dose of anxiolytics. The daily doses of antipsychotics, antiparkinsonian drugs, and anxiolytics were converted into chlorpromazine-, biperiden-, and diazepam-equivalent doses, respectively (Inada and Inagaki, 2015).

3. Results

3.1. Baseline clinical characteristics of schizophrenia patient

First, clinical variables, including levels of the measured biomarkers, were compared between the patients and healthy subjects of the present cohort (Table 1). The sex ratio

did not differ between the groups (χ^2 = 1.57, p = 0.21), but the patients were older than the healthy controls (χ^2 = -6.5, p < 0.001). In the present cohort, the pentosidine levels of the patients were significantly higher than those of healthy controls (χ^2 = -2.3, p = 0.021; after applying the Bonferroni correction to testing for two biomarkers, p < 0.025 (i.e., 0.05/2) was considered statistically significant). The pyridoxal levels in the present cohort were again significantly lower in the patients than in the healthy subjects (χ^2 = 4.5, p < 0.001).

The pentosidine and pyridoxal levels were similar in the present and previous healthy cohorts, indicating consistency in these biomarker assays between the two cohorts. After verifying as described that there were no significant differences in profiles between the two cohorts, the two patient data sets were combined (Table 2).

3.2. Relationship between the biomarkers and clinical features of schizophrenia

Of the 274 patients, 48 had not taken any antipsychotics for at least four weeks prior to admission, and 46 were given only FGA; 112, only SGA; and 68, polypharmacy of antipsychotics (at least one FGA and one SGA simultaneously). Among the 274 patients, 152 (55.5%) were taking antiparkinsonian drugs and 179 (65.3%) were taking anxiolytics (including sedatives and hypnotics). The rate of concomitant use of anticonvulsants was relatively low (41 patients, 12.6%). In addition, 36 patients (13.1%) were taking some kind of laxative, but the rates for other non-psychotropic drugs (such as antihypertensives and stomach medicine) were low (less than 5%).

In the stepwise multiple linear regression analysis with serum pentosidine level as the dependent variable and the six potential independent variables listed earlier, only two of the variables were found in the first stage of the analysis to be significant in

predicting the serum pentosidine levels: the estimated duration of medication and the daily dose of antipsychotics (p = 0.046 and p < 0.001, respectively) (Table 3). The predictive equation for serum pentosidine levels was then as follows: serum pentosidine level (ng/ml) = 32.2 + 0.358 × (daily dose of antipsychotics, mg) + 0.171 × (estimated duration of medication, years).

In the multiple linear regression analysis for serum pyridoxal levels, all six potential independent factors were excluded at the first stage of the analysis.

3.3. Impact of antipsychotics on the carbonyl stress markers

The combined 274 schizophrenia patients were grouped according to the type of antipsychotic treatment they received: None (not treated with antipsychotics at the time of admission), FGA, SGA, or polypharmacy. Some clinical variables differed significantly between the groups (Table 4). As expected, the results of post hoc analysis reflected the nature of clinical practice; for example, the FGA group were significantly older, with a longer duration of illness and longer estimated duration of medication, and the polypharmacy group were receiving a significantly higher daily dose of antipsychotics than the other groups (Table 5). The serum levels of pentosidine (but not of pyridoxal) were significantly different among the treatment groups (Table 4). Post hoc analysis suggested that antipsychotic treatments were associated with an increase in pentosidine levels (None < FGA; None < Poly) and that the relationship was more significant with polypharmacy than with monotherapy (SGA < Poly; Fig. 1 and Table 5).

3.4. Relationships between biomarkers and clinical characteristics in each

antipsychotic treatment group

The stepwise multiple linear regression analysis with serum pentosidine level as the dependent variable and the six potential independent variables listed earlier was repeated for each antipsychotic treatment group. The patients who were not receiving antipsychotic treatment (None group) showed no significant correlation between serum pentosidine levels and the severity of their symptoms or the estimated duration of medication. In the analyses for the FGA and SGA patient groups, all six independent variables were excluded at the first stage of the analysis. However, the analysis for the polypharmacy patient group again showed that the estimated duration of medication and the daily dose of antipsychotics were both significant in the first stage of the analysis in predicting the serum pentosidine levels ($p = 0.048$ and $p = 0.046$, respectively), whereas the other potential independent variables were not (Table 3). The predictive equation for serum pentosidine levels was then as follows: serum pentosidine level in the polypharmacy group (ng/ml) = 16.2 + 0.368 × (daily dose of antipsychotics, mg) + 0.365 × (estimated duration of medication, years).

4. Discussion

The present study determined the clinical significance of enhanced carbonyl stress—as shown by high peripheral pentosidine levels—for the pathophysiology of schizophrenia. The current opinions are roughly divided into the following categories: the pentosidine levels reflect the disease severity and/or the condition per se (Arai et al., 2010; Miyashita et al., 2013) or the dose of antipsychotics (Katsuta et al., 2014; Miyashita et al., 2013). A relationship was suggested between the severity of the clinical symptoms (i.e., the BPRS scores) and antipsychotic doses could be involved,

but they are not directly correlated because these drugs affect (i.e., improve) symptom severity.

The decrease in pyridoxal levels in the patients with schizophrenia in the present combined cohort of 274 patients was highly consistent with the results from other studies (Arai et al., 2010; Miyashita et al., 2013), although the clinical meaning of this decrease remains unclear. The pentosidine levels of our combined cohort were significantly higher than those in the healthy subjects, which again was consistent with previous reports (Arai et al., 2010; Miyashita et al., 2013).

The detailed analysis of the clinical characteristics of schizophrenia provided herein are derived from the largest patient cohort to date. The patients that were not taking antipsychotic drugs at the time of admission showed the highest BPRS scores (the worst symptoms), and the lowest serum pentosidine levels, suggesting that serum pentosidine levels do not represent a symptomatic marker of schizophrenia per se. However, the serum levels of pentosidine in acute schizophrenia were associated with high daily doses of antipsychotics at the time and with the estimated duration of medication over the patient's lifetime, whereas the other possible independent variables gleaned from previous studies, such as the duration of education and the severity of symptoms, showed no significant association. These results suggest the increase in serum pentosidine levels may be affected by high doses of antipsychotics and a longer exposure to these drugs. These clinical factors should be investigated in detail.

In clinical practice, antipsychotic treatment in schizophrenia is usually accompanied by the concomitant use of antiparkinsonian drugs and anxiolytics, but the doses of these major concomitant drugs did not affect the serum pentosidine levels. The relative low usage rate of other non-psychotropic drugs (used by only 13% of the

cohort) was probably due to the exclusion from enrollment of patients with physical diseases through the physical exam and blood test. Thus, the influence of these other non-psychotropic drugs could be excluded.

The serum pentosidine levels in the FGA and SGA monotherapy groups did not show any relationship with the dose of antipsychotics or the estimated duration of medication, but the pentosidine levels in the polypharmacy group again showed associations with these two variables. In addition, the polypharmacy group exhibited higher serum pentosidine levels and longer estimated durations of medication than those taking a single SGA or no antipsychotics at the time of admission. This group was also receiving higher doses of antipsychotics than the FGA and SGA single usage groups. These prominent pharmacological features of the polypharmacy group may explain the results of the multiple linear regression analysis for the whole cohort of 274 patients, which showed the same two explanatory variables for pentosidine levels.

Taken together, these findings suggest that higher daily doses of antipsychotic polypharmacy and a long duration of usage could be the most influential factors on carbonyl stress in schizophrenia. Given that higher carbonyl stress can result in worse pathophysiology for a number of diseases, including Alzheimer's disease (Takeuchi and Yamagishi, 2008; Yamagishi et al., 2005), antipsychotic monotherapy at an optimal dose may be better for the patients' long-term health.

There were some limitations to this cohort study that could not be avoided. First, we divided the antipsychotics used into the broad groups of FGA, SGA, and polypharmacy, and we did not compare the specific impact of each type of FGA and SGA on serum pentosidine levels. A more detailed analysis may have revealed differences between individual antipsychotics; for example, the FGAs thioridazine and

haloperidol have been reported to inhibit the antioxidant system, the other aging system (Otreba et al., 2015; Yang et al., 2014), in a dose-dependent manner (Otreba et al., 2015), whereas the SGA paliperidone activates the antioxidant system (Demirci et al., 2015; Yang et al., 2014). Second, a few of the patients in the present study who were not taking antipsychotic medications showed high serum pentosidine levels, but we did not investigate the known rare genetic risk factors, such as a deficit in *GLO1* (Arai et al., 2010) alone or in combination with the most debilitating rare deletion associated with schizophrenia (22q11.2), which has been suggested to affect serum levels of pentosidine (Toyosima et al., 2011). Third, it is impossible to establish accurately the duration of a patient's medication in clinical practice. For example, some patients may have stopped taking antipsychotics over periods of a few days to a few months from their onset, but this would not be known from their charts. Fourth, we used the daily dose of antipsychotics just before the patient's admission; however, in some patients the daily dose or type of antipsychotic may have been altered during the month before admission because of an expected deterioration in their symptoms.

Finally, we focused serum pentosidine levels as a carbonyl stress marker in schizophrenia, and whether this could be involved in any neurobiological pathophysiology such as the later therapeutic response and prognosis of patients with schizophrenia, in the same way that carbonyl stress can affect the renal function of diabetic patients (Gohda et al., 2008). We have previously reported that the significant increase of serum levels of glyceraldehyde-derived AGEs (the most neurotoxic AGEs known) in 61 patients with acute schizophrenia was not correlated with the dose of antipsychotics (Takeda et al., 2015). These other neurotoxic AGEs should also be investigated with a similarly large number of subjects and with detailed clinical

variables.

5. Conclusion

The present study investigated the clinical significance of elevated serum pentosidine, a marker of carbonyl stress, in patients with schizophrenia and showed that high levels of serum pentosidine were associated with high daily doses of antipsychotic drugs and the estimated duration of medication in patients treated with polypharmacy of antipsychotics. Given that higher carbonyl stress can result in worse pathophysiology for a number of diseases, antipsychotic monotherapy at an optimal dose may be better for these patients' long-term health.

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Contributors

Authors T.S. and T.O. contributed to the interpretation of the data and writing of the paper. Authors M. Takeuchi and S.Y. contributed to the experimental work and interpretation of the data. Authors E.T., Y.M., N.K., Y.T., T.N., S.N., R.H. and N.S. contributed to the clinical evaluation of patients. Authors T.G., Y.S. and Y.T. contributed to the conception of the study. Author H.A. contributed to the conception and design of the study. All authors contributed to and have approved the final manuscript.

Conflict of interest

None of the authors have any conflicts of interest to disclose pertaining to this paper.

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Figure Legends

Figure 1. Serum levels of pentosidine in schizophrenic patients treated with different types of antipsychotics in the combined cohort (N = 274). None, patients not treated with antipsychotics at the time of admission; FGA, treated with one first-generation antipsychotic drug; SGA, treated with one second-generation antipsychotic drug; Poly, treated with polypharmacy of both types (at least one of each). Values were compared by two-tailed Mann–Whitney *U*-tests. Error bars indicate mean and standard deviation.

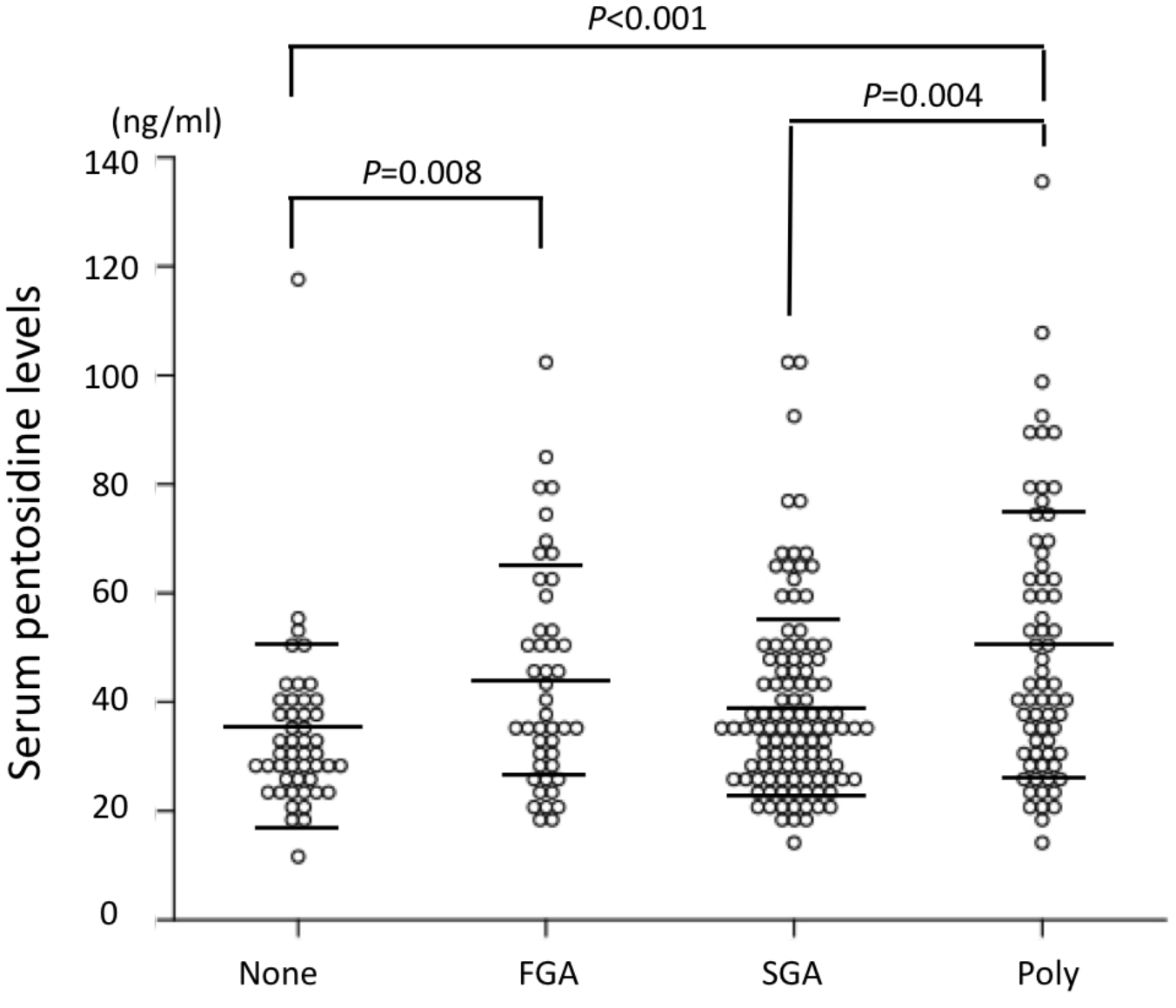
P<0.001
P=0.004
P=0.008
(ng/ml)
Serum pentosidine levels
140
120
100
80
60
40
20
0
None
FGA
SGA
Poly

Table 1. Clinical characteristics of schizophrenia patients in the present and previous cohorts

	Schizophrenia				Healthy subjects			
Variables	Present study (N = 137)	Previous study* (N = 137)	Statistical comparisons χ^2	p	Present study (N = 45)	Previous study* (N = 47)	Statistical comparisons χ^2	p
Sex, M/F	57/80	68/69	0.18	0.11	14/31	17/30	0.26	0.61
Age, mean (y)	42.0 ± 12.8 (17–73)	38.9 ± 13.9 (16–76)	-1.87	0.06	30.2 ± 5.0 (25–57)	31.0 ± 5.0 (22–48)	-1.35	0.18
Age at disease onset (y)	25.4 ± 8.6 (12–49)	23.8 ± 9.0 (12–53)	-1.40	0.16	NA	NA		
Duration of education (y)	13.0 ± 2.3 (9–18)	12.4 ± 2.5 (9–20)	-0.83	0.41	NA	NA		
Family history (Y/N)	47/90	46/91	0.23	0.42	NA	NA		
Duration of illness (y)	17.2 ± 11.8 (0–56)	17.9 ± 14.2 (0–56)	-0.55	0.58	NA	NA		
DUP (months)	30.0 ± 55.5 (0–300)	16.7 ± 32.2 (0–300)	-2.37	**0.02**	NA	NA		
Estimated duration of medication (y)	14.9 ± 12.2 (0–55.5)	14.5 ± 11.2 (0–42)	-0.04	0.97	NA	NA		
Number of admissions	2.5 ± 3.2 (1–28)	3.1 ± 2.3 (1–10)	-0.62	0.54	NA	NA		
Daily dose of antipsychotics (mg/day)**	599.4 ± 626.6 (0–3000)	654.7 ± 687.7 (0–3162)	1.82	0.07	NA	NA		
Daily dose of antiparkinsonian drugs (mg/day)**	1.1 ± 1.6 (0–9)	1.0 ± 1.9 (0–9)	-1.40	0..16	NA	NA		
Daily dose of anxiolytics (mg/day)**	13.9 ± 18.7 (0–91.7)	11.7 ± 17.6 (0–75)	-1.35	0.18	NA	NA		
BPRS (Total)	56.8 ± 13.3 (23–97)	61.5 ± 14.1 (34–96)	2.78	**0.006**	NA	NA		
(Positive)	15.1 ± 3.8 (6–26)	16.6 ± 4.6 (7–25)	2.41	**0.017**	NA	NA		
(Negative)	10.2 ± 3.2 (3–20)	10.8 ± 3.5 (3–19)	1.25	0.21	NA	NA		
pentosidine, ng/ml	47.0 ± 21.5 (17.5–117.6)	36.2 ± 17.4 (11.6–135.6)	-4.56	**<0.001**	36.8 ± 11.6 (10.1–64.4)	36.2 ± 10.2 (10.7–60.0)	0.32	0.75
pyridoxal, ng/ml	8.5 ± 7.6 (2.1–47.1)	8.8 ± 6.9 (0.1–40.7)	0.33	0.75	11.8 ± 6.3 (4.0–29.2)	11.7 ± 6.4 (3.7–29.8)	0.27	0.79

Mean ± standard deviation (range). Statistically significant p-values are in **bold** text. BPRS, Brief Psychiatric Rating Scale; DUP, duration of untreated psychosis; NA, not applicable.

* Katsuta et al., 2014. ** Given as chlorpromazine-equivalent dose (antipsychotics), biperiden-equivalent dose (antiparkinsonian drugs), and diazepam-equivalent dose (anxiolytics).

Table 2. Clinical characteristics of schizophrenia patients in the combined cohort

Variables	Combined patient cohort (N = 274)
Sex, M/F	125/149
Age, mean (y)	40.4 ± 13.4 (16–76)
Age at disease onset (y)	24.6 ± 8.8 (12–53)
Duration of education (y)	12.7 ± 2.4 (9–20)
Family history (Y/N)	93/181
Duration of illness (y)	17.6 ± 11.7 (0–56)
DUP (months)	23.0 ± 45.2 (0–300)
Estimated duration of medication (y)	14.7 ± 11.7 (0–55.5)
Number of admissions	2.8 ± 2.9 (1–28)
Daily dose of antipsychotics (mg/day)*	652.2 ± 662.5 (0–3162)
Daily dose of antiparkinsonian drugs (mg/day)*	1.0 ± 1.7 (0–9)
Daily dose of anxiolytics (mg/day)*	12.9 ± 18.2 (0–91.7)
BPRS (Total)	59.3 ± 13.9 (23–97)
(Positive)	15.9 ± 4.3 (6–26)
(Negative)	10.5 ± 3.4 (3–20)
pentosidine, ng/ml	41.6 ± 20.3 (11.6–135.6)
pyridoxal, ng/ml	8.7 ± 7.3 (0.1–47.1)

Mean ± standard deviation (range). BPRS, Brief Psychiatric Rating Scale; DUP, duration of untreated psychosis.

* Given as chlorpromazine-equivalent dose (antipsychotics), biperiden-equivalent dose (antiparkinsonian drugs), and diazepam-equivalent dose (anxiolytics).

Table 3. Multiple linear regression analysis with six possible explanatory variables for the serum pentosidine levels, for all 274 patients and for the polypharmacy treatment group.

	Total (N = 274)		Poly (N = 68)	
Independent variables:	*b* (SE)	β	*b* (SE)	β
Duration of education	-	-	-	-
Total BPRS score	-	-	-	-
Estimated duration of medication	0.317 (0.157)	0.171*	0.900 (0.443)	0.365*
Daily dose of antipsychotics	0.012 (0.003)	0.358***	0.014 (0.007)	0.368*
Daily dose of antiparkinsonian drugs	-	-	-	-
Daily dose of anxiolytics	-	-	-	-
constant	32.2		16.2	
R^2	0.190***		0.407***	

BPRS, Brief Psychiatric Rating Scale; Poly, polypharmacy treatment group, treated with both types of antipsychotics (at least one of each); SE; standard error

Statistical values of independent factors excluded from the first step of the equation are not shown.

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Table 4. Clinical characteristics and biomarkers of the schizophrenia patients (N = 274) treated with no antipsychotics or first-generation, second-generation, or combinations of antipsychotics

Variables	None (N = 48)	FGA (N = 46)	SGA (N = 112)	Poly (N = 68)	Statistical comparisons χ^2	*p*
Sex, M/F	18/30	22/24	56/55	33/35	NS	
Age, mean (y)	41.5 ± 11.8 (22–65)	47.5 ± 14.0 (25–73)	38.0 ± 13.0 (16–76)	41.1 ± 14.6 (18–76)	13.6	0.004
Age at disease onset (y)	29.8 ± 9.8 (16–53)	24.2 ± 7.8 (12–45)	24.0 ± 8.3 (12–53)	22.8 ± 8.7 (13–49)	19.2	< 0.001
Duration of education (y)	14.1 ± 2.1 (9–18)	12.9 ± 2.4 (9–20)	12.8 ± 2.3 (9–18)	12.3 ± 2.4 (9–18)	11.3	0.010
Family history (Y/N)	10/38	11/35	39/73	25/43	NS	
Duration of illness (y)	13.3 ± 11.2 (0–41)	23.4 ± 12.1 (1–50)	14.3 ± 11.2 (1–56)	19.5 ± 11.9 (1–56)	27.6	< 0.001
DUP (months)	38.7 ± 70.7 (0–300)	17.5 ± 38.3 (0–240)	19.6 ± 36.0 (0–300)	18.2 ± 35.6 (0–180)	NS	
Estimated duration of medication (y)	9.7 ± 9.4 (0–37)	21.6 ± 12.1 (0–45)	12.3 ± 10.8 (0–55)	17.4 ± 11.6 (0–48)	35.1	< 0.001
Number of admissions	1.5 ± 1.0 (1–5)	2.6 ± 2.5 (1–10)	1.8 ± 1.5 (1–9)	3.9 ± 4.9 (1–28)	21.1	< 0.001
Daily dose of antipsychotics dose (mg/day)*	0	548.5 ± 490.8 (20–2025)	580.4 ± 467.9 (19–2350)	1304.9 ± 708.8 (300–3163)	62.7	< 0.001
Daily dose of antiparkinsonian drugs (mg/day)*	0	1.2 ± 1.7 (0–6)	1.0 ± 1.7 (0–9)	0.9 ± 1.6 (0–7)	NS	
Daily dose of anxiolytics (mg/day)*	0	11.6 ± 16.0 (0–75)	13.0 ± 18.7 (0–92)	10.0 ± 14.2 (0–70)	NS	
BPRS (Total)	61.0 ± 13.7 (23–96)	56.5 ± 10.1 (39–80)	61.0 ± 14.7 (30–97)	57.0 ± 14.5 (24–89)	NS	
(Positive)	16.8 ± 4.5 (7–25)	15.2 ± 3.3 (9–23)	16.5 ± 4.4 (6–26)	15.2 ± 4.6 (7–25)	NS	
(Negative)	10.0 ± 3.9 (3–20)	10.5 ± 3.1 (5–17)	11.1 ± 3.5 (3–19)	10.1 ± 3.0 (3–18)	NS	
pentosidine, ng/ml	33.9 ± 15.7 (11.6–117.6)	44.3 ± 20.4 (17.5–103.4)	39.1 ± 17.0 (14.1–102.6)	50.0 ± 25.0 (14.2–135.6)	17.6	0.001
pyridoxal, ng/ml	9.4 ± 8.9 (2.1–47.1)	10.7 ± 10.7 (0.1–45.3)	8.3 ± 5.8 (0.1–40.7)	7.6 ± 4.9 (2.3–27.0)	NS	

Data are presented as mean ± standard deviation (range). BPRS, Brief Psychiatric Rating Scale; DUP, duration of untreated psychosis; FGA, treated with one first-generation antipsychotic drug; NA, not applicable; None, not taking any antipsychotic drug; NS, not statistically significant; Poly, treated with both types of antipsychotics (at least one of each); SGA, treated with one second-generation antipsychotic drug.

* Given as chlorpromazine-equivalent dose (antipsychotics), biperiden-equivalent dose (antiparkinsonian drugs), and diazepam-equivalent dose (anxiolytics).

Table 5. Post hoc analysis of the clinical features where significant differences were found in the patients in the combined cohort (N = 274) treated with no antipsychotics, FGA, SGA, or polypharmacy

Variables	Significant differences found between the groups	Statistical comparisons	
		Z	*p*
Sex, M/F		NA	
Age, mean (y)	FGA > SGA	-3.6	<0.001
Age at onset (y)	None > FGA	-2.8	0.006
	None > SGA	-3.5	0.001
	None > Poly	-4.2	<0.001
Duration of education (y)	None > Poly	-3.1	0.002
Family history (Y/N)		NA	
Duration of illness (y)	None < FGA	4.0	<0.001
	None < Poly	3.0	0.003
	FGA > SGA	-4.3	<0.001
	SGA < Poly	3.0	0.002
DUP (months)		NA	
Estimated duration of medication (y)	None < FGA	4.7	<0.001
	None < Poly	3.8	<0.001
	FGA > SGA	-4.5	<0.001
	SGA < Poly	3.2	<0.001
Number of admissions	None < Poly	3.9	<0.001
	SGA < Poly	3.7	<0.001
Daily dose of antipsychotics dose (mg/day)*	FGA < Poly	6.0	<0.001
	SGA < Poly	7.5	<0.001
Daily dose of antiparkinsonian drugs (mg/day)*		NA	
Daily dose of anxiolytics (mg/day)*		NA	
BPRS (Total)		NA	
(Positive)		NA	
(Negative)		NA	
pentosidine, ng/ml	None < FGA	2.6	0.008
	None < Poly	3.8	<0.001
	SGA < Poly	2.8	0.004
pyridoxal, ng/ml		NA	

BPRS, Brief Psychiatric Rating Scale; DUP, duration of untreated psychosis; NA, not applicable (no significant difference found); None, not treated with any antipsychotics at admission; FGA, treated with one type of first-generation antipsychotic; SGA, treated with one type of second-generation antipsychotic; Poly, treated with both types of antipsychotics (at least one of each).

$p < 0.0083$ (0.05/6) was considered as statistical significance in multiple comparisons.

* Given as chlorpromazine-equivalent dose (antipsychotics), biperiden-equivalent dose (antiparkinsonian drugs), and diazepam-equivalent dose (anxiolytics).