Regular article

High salivary alpha-amylase levels in patients with schizophrenia: A pilot study

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ABSTRACT

Previous studies have demonstrated the autonomic dysregulation in patients with schizophrenia using electrophysiological methods, such as electrodermal measures and heart rate analysis. Several theories have been proposed to explain the underlying mechanisms of schizophrenia and its autonomic function. Recently, the measurement of salivary alpha-amylase has been considered to be a useful tool for evaluating the sympathetic-adrenal- medullary (SAM) system. Psychosocial stress increases the release of salivary alpha-amylase. Although some studies have evaluated salivary alpha-amylase under psychosocial stress, no studies have demonstrated the change in the salivary alpha-amylase (sAA) activity level in schizophrenic patients. We examined the relationship between sAA level and psychiatric state in patients with schizophrenia (n=54) using a portable and rapid hand-held monitor to investigate sAA. The sAA activity in the patients was significantly higher than that in the control subjects (n=55)($p<0.01$). The correlation between amylase level and psychiatric symptoms was highly significant($r=0.37$, $p<0.01$). These findings indicate that higher increases in sAA may indicate severe psychiatric symptoms. These results indicate a predominant role of the sympathetic nervous system in the secretion of sAA, together with parasympathetic

withdrawal, under psychosocial stress.

Key words: autonomic function, psychological stress, salivary alpha-amylase, schizophrenia, sympathetic-adrenal-medullary system

1. Introduction

Reliable biological indicators of stress reactions and disease are valuable markers for both psychophysiological research and clinical practice. In patients with schizophrenia, previous studies have shown alteration in autonomic function using electrophysiological methods, such as electrodermal measures and heart rate analysis (Bär et al., 2005; Schell et al.,2005). In this study, we were interested in the relationship of schizophrenia with autonomic nervous system (ANS) and proposed to evaluate ANS activity by means of salivary alpha -amylase (sAA) measurement as a biomarker. No studies have demonstrated the importance of sAA activity in patients with schizophrenia.

As for biomarkers, the measurement of them was reported to be useful for assessing mental stress (Nater et al., 2005; Nater et al.,2009). To date, a number of biomarkers, such as cortisol and catecholamines, have been found to reliably indicate the reactivity of physiological stress systems, e.g. the hypothalamic-pituitary-adrenal (HPA) and sympathetic-adrenal-medullary (SAM) systems. Measurement of salivary cortisol or sAA, which can be sampled noninvasively, have been evaluated as stress biomarkers (Noto et al., 2005; Nater et al.,2005). Salivary sampling has the advantage that it is non invasive, making multiple sampling easy and

stress-free (Takai et al.,2004). Previous reports have demonstrated that salivary cortisol and sAA displayed different reaction profiles in response to psychosocial stressors. Salivary alpha-amylase reacted and recovered more quickly than did cortisol. The levels of sAA were significantly more increased and reacted more rapidly than those of cortisol by psychological stressors (Takai et al., 2004; Gordis et al., 2006; Nater et al., 2009). Salivary alpha-amylase has been proposed to indicate stress-reactive bodily changes. Numerous studies have also shown that changes in sAA are indeed dependent on stressful stimuli, either physiological or psychological in nature (Nater et al., 2009).

Several reports have suggested that psychosocial stress increases the release of sAA, which reflects the activity of the SAM system, and revealed marked increases in sAA following psychosocial stress, indicating the stress-dependent activation of sAA. Therefore, it is supposed that the measurement of sAA is a useful tool for evaluating the SAM system (Chatterton et al.,1996; Takai et al.,2004; Nater et al.,2005; Nater et al.,2006; Gordis et al.,2006; Granger et al.,2007; Shirasaki et al., 2007; Nater et al.,2009). In addition, previous studies that examined the response of sAA to the activity of SAM system showed that increased sAA levels were correlated with increased plasma catecholamine

(norepinephrine), indicating sympathetic nervous system activation(Rohlender et al., 2004; Gordis et al., 2006; Yamaguchi et al.,2006).

To the best of our knowledge, although some studies have evaluated sAA under acute or subacute psychosocial stress(Takai et al.,2004; Nater et al.,2005; Nater et al.,2006; van Stegeren et al.,2006; Grillon et al.,2007), few studies have looked into the relationship of psychosis with sAA.

In schizophrenic patients, several studies reported that autonomic dysregulation occurred and altered cardiac autonomic function might to some extent account for the elevated cardiovascular mortality rate using heart rate variability (HRV)analysis(Jindal et al.,2005; Bär et al.2008a). As to relationship between psychiatric symptoms and autonomic nervous system (ANS), schizophrenic patients displaying stronger psychotic symptoms exhibited more severe cardiac autonomic nervous system disturbances. (Zahn et al., 2005; Bär et al., 2008b; Fujibayashi et al.,2009)

The aim of this pilot study was therefore to compare the sAA changes between schizophrenic patients and normal control subjects. Furthermore, we examined the relationship of sAA level with psychiatric state in patients with schizophrenia. For this purpose, we used a portable and rapid hand-held monitor to investigate sAA (Yamaguchi et al.,2004 ; Yamaguchi et

al.,2006) .

2. Methods and Materials

2.1 Subjects

The study subjects consisted of schizophrenic out-patients at the Department of Psychiatry of Shimane University School of Medicine and a private psychiatric clinic (Asahi Clinic) and healthy control subjects who were recruited between November, 2008, and March, 2009. Fifty-four out-patients (mean age:44.15±11.35 years; 23 males ; 31 females) who fulfilled the fourth-edition Diagnostic and Statistical Manual of Mental Disorders(DSM-IV) criteria for schizophrenia were included. Of the 54 out-patients, 49 were being treated with standardized antipsychotic medications, and 5 were drug-free. Among the 49 treated patients, 34 were taking atypical neuroleptics, 10 were taking first generation neuroleptics, and 5 were taking both types. These patients were stabilized and had not experienced acute episodes during the last month. We excluded the patients having serious psychosocial condition. Schizophrenic symptoms were assessed using the Brief Psychiatric Rating Scale (BPRS;Overall and Gorham, 1962)). In present study, we focused on positive symptoms of schizophrenic patients.

The patients' data were compared with those obtained from 55 healthy controls(mean age:41.06±11.01 years; 28 males; 27

females). The 54 out-patients with chronic schizophrenia and 55 healthy control subjects gave their written informed consent before participating in this study after the purpose and procedure had been fully explained to them, and both sets of subjects were free of neurological and cardiovascular diseases that cause autonomic dysfunction, chronic diseases such as cancer or diabetes mellitus, and drug/alcohol abuse. None of the control subjects had any history of psychiatric illness, and none were taking any medication. We could not fully obtain the information about smoking, education and body mass index (BMI).

2.2 Measurement of salivary alpha-amylase

We used a hand-held monitor (Nipro Co., Japan) to measure the enzymatic sAA activity automatically using reagent paper. The hand-held monitor consisted of a disposable test-strip and a monitor designed by Yamaguchi et al.(Yamaguchi et al.,2004 ; Yamaguchi et al.,2006). The monitor was equipped with a saliva transfer device and an optical device. The collecting paper was directory inserted into the oral cavity, and approximately 20-30 μl of whole saliva were collected from under the tongue in about 30 seconds. Immediately after saliva collection, the test-strip was placed onto the automatic saliva transfer device. The test-strip paper contained 2-chloro-4-nitrophenyl-4-o-β-D-galactopyranosylmaltoside

(GAL-G2-CNP, Toyobo Co. Ltd., Japan), and when Gal-G2-CNP is hydrolyzed by salivary amylase, the hydrolyzed product develops a yellow color over time by the following reaction. Using this hand-held monitor, it took 30 seconds for saliva sampling and 30 seconds for saliva transfer and measurement, and so a total of one minute was sufficient for measuring sAA activity. This methodology for analyzing sAA with a hand held device was evaluated previously (Yamaguchi et al., 2008). Higashi et al.(2005) evaluated this hand held device as the nonverbal communication procedure for children with severe motor and intellectual disabilities.

This measurement method can be performed easily and quickly, and is therefore convenient and useful for psychiatry practice and research. Using previously developed method, one-time sampling of saliva collection was conducted in the morning (10:00-12:00 AM), as sAA activity shows a diurnal pattern with a steady increase in activity during the course of the day (Rohlender et al., 2004; Nater et al.,2007). We did not investigate sAA activity at multiple time points. Saliva was collected two or more hours after the last meal in order to cancel out the influence of meal and drink on the sAA activity (Yamaguchi et al.,2006). At least 60 minutes before the measurement, the subjects were told to refrain from brushing their teeth or eating (Nater et al.,2006). All subjects were

requested to rest in a chair for at least 10 minutes (Noto et al., 2005).

2.3 Cardiovascular measures

For the assessment of autonomic functional changes, resting heart rate (HR) was measured in all subjects in a recumbent position during the same time interval. Heart rate is thought to be an indicator of autonomic (sympathetic and parasympathetic) function.

2.4 Statistics

The results are expressed as means ± SD. To compare the clinical variables between the patients and controls, the unpaired t-test or Chi-square test was used. The sAA data were not sampled from Gaussian distribution, we therefore analyzed these data with a non-parametric test (the Mann-Whitney U-test). Correlations between variables were calculated using Spearman's correlation coefficients using Statview 5.01 (SAS Institute Inc., USA). The level of significance was set at $P<0.05$.

3. Results

Table 1 shows the characteristics of both schizophrenic out-patients and healthy control subjects.

3.1 Comparison between schizophrenic out-patients and normal controls

The patients and control subjects were individually matched for age and gender. The mean levels of sAA in the schizophrenic patients and control subjects were 89.70±71.16 (kU/l)(range,15-345) and 38.91±24.17 (kU/l)(range,10-118), respectively. The sAA activity in the patients was significantly higher than that in the control subjects ($p<0.001$), and there were no significant differences in HR between the patients and healthy controls ($p=0.1$). We found no significant difference of sAA level in gender (23 male and 31 female, $p=0.06$ in patients, 28 male and 27 female, $p=0.49$ in controls). We further divided the patients into two groups based on the dose of antipsychotics: over 200mg chlorpromazine equivalents (CPZeq) (n=27), and under 200mg CPZeq (n=22), and based on the duration of schizophrenia: over 10 years (n=37), and under 10 years (n=17). There were no significant differences of sAA levels both in the dose of antipsychotics ($p=0.19$) and in the duration of schizophrenia($p=0.68$), respectively.

3.2 Relationship between the sAA level and BPRS score

Fig 1 shows the relationship between the sAA level and the BPRS score in schizophrenic patients. The correlation between sAA level and the BPRS score was highly significant($r=0.37$, $P<0.01$).

The sAA level of high BPRS score group (over 30 points, n=27) was significantly higher than that of low BPRS score group

(under 29 points, n=27) (111.89±69.82 (kU/l) vs 67.52±66.51 (kU/l), p=0.021).

4. Discussion

To the best of our knowledge, this is the first report to show the association between sAA changes and psychiatric state in schizophrenic patients. We found that the sAA levels in schizophrenic out-patients were significantly higher than those of normal healthy controls. In patient group, there is no significant difference of sAA level in gender (p value=0.06). However, the p value (0.06) may be identified as a trend and may suggest the possibility of gender differences in schizophrenia. Although previous study showed no gender differences in salivary biomarker responses to acute psychological stress (Takai et al.,2007), no studies demonstrated the gender differences in patients with schizophrenia. Gender differences of schizophrenia remain to be elucidated.

As for the effect of medication, the potentially confounding impact of the intake of antipsychotic drugs on sAA activity needs mentioning. We tried to compare the sAA levels between the five unmedicated five patients and 20 randomly sampled patients from the 49 medicated patients in this study. The mean levels of sAA in the unmedicated patients

and medicated patients were 99.8±72.5 (kU/l) and 69.7±51.6 (kU/l) respectively, and there were no significant differences between these groups (p=0.06). Comparing high dose antipsychotics group and low dose one, there was no significant difference of sAA level (p=0.19). Although the sample sizes were small, these results suggested that medication does not affect sAA level.

Previous studies revealed marked increases in sAA following psychosocial stress, indicating a stress-dependent activation of sAA and that high stress levels were associated with higher overall sAA levels(Takai et al.,2004; Nater et al.,2005; Nater et al.,2006; van Stegeren et al.,2006). Salivary alpha-amylase is a candidate substance for indicating autonomic activity since salivary gland secretion occurs in response to neurotransmitter stimulation, and salivary glands are innervated by both sympathetic and parasympathetic nerves (Nater et al., 2005). The two autonomic nerve systems work together harmoniously to evoke salivary secretion (Proctor et al., 2007) The results of previous studies into sAA reactivity to psychological stimuli have suggested it as a potential direct marker and have used it as a good measure of SAM activity (Nater et al., 2005; van Stegeren et al., 2006; Shirasaki et al., 2007) ; i.e., autonomic activation.

There is little evidence as to which branch (sympathetic

or parasympathetic) of the autonomic nervous system is predominant in the increases in sAA during psychological stress. Nater et al.(2006) reported a positive relationship between sAA and sympathetic tone, which was assessed using heart rate variability (HRV) parameters during stress. Previous reports indicated a predominant role of the sympathetic nervous system in the secretion of sAA, together with parasympathetic withdrawal, under psychosocial stress (Gordis et al.,2006; Nater et al.,2009).

Interestingly, we did not observe a rise in HR as a marker of autonomic function, which suggests that salivary amylase is more sensitive to subtle psychological stress than heart rate(van Stegeren et al.,2006). van Stegeren et al.(2006) also concluded that, compared with HR, amylase was a more sensitive indicator of sympathetic drive. Therefore, our results suggest that high levels of sAA are associated more strongly with increased sympathetic function than parasympathetic function in this out-patients group.

Several studies have reported an association between autonomic function and schizophrenic patients. Although autonomic dysregulation in patients with schizophrenia has been reported, the exact mechanisms underlying the neurobiology of the schizophrenia related autonomic nervous system (ANS) are still unclear (Toichi et al.,1999;, Bär et al.,2008a;, Bär et

al.,2008b;, Fujibayashi et al.,2009). Bär et al.(2005) showed low parasympathetic activity (reduced HRV) in acute schizophrenic patients and suggested that schizophrenia was accompanied by a loss of vagal efferent activity, probably due to disturbance of the cortical-subcortical circuits modulating the autonomic nervous system. Fujibayashi et al.(2009) also suggested the schizophrenic patients possessed markedly depressed ANS activity. Toichi et al.(1999) revealed that the parasympathetic index was significantly decreased without significant changes in the sympathetic index and demonstrated that psychotic state could affect the autonomic nervous system, presumably mediated through the parasympathetic nervous system, in chronic schizophrenic patients receiving neuroleptic treatment, and they also demonstrated that psychotic states might act as mental stressors, suggesting a relationship between cerebral cognition and peripheral autonomic nervous function. Williams et al.(2004) described reduced activity in amygdale-medial prefrontal circuits in schizophrenic patients. This functional disconnection of the autonomic and central systems might influence brainstem neurons and the sympatho-parasymapathetic balance consecutively (Bär et al.,2008b).

In the present study, we found that the sAA level increased significantly with psychotic state. This result suggests that

higher increases in sAA may indicate severe psychiatric symptoms. This is the first pilot study to suggest that sAA might correlate with psychiatric symptoms in schizophrenic patients.

The methodological limitations of the study were that only one measurement time point was used and that we did not fully discuss the potentially confounding impact of antipsychotic medication on sAA. The explanatory power of our data on drug effects is limited. Investigation of the association between medication and the sAA level, and those between the different types and dosages of antipsychotics and sAA level is warranted in future study.

Although we evaluated the patients using BPRS in this study, more comprehensive rating scale (e.g. the positive and negative syndrome scale:PANSS) should be used in future study. We also did not examine autonomic function except by measuring HR. The physiological mechanism by which psychotic state affects autonomic nervous function, and especially sAA, remains unclear. Further research is needed to elucidate this.

5. Conclusion

We examined the association of sAA changes with schizophrenia using a portable and rapid hand-held monitor. We speculate that parasympathetic nervous activity might be

suppressed in schizophrenic patients and that the sympathetic nervous system shows comparatively high activity in schizophrenic patients. As a result, schizophrenic patients are expected to show high levels of sAA accompanied with severe psychiatric symptoms.

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Figure legend

Correlation between salivary alpha-amylase activity and BPRS score

The correlation between amylase level and the BPRS score was highly significant(r=0.37, P=0.0061)

Figure 1

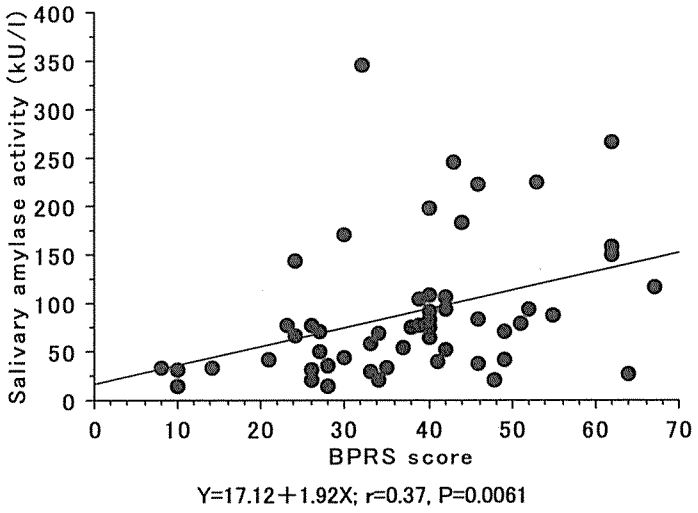
Salivary amylase activity (kU/l)
400
350
300
250
200
150
100
50
0
0
10
20
30
40
50
60
70
BPRS score
Y=17.12+1.92X; r=0.37, P=0.0061

Table 1

Subject characteristics

	Schizophrenia	Controls	P-value	
No. of patients	54	55		
Gender (Male/Female)	23/31	28/27	0.384	†
Age (years)	44.15±11.35	41.06±11.01	0.152	*
Salivary alpha-amylase level (kU/l)	89.70±71.16	38.91±24.17	<0.001	*
Heart rates	70.26±10.60	73.62±10.50	0.1	*
Duration of illness (years)	14.48±9.29	-		
Dosage of antipsychotics (mg/day)	348.74±338.19	-		
BPRS score	37.81±13.65	-		

Participants data (mean±SD)
Statistical analysis was conducted using the unpaired t-test, Mann-Whitney U-test and χ^2 test.
BPRS: Brief Psychiatric Rating Scale