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Salivary Alpha Amylase Enzyme and Salivary Cortisol Level in Depression after Treatment with Fluoxetine

Andi Jayalangkara Tanra, Hawaidah Madeali, Mayamariska Sanusi*, Saidah Syamsuddin, Sonny Teddy Lisal

Department of Psychiatry, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia

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***Correspondence:** Mayamariska Sanusi, Department of Psychiatry, Faculty of Medicine, Hasanuddin University, Makassar 90245, Indonesia.
E-mail: goncingang_makassar@yahoo.com
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Abstract

BACKGROUND: Hypothalamic-pituitary-adrenal axis and its end product cortisol have been extensively investigated in patients with depressive disorders for many years. Recently, salivary alpha-amylase (sAA) had emerged as a new biomarker with non-invasive and more convenience protocol for measuring sympathetic activity which were also associated with depression. Selective Serotonin Reuptake Inhibitor is antidepressant drug extensively used to treat depression.

AIM: The aim of this study was to determine whether decrease of sAA and salivary cortisol levels could be observed in subjects with depression who were treated by fluoxetine.

METHODS: The total subjects were 25 depressed subjects and ten healthy controls. sAA was examined before therapy, and after 2, 4, and 6 weeks of fluoxetine administration using a portable cocorometer. Salivary cortisol was examined before therapy, after 4 and 6 weeks of fluoxetine administration with Elisa method. The therapeutic effect was assessed with Hamilton Depression Rating Scale (HDRS).

RESULTS: sAA and cortisol levels were significantly decreased after fluoxetine administration ($p < 0.001$), followed by at least 50% reduction of HDRS scores after 6 weeks of fluoxetine administration. Levels of sAA and cortisol were higher in the depression group than in the healthy control.

CONCLUSIONS: Measurement of sAA levels can be used as a potential biomarker of therapeutic response in depressed patients in addition to salivary cortisol.

Introduction

Major depressive disorder (MDD) is the leading cause of disability worldwide and is a major contributor to the overall global burden of disease [1]. In Indonesia, the prevalence of mental disorders indicated by symptoms of depression and anxiety increased from 6% in 2013 to 6.1% in 2018 [2]. The high level of stress was thought responsible for the increase rate of mental disorder. Stress is defined as a state of threatened homeostasis following exposure to extrinsic or intrinsic adverse forces. Acute stress refers to stress that occurs in minutes or hours, whereas chronic stress persists for days, weeks, or months. Stress was associated with depression and could precipitate another depressive episode including severity, duration, and the natural course of the disorder [3], [4]. In response to a stressor, two major biological stress systems are activated: The Sympathetic Nervous System (SNS) and the hypothalamic-pituitary-adrenal (HPA) axis. Across life, the repetition or chronic activation of these systems could lead to changes in functioning, impaired or inadequate responses to subsequent challenges [5], [6].

At present, the diagnosis of MDD mainly relies on clinical examination and subjective evaluation of depressive symptoms. At present, there is no specific biomarker for MDD have been identified to date. However, biomarker can be helpful in the treatment choice and in predicting the course of the disorder during the early stages [7], [8].

Cortisol and alpha-amylase are both markers of the human stress system, but have different mechanism. Over the past years, the functioning of the HPA axis and its end-product cortisol had received much attention. Salivary cortisol, a commonly used biomarker of the stress response has been used in a range of clinical study of stress-related diseases including depression [9], [10]. In a meta-analysis, Stetler and Miller summarized four decades of research into HPA axis activation and depression, and concluded that there was a tendency for an increase of HPA axis activation in depressed subjects. They also noted that results varied considerably across studies and that several studies showed decreased instead of increased activation of the HPA axis [11]. Salivary alpha-amylase (sAA) is an enzyme that hydrolyses starch in oral cavity; secreted by parotid gland under autonomic regulation and highly sensitive to stress-related changes. sAA has emerged as a new biomarker for responses to psychosocial stress within the SNS. Activation of the SNS results in norepinephrine (NE) release that may subsequently

elicit the release of sAA by the salivary glands. NE is involved intrinsically with the stress response system, and chronic stress in depression would trigger release of NE from SNS. Few studies that examined sAA level in relation to depressive symptoms show a tendency toward increased sAA level in depressed versus non-depressed group [10], [11], [12].

Ishitobi *et al*. (2010) compared basal level of salivary cortisol and sAA in unremitted and remitted MDD patients as well as in healthy controls. It was found that sAA and cortisol levels in unremitted patients were significantly elevated compared to controls and remitted patients. It suggested that sAA may be a state-dependent marker of MDD in addition to salivary cortisol. Bauduin *et al*. (2018) suggest that sAA at awakening may be a valuable candidate biomarker specifically for MDD [13], [14].

Selective Serotonin Reuptake Inhibitors (SSRIs) are antidepressant drugs extensively used in MDD. Interestingly, besides increasing serotonergic neurotransmission, SSRIs might also altered HPA-axis disturbances [15]. In MDD, the SSRI fluoxetine decreased corticotropin-releasing hormone [16]. Effects of antidepressants, including SSRIs on the HPA-axis occurred mainly in MDD-patients who are responsive to treatment. Therefore, it has been suggested that resolving HPA-axis abnormalities during MDD treatment indicates SSRI response [17], [18].

Study by Piwowarska *et al*. concluded that patients who were successfully treated with fluoxetine showed a significant decrease in Hamilton Depression Rating Scale (HDRS) scores and decreased cortisol secretion after 6 weeks of treatment [19]. However, after 5 weeks of treatment with SSRI or the non-SSRI, HPA axis activities only decrease in non-SSRI responders [17]. Another study showed that the administration of SSRIs did not show any changes in sAA levels [20].

Evaluation of cortisol levels is mostly done for research purposes, it is still rarely used in daily clinical practice considering that not all health facilities can carry out cortisol measurements and the costs needed are relatively more expensive. The measurement of sAA level using optical detection method through saliva was introduced as a non-invasive and more convenience protocol. It does not make the subjects to be stress as it gives ease in taking sample besides time needed is relatively short, easy to do anywhere and anytime without being reliant on the assistance of laboratory staff.

In a previous study at our department shown that sAA level in subject with depression was significantly higher than the normal population. In addition, an increase in HDRS scores in patients with depression was followed by a significant increase in the levels of the sAA enzyme [21]. In this study, we compared the level of sAA and salivary cortisol in depressed subject before and after treatment with fluoxetine. We hypothesized that sAA enzyme and salivary cortisol would be lower after treatment with fluoxetine.

Methods

Patient sample

The study included 25 subjects with MDD either inpatient or outpatient who were admitted for the 1st time to Psychiatric clinic at Wahidin Sudirohusodo Hospital, Makassar, Indonesia, and its network. The MDD diagnosed based on criteria of the Diagnostic and Statistical Manual of mental Disorder, 5th edition (DSM-V). The ages of subjects were 18–45 years old. Exclusion criteria were comprised of the following: Chronic metabolic and oral disease, required adrenergic agonist or antagonist drugs, required corticosteroid therapy, and comorbidity with other psychiatric disorders. The healthy controls (ten subjects) were civil servant in health service center, 18–45 years old with no history of mental disorder and met the same exclusion criteria with depressed subjects. All subjects were provided with complete written and oral descriptions of the study, written informed consent was also obtained. The protocol was approved by the local ethics committee.

Procedures

To control for variations of sAA and cortisol levels, salivary collection was performed between 09:00 AM and 14:00 PM. We measured saliva samples 4 times, before treatment, 2, 4, and 6 weeks after treatment. sAA activity was measured using the cocorometer (Nipro Corp., Japan) according to the manufacturer's protocol. Saliva was sampled by directly immersing a saliva-sampling strip in saliva under the tongue for 30 s, and then the test strip was inserted into the monitor which revealed the nominal of sAA enzyme levels. It took only 1 min to measure the sAA enzyme level. The measurement of salivary alpha-amylase enzyme by this tool was done by using optical detection method. The substrate (saliva) was collected using strip which contain a strip reagent so that if mixed: Gal-G2 (Galaktospyranosylmatosa) as the substrate will bind to CNP (Chloro-Nitrophenyl) chromogen compound to become Gal-G2-CNP (2-Chloro-4-nitrophenyl-4-O-ßD-galactospyranosylmaltoside). This 2-Chloro-4Nitrophenyl (CNP) reagent strip will hydrolyze the substrate to detect the sAA enzyme by producing a yellow product. Changes in the intensity of the color were in accordance with changes in activity of the salivary alpha amylase enzyme; the higher the intensity of the yellow color meant the higher the sAA enzyme levels. Then, the reagent strip was inserted into the optical analyzer, in which the device was capable of

measuring the activity of sAA enzyme.

The concentration of salivary cortisol was measured using DBC Cortisol kit and analyzed by ELISA assay. The absorbance measured on a microtiter plate reader. Saliva samples were collected immediately using passive drooling technique. The tubes were placed in a refrigerator at –80°C and then thawed for analysis. Salivary cortisol was measured 3 times, before treatment, 4, and 6 weeks after treatment. Subjects were refrained from eating, drinking, smoking, and teeth brushing at least 30 min before sampling to minimize the effect of food or drink on the activity of the sAA and cortisol level, rinsing with mineral water and expected to rest for about 10 min before sampling.

We assessed subjects with the HDRS for psychological status and efficacy of antidepressant therapy before treatment, 2, 4, and 6 weeks after treatment. Subjects were given 20–40 mg of fluoxetine and benzodiazepine (alprazolam and lorazepam in equivalent dose for sedation). Control group was examined for sAA and salivary cortisol only 1 time, measurement results as a standard level for sAA and cortisol value.

The data were analyzed using IBM SPSS statistic for windows version 22 and presented as tables and graphs. Chi-square was used to assess the distribution of characteristics according to the sample group, Mann–Whitney was used to assess differences in initial cortisol levels and initial sAA between depressed and control groups. Wilcoxon Signed-Rank was used to assess differences in enzyme levels and HDRS scores between pre- and post-therapy. The test results are statistically significant if the value is $p < 0.05$.

Results

Thirty-five subjects participated in the study. The subjects were divided in two groups, namely, depressed group and control group. Ages of the subjects range between 22 and 45 years old. The mean age of subjects was 33.5 years (SD = 7.5). There were no significant differences in age, sex, and education level ($p > 0.05$). There was a difference in employment status where all the healthy control are employee ($p = 0.002$).

Table 1: Comparison of initial sAA level and salivary cortisol level with healthy control

Variable	Group	n	Mean	SD	p
sAA 0	Depression	25	52.0	16.8	0.000
	Control	10	13.4	6.2	
CORTISOL 0	Depression	25	24.1	14.5	0.000
	Control	10	7.9	3.2	

sAA: Salivary alpha-amylase.

Based on the collected data, sAA_0 (sAA initial) and cortisol_0 (cortisol initial) were taken before treatment. Significant initial sAA levels were observed higher in depressed group (52.0 kU/L) compared to control group (13.4 kU/L) with $p < 0.001$. Initial cortisol levels were observed higher in depressed group (24.1 ng/dl) compared to control group (7.9 ng/dl) with $p < 0.001$, as shown in Table 1. Comparison of the sAA levels in every measurement compared with healthy control is shown in Figure 1, while comparison of salivary cortisol levels in every measurement compared with healthy control is shown in Figure 2.

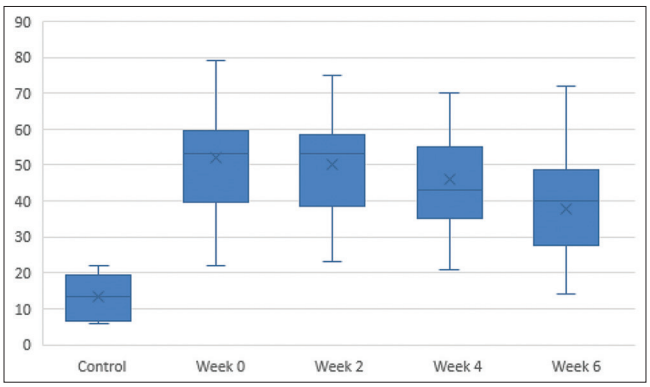

Figure 1: Comparison of salivary alpha-amylase level with healthy control

Comparison of the sAA levels in every measurement is shown in Figure 3. The sAA levels were found to be significantly decreased consistently after 2 weeks (50.28) kU/L, 4 weeks (46.0) kU/L, and 6 weeks (37.96) kU/L of treatment. There was a significant reduction in sAA level after fluoxetine administration with $p < 0.001$.

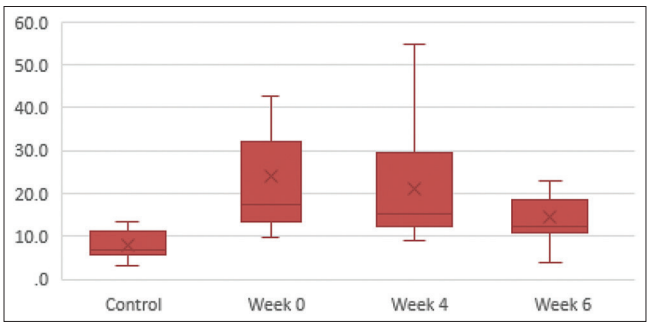

Figure 2: Comparison of salivary cortisol level with healthy control

Comparison of salivary cortisol levels in every measurement is shown in Figure 4. The level of salivary cortisol was found to be significantly decreased

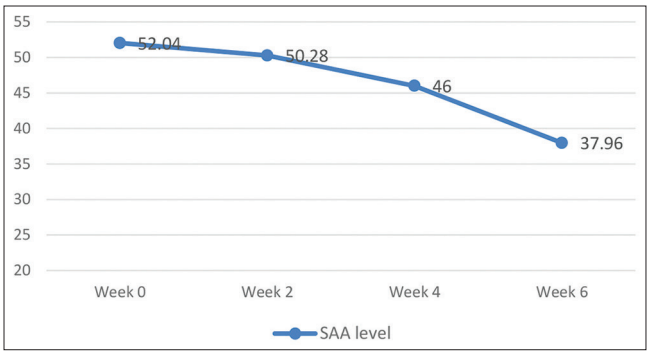

Figure 3: Comparison of salivary alpha-amylase level weeks 0, 2, 4, and 6

consistently after 4 weeks (19.3–2.0) ng/dL and after 6 weeks (14.5) ng/dL of treatment. There was a significant reduction in salivary cortisol level after fluoxetine administration with $p < 0.001$.

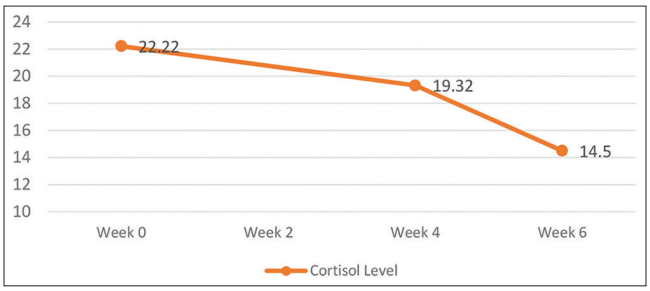

Figure 4: Comparison of cortisol level weeks 0, 4, and 6

The therapeutic effect was assessed with analysis of the percentage of the reduction of HDRS score compared to the initial values. HDRS score were found to be significantly decreased consistently after 2 weeks (19.08), 4 weeks (15.6), and 6 weeks (12.12) of treatment. There is significant reduction in HDRS score after fluoxetine administration with $p < 0.001$. Six weeks were needed for the reduction by at least 50% (52.36%). Comparison of HDRS score in every measurement is shown in Figure 5.

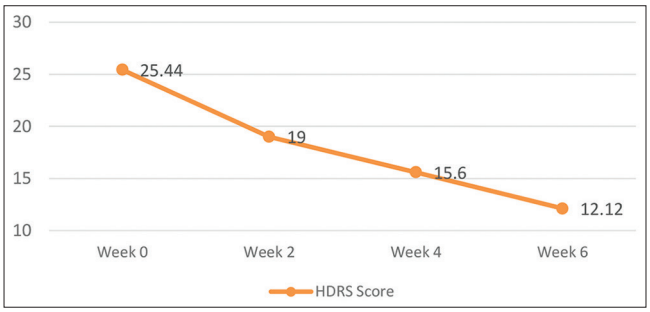

Figure 5: Comparison of Hamilton Depression Rating Scale score weeks 0, 2, 4, and 6

Discussion

This study was conducted to determine if there were differences in the sAA enzyme levels and the cortisol levels in depressed subjects before and after fluoxetine administration. We found that sAA levels were significantly higher in depressed subjects (52.04 ± 16.84) compared to healthy controls (13.40 ± 6.24). The mean value of the initial sAA level in depressed subject is slightly higher than the previous research in our department which showed the average value 35.17 ± 11.92 KU/L but lower than the average value in anxiety group that is 63.57 ± 32.21 [21]. It was suggested that sAA could be used as a marker for measuring sympathetic activity which associated with depression. Patients with depression might have elevation in sAA level [13], [14].

After 2 weeks administration of fluoxetine, the measurement showed a significant reduction of sAA levels. The measurement in the 4th week and the 6th week also showed a significant reduction ($p < 0.001$). Decrease of the enzyme levels of sAA was noticeable after fluoxetine administration. The minimum levels of sAA enzymes in depressed subjects before therapy were 22 kU/L and the maximum was 99 kU/L, indicating a considerable wide range of value. The difference in the levels of sAA enzymes among the subjects could be due to the sampling of sAA (09.00 Until 14.00) so that various factors could affect the level of the sAA enzyme, including the waiting time, hunger conditions, eating or drinking products that could increase the concentration of sAA, smoking, etc. [10]. This could lead to a bias of the results and become the limitation on this study.

Our data also showed that salivary cortisol levels in depressed subjects were higher compared to healthy controls. Our finding was consistent with those from Ishitobi *et al*. (2010) which found that sAA and cortisol levels in unremitted patients were significantly elevated compared to controls and remitted patients [13]. The mean value of salivary cortisol showed a reduction in the 4th week and the 6th week of measurements. So that it could be concluded that there was a significant reduction of salivary cortisol with $p < 0.001$ after fluoxetine administration. The molecular mechanisms underlying the SSRI function were unknown. However, SSRIs can affect the endocrine and immune system of patients with depression. *In vitro* studies had shown that SSRIs enhance transcription of the mediated GR in the presence of cortisol and proposed that the antidepressant inhibits the transporter of steroid-bound membranes, increasing the intracellular concentration of glucocorticoids, which, in turn, increase the expression and function of GR and restore negative feedback by cortisol [22], [23].

Similarly, HDRS scores were significantly decreased in the 2nd, the 4th, and the 6th weeks with $p < 0.001$.The effectiveness of fluoxetine administration was confirmed by the reduction of HDRS score compared to initial values. There was a 52.36% decline of initial HDRS score after 6 treatments. This is in line with the study by Piwowarska *et al*. (2012) which showed reduction in the HDRS score which could be seen starting from the 2nd week. For the patient who successfully treated with fluoxetine showed a decline of 50% HDRS score and decreased secretion of cortisol in the 6th and 8th weeks [19].

This study tried to minimize factors that could affect the levels of the sAA enzyme and salivary cortisol including chronic metabolic diseases, oral diseases, the use of drug agonists or adrenergic antagonists, corticosteroid therapies, and comorbidities with other psychiatric disorders. There were still many things that could cause bias on this study that has been mentioned previously.

This study used saliva cortisol with consideration that it is easy; inexpensive, the subject

can take its own sample. The blood serum is not carried out with the reason of additional stress, which can affect the measurement of sAA level which is also done in the same time. We used passive drooling technique into sterile pots. This method is able to provide samples in large quantities and is easy to do but the weaknesses are containing mucin and caused unpleasant smell. The average study uses a saliva retrieval method with an absorbent material such as Salivette® but is not currently available in Indonesia.

This study should be replicated using another group of antidepressants because it is cheap and easily to compare to another method. In the future, objective examination of sAA enzyme levels was expected to be an additional routine that could help determine depression level and monitoring the progress of treatment.

Conclusions

Our findings support previous studies that the sAA levels and the salivary cortisol levels were higher in depression compared to healthy controls. The SAA and the cortisol level were significantly decreased after fluoxetine administration followed by at least 50% reduction of HDRS scores after 6 weeks of fluoxetine administration. Measurement of sAA levels could be used as a potential biomarker of therapeutic response in depressed patients in addition to salivary cortisol

Authors' Contributions

All the authors were involved in the conception of the study. AJT, HM, and MS were involved in interpretation of the research findings. SS and STL were contributed to the drafting of the manuscript. All authors read and approved the final manuscript.

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