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Preliminary communication

# Low baseline salivary alpha-amylase in drug-naïve patients with short-illness-duration first episode major depressive disorder

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ABSTRACT

*Background:* Altered monoamine neurotransmission accompanied by hypothalamic–pituitary–adrenal axis dysfunction and autonomic nervous system hyperactivity have been associated with major depressive disorder (MDD). Salivary α-amylase (sAA) is indicative of autonomic activation and reflects central noradrenergic activity. Scarce studies on sAA in MDD produce confounded results and no data is available regarding baseline sAA activity.
*Methods:* The basal, non-stimulated sAA activity was studied in this cross-sectional case-control study on 20 non-late-life adult, short-illness-duration first-episode, treatment-naïve MDD patients and in 20 age- and sex-matched healthy controls. Depressed patients showed a basal score in the Hamilton Rating Scale for Depression (HAMD-17) higher than 20.
*Results:* The sAA was significantly lower in depressed individuals as compared to controls ($p=0.011$). In post hoc analysis significantly lower sAA was present in melancholic MDD ($p=0.016$) as related to controls whereas no difference was seen between non-melancholic MDD patients and controls. The sAA activity was not significantly correlated neither with duration nor the severity of depressive symptoms as measured by the total HAMD-17 score.
*Limitations:* The current study is limited by its cross-sectional design, small sample size, and factors related to saliva sampling methodology.
*Conclusion:* Low baseline sAA levels were found in MDD in basal, non-stimulated conditions. The study provides no support for elevated sAA in drug-naïve patients with short-illness-duration first episode MDD. The results support the evidence for decreased central noradrenergic transmission in MDD when sAA activity is considered indicative of central noradrenergic function.


## 1. Introduction

In major depressive disorder (MDD) altered monoamine neurotransmission accompanied by endocrine abnormalities with hypothalamic–pituitary–adrenal (HPA) axis dysfunction and autonomic nervous system (ANS) maladaptive activation are observed (Veith et al., 1994; Chopra et al., 2011).

Studies on ANS in MDD point out to a dysfunction involving brain noradrenergic mechanisms which control autonomic output. Sympathetic nervous system (SNS) activity is elevated in MDD with concomitant impairment of parasympathetic function and higher levels of catecholamines are seen (Veith et al., 1994; Guinjoan et al., 1995; Lehofer et al., 1997; Carney et al., 2005).

Salivary α-amylase (sAA) has been adopted as a marker of the ANS activity in an array of salivary measures offering a non-invasive and stress-free sampling method. The salivary glands receive both sympathetic and parasympathetic innervations of the ANS. The secretion of sAA occurs via α- and β-adrenergic mechanisms and its activity is indirectly indicative of autonomic activation and reflects central noradrenergic activity (Nater and Rohleder, 2009).

Studies on sAA in stress-related disorders are confounding. The direct exposure to physical exercise appears to acutely elevate sAA and no effect is seen with regard to regular physical activity. Drugs affecting ANS influence sAA in accordance with their pharmacodynamics. Interestingly, antidepressants generally produce major sAA elevation. Scarce data indicate no effect of the menstrual cycle nor the use of oral contraceptives on sAA. Baseline sAA levels are relatively independent of age, gender, body mass index (BMI), smoking, eating and drinking but significantly associated with chronic stress and stress reactivity in healthy individuals (Nater and Rohleder, 2009; Veen et al., 2012, 2013). Four studies on sAA in MDD were published so far and data is sparse and inconclusive (Schumacher et al., 2013). It is hypothesised that the elevated sAA is present in MDD being indicative of an increased ANS activity. There is, however, considerable inconsistency in the results

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**Table 1**
Demographic and clinical variables.

| | | Control | MDD | MDD | |
|---|---|---|---|---|---|
| | | | | Melancholic | Non-melancholic |
| *N* | | | 20 | 9 | 11 |
| Women (%) | | 60 | 55 | 55 | 54 |
| Age (years)* | Median (IQR) | 33.5 (30.3, 35.8) | 30.5 (24.5, 37.5) | 30** (25, 31) | 31 (24, 44) |
| BMI | Mean (95% CI) | 23.9 (22.6, 25.3) | 22.8 (21.4, 24.1) | 21.5*** (19.9, 23.2) | 23.8 (21.6, 25.9) |
| WHR | Mean (95% CI) | 0.82 (0.79, 0.86) | 0.82 (0.73, 0.85) | 0.82 (0.77, 0.87) | 0.82 (0.77, 0.87) |
| Episode duration (weeks) | Mean (95% CI) | – | 14.5 (12.2, 16.7) | 14.9 (11.1, 18.7) | 14.1 (10.9, 17.3) |
| HAMD-17* | Median (IQR) | 1 (0, 2) | 22.5 (21, 24) | 24**** (23, 25) | 21 (20, 22) |
| Salivary α-amylase* (daIU/mL) | Median (IQR) | 6.0 (4.4, 10.7) | 3.7$ (2.7, 4.9) | 3.8$$ (3.2, 4.6) | 3.5 (2.4, 8.0) |

* Shapiro–Wilk W $p < 0.05$.
** vs. Control: $p=0.015$, Mann–Whitney *U*-test, median difference (95%CI)= −5 (−10, −1).
*** vs Control: $p=0.035$, two-tailed, unpaired *t*-test, mean difference (95%CI)= −2.4 (−4.60, −0.17).
**** vs. Non-melancholic: $p=0.002$, Mann–Whitney *U*-test, median difference (95%CI)=3 (2, 4).
$ vs. Control: $p=0.011$, Mann–Whitney *U*-test, median difference (95%CI)= −2.2 (−4.5, −0.6).
$$ vs. Control: $p=0.016$, Mann–Whitney *U*-test, median difference (95%CI)= −2.3 (−5.8, −0.6).

attributing sAA elevation to gender, illness stage and severity or exposure to antidepressants (Ishitobi et al., 2010; Bagley et al., 2011; Tanaka et al., 2012; Veen et al., 2013) with substantial heterogeneity of MDD patients studied. Finally, to our best knowledge no data is available with regard to baseline sAA levels and a potentially associated ANS dysregulation in MDD.

To address these factors, a case-control study was designed to examine sAA activity in a well defined cohort of first-episode, drug-naïve, short-illness-duration MDD patients and healthy subjects in baseline non-stimulated conditions. It was hypothesized that the sAA elevation representing ANS hyperactivity would show in MDD in non-challenging and stress-free setting.

## 2. Method

### 2.1. Subjects

Twenty MDD patients diagnosed with the structured clinical interview for DSM-IV Axis I disorders (First et al., 1997) were recruited from the outpatient unit of a university hospital. The severity of depression was evaluated with 17-item Hamilton rating scale for depression (HAMD-17) (Hamilton, 1960). Subjects with HAMD-17 score of $\geq 20$ with first-episode major depression being drug-naïve for any psychotropic medication and episode duration $\leq 24$ weeks were eligible for the study. All patients underwent routine physical examination. Exclusion criteria were: any other Axis I disorder, any unstable medical condition, any history of endocrine, inflammatory, autoimmune, oral/dental health problems including salivary gland disorders or neurological disease, inflammatory responses in the previous 2 weeks, pregnancy or lactation, alcohol or drug abuse in the past 12 month, tobacco smoking exceeding 25 cigarettes a day, BMI $\leq 18$ and $\geq 30$, age $< 18$ and $> 55$ years. Women had not received hormonal contraception for at least 12 month.

The control group consisted of 20 healthy subjects matched by age and sex, menopausal status, and metabolic parameters. They were interviewed using the structured clinical interview for DSM-IV, nonpatient edition (First et al., 1997). All subjects underwent routine physical examination. None of them had a history of serious medical or neuropsychiatric illness or a family history of major psychiatric or neurological illness in their first-degree relatives. A score $\leq 5$ in the HAMD-17 was required for inclusion. Exclusion criteria were: positive history of any exposure to psychotropic medication, unstable medical condition, oral/dental health problems including salivary gland disorders, inflammatory responses in the previous 2 weeks, pregnancy or lactation, alcohol or drug abuse in the past 12 month. Women received no hormonal contraceptives.

The study was carried out in accordance with the Declaration of Helsinki with the approval of the Ethic Research Committee of the Institution. For each participant, written consent was obtained.

### 2.2. Study protocol

The study followed a cross-sectional, case-control design. All subjects fasted from midnight before the test day and arrived at the laboratory at 07:00 a.m. After the subjects had sat quietly for 45 min, saliva samples were taken for the assay of sAA at 08:20, 08:40, and 09:00 a.m. using Salivettes (Sarstedt, Germany) placed in mouth and moved around in a circular pattern for 2 min. Collected samples were immediately stored at −80 °C with centrifugation at 3000 rpm for 15 min after unfreezing for batch analysis. Sampling was performed between days 3 and 10 of the menstrual cycle in premenopausal women. The mean value from three samples was taken for analysis. Three samples were significantly correlated with each other (1st and 2nd: $r=0.60$, $p < 0.0001$; 1st and 3rd: $r=0.51$, $p=0.0077$; 2nd and 3rd: $r=0.59$, $p < 0.0001$). No significant median differences (Mann–Whitney *U*-test) between three samples were found in controls and MDD patients.

### 2.3. Assays

The sAA activity was measured by an enzyme-linked immunoassay using an ELISA kit (Salivary α-Amylase ELISA Kit, Salimetrics LLC, USA). The assays were run in duplicates. The inter- and intra-assay variations were below 5.8% and 7.2%, respectively. Results are computed in deca-international units per milliliter (daIU/mL) of sAA at 37 °C.

### 2.4. Statistical analysis

Statistical procedures were performed using StatsDirect v2.7.9. Shapiro–Wilk test was used to assess normal distribution of continuous data. Normally distributed variables were compared using Student's *t*-test, all other continuous data were compared with nonparametric Mann–Whitney *U*-test. The Spearman rank correlation coefficient was used to assess correlations between the obtained variables. All tests were two-tailed with an alpha=0.05.