Bachelor Thesis

Ratio of baseline salivary alpha amylase to salivary cortisol and the relationship with depression

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1. Abstract

Background A dysregulation of the hypothalamic-pituitary-adrenal axis and sympathetic nervous system in patients with Major Depressive Disorder (MDD) has been noted in numerous studies. A recent study has highlighted that the ratio of reactive salivary alpha amylase to salivary cortisol (AOC) may be a better indicator of MDD than either biomarker alone[1]. Yet no studies thus far have examined whether this ratio can be used with baseline values instead of reactive measurements. This study will investigate the relationship between MDD or depressive symptoms and baseline AOC in patients with symptomatic atherosclerotic disease.

Methods Data for this study was gathered from the SMART-Medea study, consisting of 614 subjects with symptomatic atherosclerotic disease. Detailed information was gathered on MDD status, through the Patient Health Questionnaire-9 (PHQ-9) and the Composites International Depression Interview. Samples of salivary alpha amylase and salivary cortisol were taken at several time points in a single day[2]. The SMART-Medea study gathered detailed demographic and lifestyle information allowing for extensive use of demographic, sampling, lifestyle and medication covariate use in this study. This study firstly examined the relationship between PHQ-9 score and AOC through a linear regression analysis. Secondly the difference in AOC values among the three MDD groups (no MDD, subthreshold MDD and MDD) was gathered through an Analysis of Covariance (ANCOVA).

Results The linear regression with adjustments for age, gender, level of education, smoking, stress, alcohol use, and medication use showed that PHQ-9 score had a significant positive association with AOC at the time points 45 minutes after awakening and 60 minutes after awakening. The other time points do not show a significant association between PHQ-9 score and AOC. Results of the ANCOVA do not show a significant difference in AOC among the no MDD, subthreshold and MDD group for any of the time points or the area under the curve values.

Conclusion This study is the first to study the effects of MDD and MDD symptoms on the ratio of baseline salivary alpha amylase to salivary cortisol in subjects with symptomatic atherosclerotic disease. Results of this study indicate that AOC is associated with PHQ-9, as a marker for MDD symptom expression, at several time points during the day. An association between MDD group and AOC was not found in this study.

2. Introduction

Major Depressive Disorder (MDD), as characterized by the Diagnostic Statistical Manual of Mental Disorders (DSM), has a frequent occurrence, with a 12-month prevalence ranging from 2 to 10 percent[3,4,5]. According to the DSM-V a diagnosis of MDD entails that individuals have a depressed mood and/or anhedonia, combined with at least four other associated symptoms[6]. Individuals with MDD often experience loss of quality of life and functional disability[6,7]. Research has found that despite efforts, there is still a high rate (60-70%) of underdiagnosis of MDD[6]. A better understanding of the etiology and underlying mechanisms of MDD can provide aid in the development of further diagnostic mechanisms, treatments and prevention of the illness. This study therefore aims to investigate the relationship between the ratio of baseline salivary alpha amylase to salivary cortisol (AOC) and MDD.

MDD is categorized as a stress-related disorder, and previous research has frequently noted the significance of a deregulated stress response of the hypothalamic-pituitary-adrenal (HPA) axis and particularly cortisol, in MDD[8,9,10]. It has been found that, due to increased resistance to the negative feedback of cortisol on the HPA axis in MDD patients, the diurnal pattern of MDD patients is disturbed[8]. Specifically, a meta-analysis of four decades of studies examining the relationship between cortisol and MDD demonstrated an elevated cortisol level in 73% of MDD subjects[11].

Recently research has also found dysregulation of salivary alpha-amylase (sAA) in MDD patients[1,12,13]. sAA, a marker for the sympathetic nervous system (SNS), tends to be present in higher levels in individuals with MDD[15]. A study by Veen et al. (2013)[14] found the mean sAA levels in subjects with MDD to be 230 U/l, while remitted MDD subjects showed mean sAA levels of 221 U/l and controls 214 U/l; after controlling for age, gender and daily alcohol intake this formed a borderline significance ($p=0.06$)[14].

Additionally Tanaka et al. (2012)[15] published an article demonstrating that MDD patients show significantly ($p<0.01$) higher reactive sAA levels. Specifically women showed significant difference ($p<0.05$) in sAA levels both before and after stress stimulation, while men showed

slight significant values (p=0.07). The stress stimulus was an electrical current in the right median nerve at the greatest amplitude tolerable for the subject[15]. A study by Nater (2007) similarly found that chronic stress and reactive stress led to higher levels of baseline sAA levels[16].

Not only has an association been found between sAA and MDD and cortisol and MDD, recent research is also highlighting an association between sAA and salivary cortisol per se[17,18,19,20]. This is exemplified through a study by Engert et al. (2011)[17] whom measured sAA and salivary cortisol at frequent intervals of two minutes after administration of a psychological laboratory stressor in 50 males. Both markers were found to be significantly associated although with a time lag of around 13.5 minutes for salivary cortisol[17]. The time lag found in this experiment was also seen in the research by Ali et al. (2012)[1].

Recent studies have found that this association between sAA and salivary cortisol can be disturbed by stress-related disorders such as MDD[1,18.21]. The balance between the SNS and HPA stress response system is broken down as illustrated by Ali et al. (2012)[1]. Their research proposed that the ratio of sAA to salivary cortisol (the AOC ratio) may be a better indicator of a stress response attenuation then sAA or salivary cortisol alone. They tested this hypothesis through comparing subjects with and without early life adversities. All subjects were exposed to the Trier Social Stress Test and through analysis of the AOC they found that systemically there was a stronger positive relationship between stress related disorders such as MDD and the AOC ratio than to either biomarker alone. This indicates that the AOC ratio may be a satisfactory marker of a dysregulation of the stress response system[1].

A vast amount of research has to this date been performed on the effect of MDD on the dysregulation of the HPA axis and the SNS. The strong association that Ali et al. (2012) found between the AOC and MDD, is indicative of a blunted HPA axis and SNS response, and this ratio may therefore provide a better marker for MDD than either marker alone. As of yet no research has been performed to examine this ratio in baseline levels of these markers[22]. This research will therefore examine whether there is a relationship between the ratio of baseline sAA to baseline salivary cortisol (AOC) and MDD or MDD symptoms.

3. Methods

3.1 Subjects

Data were gathered from the Second Manifestations of ARTerial disease – Memory, Depression and Aging (SMART-Medea) study. The SMART-Medea study is a prospective cohort study aimed to investigate the association between brain changes and psychosocial vulnerability and stress. 614 older subjects with manifest arterial disease participated in this study. Data collected included sAA and salivary cortisol, blood samples and questionnaires concerning depression and medical history among others. All patients provided written consent and the SMART-Medea study was approved by the ethics committee of University Medical Center Utrecht[2].

3.2 Analytical sample

Several inclusion criteria were implemented in order to ensure that the subjects included in the study have sufficient data measurements available, as illustrated in figure 1. Only subjects whom had at least one measurement of sAA and cortisol were included. If no salivary measurements were recorded for a subject, the subject would be excluded from the analytic sample. 76 cases were excluded based on these criteria.

Subsequently subjects whom did not have a result for the Composites International Depression Interview were excluded from the analytic sample. 12 subjects did not have a result for the Composites International Depression Interview. Therefore a total of 88 cases were excluded from the analytic sample based on a limited availability of MDD diagnosis data, leaving an analytic sample of 614 subjects.

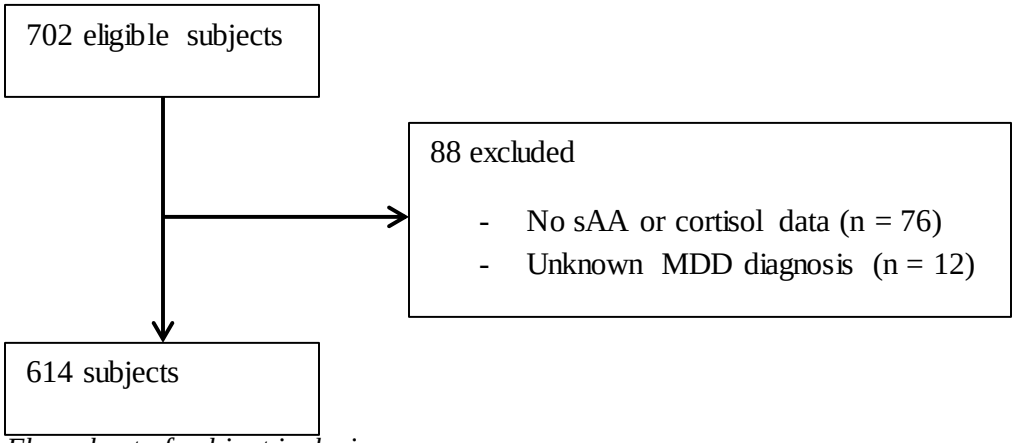


Figure 1 – Flow chart of subject inclusion.

3.3 Depression

MDD was measured in terms of absence or presence of a diagnosis in the past 12 months using the Composites International Depression Interview. This interview assesses the participants according the nine DSM MDD criteria. Symptom severity was measured through the Patient Health Questionnaire-9 (PHQ-9), likewise assessing the nine DMS MDD criteria. The answers are recorded on a four point scale, where 0 equals "not at all" and 3 equals "nearly every day". The answers per question lead to the ultimate defining of the PHQ-9 score where a score of 6 or above is considered as depressive symptomology[23].

Based on a MDD diagnosis using the Composites International Depression Interview and MDD symptom expression using the PHQ-9, three groups were formed. Firstly the "no MDD" group who do not have a MDD diagnosis and a PHQ-9 score less than 6. Secondly the "subthreshold MDD" group where subjects are placed if they do not have a MDD diagnosis but have a PHQ-9 score of 6 or above. Finally the "MDD" group which contains subjects with a MDD diagnosis regardless of their PHQ-9 score.

3.4 Alpha-amylase and cortisol

The collection of saliva was performed at home on a regular weekday. The subjects took samples at seven set time points throughout the day (awakening, 30, 45 and 60 minutes after awakening and at 10PM and 11PM). Cotton bolls (Salivette, Sarstedt, Numbrecht, Germany) were chewed on for at least two minutes and stored in the freezer until the subjects next hospital visit, which did not exceed two weeks. Participants were asked not to smoke, drink caffeine, eat or brush their teeth within 30 minutes prior to taking the sample[2].

In the laboratory, the saliva samples were centrifuged for 10 minutes at 3000 rpm and then stored at 20°C until assessments were performed. Cortisol measurements were performed using competitive radioimmunoassay with the aid of a polyclonal anticortisol-antibody (K7348). The tracer used in this method was the [1,2-3H(N)]-Hydrocortisone (NET185, NEN-DuPont, Dreiech, Germany). The assessment of sAA was done using the Beckman-Coulter Unicel DxC 800 chemistry analyzer (Beckman-Coulter Inc, Brea, CA). The samples of sAA samples were

diluted at 25x, 250x or 2500x with 0.2% BSA in phosphate buffer pH 7.0. The dilution depended on the concentration of the amylase in the sample[2].

3.5 Data analysis

The AOC values were calculated by dividing the sAA over the salivary cortisol. The distribution of the resulting AOC ratio was strongly positively skewed. Therefore natural log transformations were applied to the AOC ratio and formed a normal distribution curve. To standardize the unit of the log transformed AOC ratio, z transformations were imposed on these values. The resulting z-score of the log AOC values were used for the statistical analyses.

The statistical analyses will be preceded by an evaluation of the data set characteristics. Characteristics that will be determined include age, gender, stress level, cardiovascular disease status, smoking, alcohol use, antidepressant and beta-blocker use or use of corticosteroids.

An Analysis of Covariance (ANCOVA) will be performed to determine whether there is a significant difference between the AOC values in the three specified MDD groups (no MDD, subthreshold MDD or MDD). This will be performed for all time points as well as using the ratio of the area under the curve (AUC). Next a linear regression analysis will be performed to determine the presence and strength of a relationship between AOC and PHQ-9 score. The linear regression will likewise be performed for all time points and for the AUC level. All analyses are performed using SPSS version 22.0 (SPSS Inc, Chicago, Illinois).

3.6 Covariates

Several variables influence the salivary cortisol production and should be considered as confounding variables in this research. Age, gender and smoking are such variables[24]. Likewise variables affecting the production of sAA include lifestyle factors such as higher than average daily alcohol intake, stress or use of medication such as corticosteroids or beta-blockers[25]. Usage of antidepressant medication will likewise be taken into account as a covariate.

4. Results

4.1 Sample population characteristics

Table 1 presents the baseline characteristics of the sample population of 614 subjects. 485 subjects fell in the category "no MDD" (79.0%), 79 fitted in the "subthreshold MDD" group (12.9%) and 44 were placed in the category "MDD" (7.2%). Within these three groups the percentage of males was much larger than the percentage females. 81% of the total sample population was male, with similar male to female ratios in the individual MDD groups. The mean age of the subjects varied from 59 years (MDD) to 62 (no MDD) averaging around 62 years of age (SD=9.5 years) for all subjects. The average level of education for all three groups was MBO and the average stress level on a scale of 1 to 10 in all three groups on the day their samples were taken was a 3.

Table 1 - Baseline characteristics of sample population presented as means unless otherwise specified.

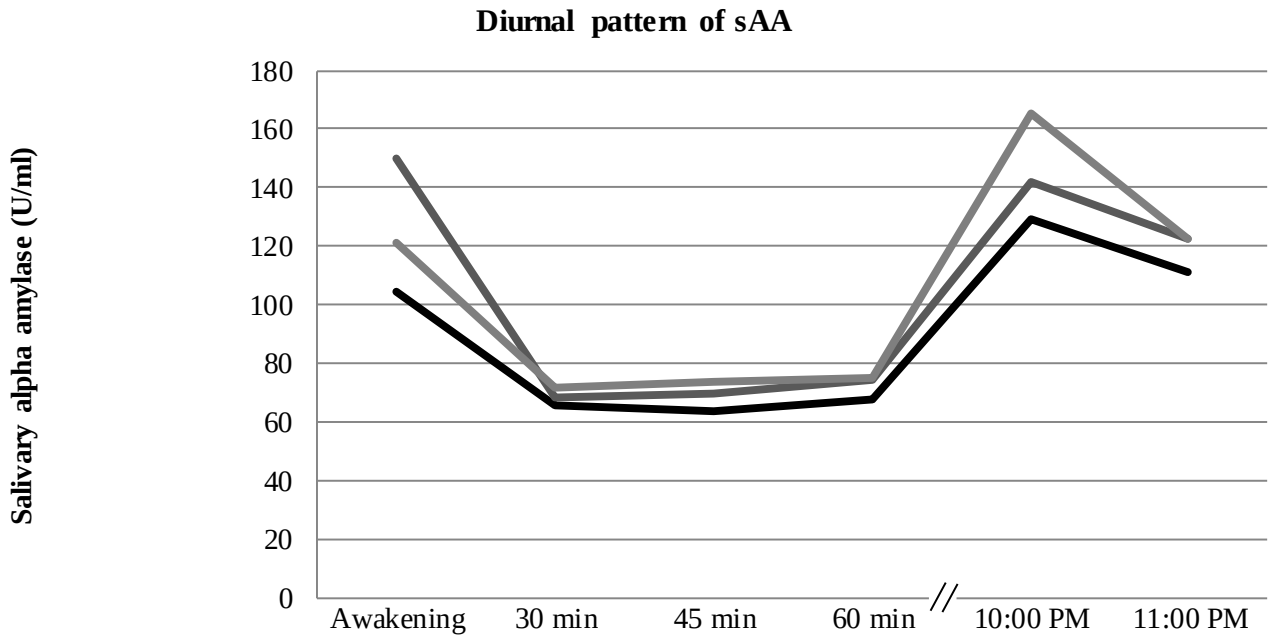
		MDD			Total
		No MDD	Subthreshold MDD	MDD	
Subjects (number)		485	79	44	614
Gender (% males)		83	72	77	81
Smoking (pack years)		21	26	23	22
Level of education*		MBO	MO	MO	MBO
Work on test day (%)		39	35	48	39
Stress on sampling day (1-10)		3	4	4	3
Age (years)		62	60	59	62
Disease (%)	Cerebrovascular	23	34	34	25
	Cardiovascular	66	58	71	66
	Peripheral arterial	20	23	21	20
	Abdominal aneurysm	7	5	5	7
Alcohol (%)	<1 drink per week	29	38	41	31
	1-10 drinks per week	40	35	34	39
	11-20 drinks per week	18	17	18	18
	>20 drinks per week	13	10	7	12
Medication (%)	Beta-blocker	55	37	36	52
	Corticosteroid	7	12	9	8
	Antidepressant	5	13	25	8

**MBO = Middelbaar beroeps onderwijs, MO = Middelbaar onderwijs*

4.2 Diurnal pattern

The diurnal patterns and mean values of sAA and salivary cortisol are presented in figures 2 and 3 respectively. The diurnal pattern is formed based on average sAA and salivary cortisol levels found in all subjects within one specified MDD group. As figure 2 illustrates, sAA levels drop

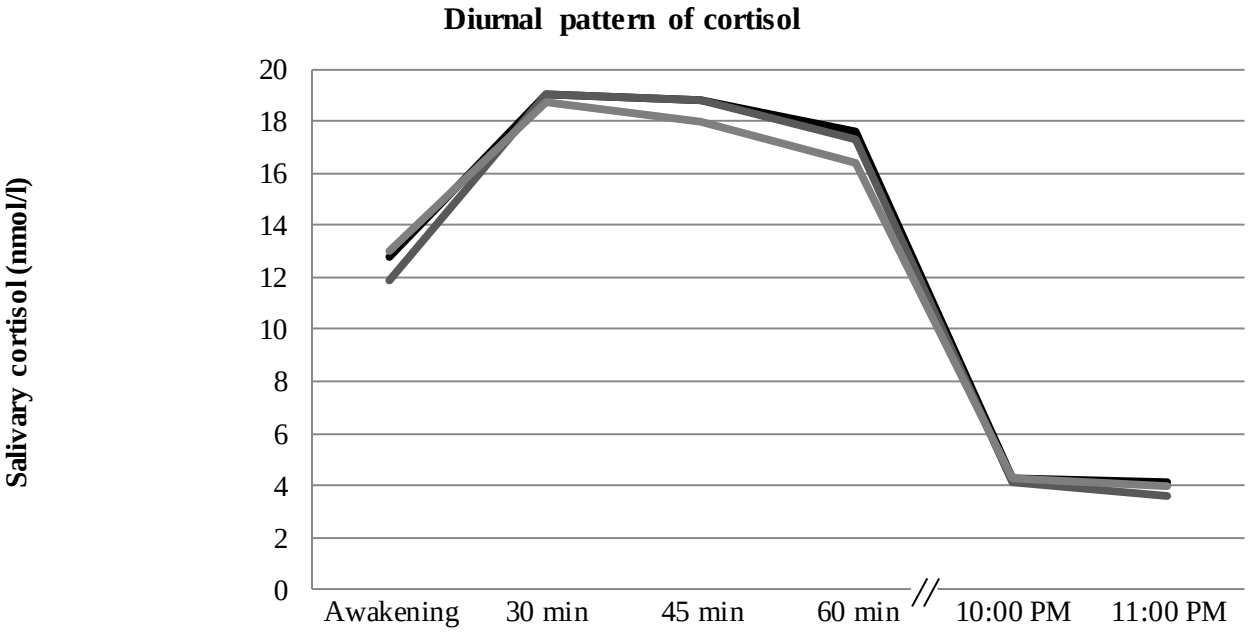
within the first 30 minutes after awakening after which it remains stable through 60 minutes after awakening. At 10 PM, sAA has risen compared to the morning and a slight drop from 10 PM to 11 PM is visible. Figure 2 illustrates that the MDD group presents with a mean value of sAA that is higher at all time points except awakening. At time point awakening the mean sAA for the MDD group, *M = 121.3 (CI 95% [84.3, 158.2])*, is lower than the mean for the subthreshold MDD group, *M = 150.0 (CI 95% [123.1, 176.9])*. Both are nonetheless higher than the mean for the no MDD group, *M = 105.5 (CI 95% [94.9, 116.2])*. The largest differences among the means is found at time-point 10PM at which time the mean of the MDD group, *M = 162.9 (CI 95% [124.1, 201.7])*, is higher than the mean of the subthreshold MDD group, *M = 137.1 (CI 95% [109.8, 164.3])*, which is also higher than the mean of the no MDD group, *M = 128.9 (CI 95% [117.6, 140.2])*.



	Awakening	30 min	45 min	60 min	10:00 PM	11:00 PM
No MDD	104.7	65.8	63.4	67.6	129.0	111.1
Subthreshold MDD	150.0	68.5	69.4	74.3	142.0	122.2
MDD	121.3	71.7	73.7	74.9	165.4	122.6

Figure 2 – Diurnal pattern of sAA per MDD group and table of mean values of sAA per MDD group at all time points.

The diurnal pattern of cortisol shows an increase within the first 30 minutes after awakening after which it gradually declines through 60 minutes after awakening, as demonstrated in figure 3. At 10 PM, cortisol levels have dropped and remain stable until the final measurement at 11 PM. Overall cortisol displays a diurnal pattern that presents as a mirror image of the diurnal pattern shown by sAA. It can be seen when comparing figure 2 to figure 3, that cortisol does not display as big a difference among the three MDD groups as sAA. At awakening the mean level of cortisol for the no MDD group, *M = 12.9 (CI 95% [12.3, 13.4])*, is higher than the mean of the subthreshold MDD group, *M = 11.6 (CI 95% [10.3, 12.9])*. Nonetheless the mean of the MDD group, *M = 13.0 (CI 95% [11.2, 14.8])*, is highest. At 11 PM the mean values lie very close together. The mean of the no MDD group, *M = 4.2 (CI 95% [2.5, 4.6])*, is very close to the mean of the MDD group, *M = 4.1 (CI 95% [2.5, 5.7])*. The subthreshold MDD group has a slightly lower mean, *M = 3.6 (CI 95% [2.5, 4.7])*.



	Awakening	30 min	45 min	60 min	10:00 PM	11:00 PM
No MDD	12.8	19.0	18.8	17.6	4.3	4.1
Subthreshold MDD	11.9	19.0	18.8	17.3	4.1	3.6
MDD	13.0	18.7	18.0	16.4	4.3	4.0

Figure 3 – Diurnal pattern of cortisol per MDD group and table of mean values of salivary cortisol per MDD group at all time points.

4.3 Association between MDD group and AOC values

A one-way ANCOVA was performed and used to compare the AOC level at different time points among the subjects within three different MDD groups (no MDD, subthreshold MDD, and MDD). Covariates included in the analysis were gender, age, stress, level of education, smoking, alcohol intake and medication use (beta-blocker, corticosteroids and antidepressants). All assumptions for the ANCOVA were met for all time point analyses, allowing the data to be interpreted using the parametric tests.

The results of the ANCOVA, as presented in table 2, indicate that there was no statistically significant effect of MDD group on the AOC of the subjects for awakening, $F(2,478) = .959$, $p = .505$, or the following time points throughout the day. 30 minutes after awakening had a significance level of $F(2, 473) = .056$, $p = .946$, time point 45 minutes after awakening $F(2,468) = .844$, $p = .431$ and time point 60 minutes after awakening $F(2,465) = .926$, $p = .397$. The evening time points were also insignificant, with 10 PM showing a significance level of $F(2, 485) = .712$, $p = .491$ and 11 PM, $F(4, 486) = .951$, $p = .387$. Examining the ANCOVA for the AUC also demonstrated no statistically significant effect, $F(2,441) = .098$, $p = .907$.

Table 2 – Association of MDD group and AOC per time point.

	Significance	**F**	**Group**	**Mean****	**CI 95%**
Awakening	.686	.377	No MDD	-.031	[-.132, .070]
			Subthreshold	.052	[-.209, .312]
			MDD	-.133	[-.475, .210]
30 min	.946	.056	No MDD	-.007	[-.107, .093]
			Subthreshold	.021	[-.236, .279]
			MDD	.047	[-.294, .387]
45 min	.431	.844	No MDD	-.030	[-.132, .072]
			Subthreshold	.160	[-.109, .430]
			MDD	.034	[-.208, .375]
60 min	.397	.926	No MDD	-.030	[-.134, .074]
			Subthreshold	.174	[-.100, .448]
			MDD	.014	[-.367, .339]
10 PM	.491	.712	No MDD	-.016	[-.118, .085]
			Subthreshold	.131	[-.135 .397]
			MDD	-.108	[-.456, .241]
11 PM	.387	.951	No MDD	-.022	[-.123, .079]
			Subthreshold	.112	[-.155, .379]

			MDD	-.183	[-.521, .155]
AUC	.925	.078	No MDD	-.015	[-.120, .090]
			Subthreshold	.037	[-.236, .310]
			MDD	.032	[-.328, .392]

** $p < .05$*

*** Mean z score of log AOC values.*

Figure 4 presents the mean z score of the log AOC at all the time points. Although no significant difference was found at any of the time points between the groups, the figure does show that MDD tends to present with z-score of log AOC that is lower than the other two groups, particularly at the time points awakening, 10 PM and 11 PM.

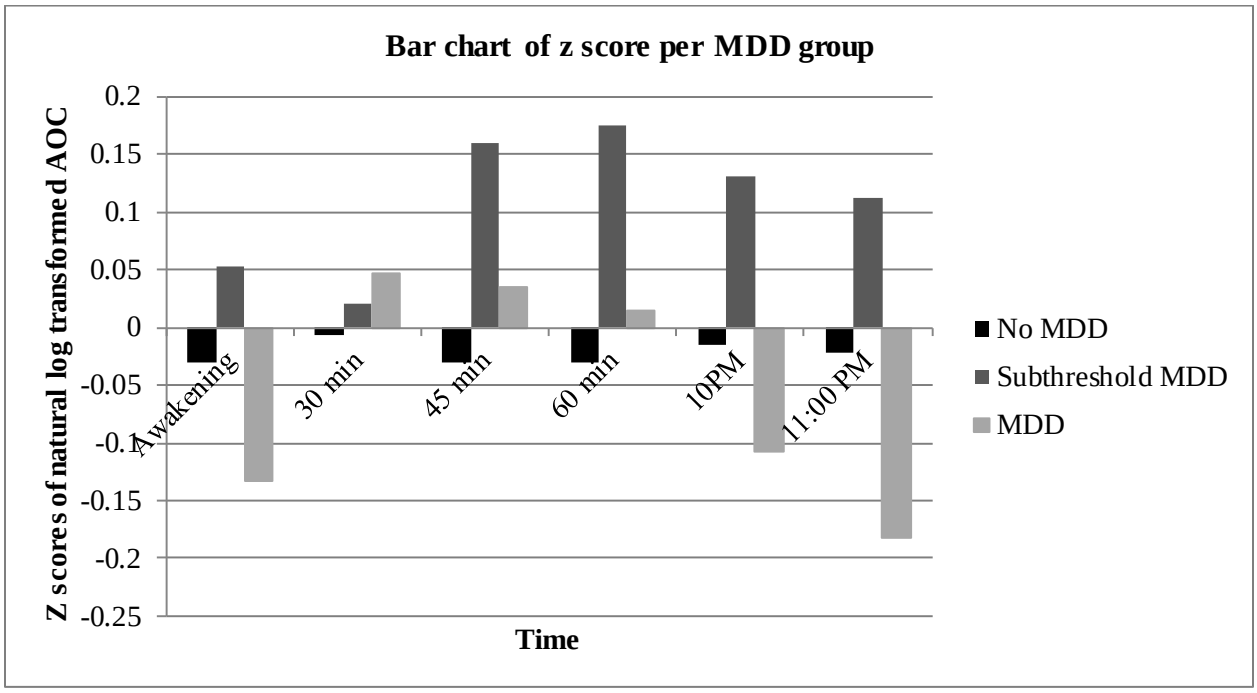


Figure 4 – Bar graph of z score of log AOC at different time points per MDD group.

4.4 Relationship between MDD symptoms (PHQ-9 score) and AOC values

To estimate the effect of PHQ-9 score on AOC, a linear regression was performed. The assumptions for the linear regression were met and thus there is no interference with the interpretation of the data of the linear regression.

Table 3 provides an overview of unstandardized regression coefficients, significance levels and confidence intervals for all time points. At the time point of 45 minutes after awakening a significant unstandardized regression coefficient is found, *B = 0.042, p = 0.004, CI 95% [.014, .071]*. This is likewise for the time point 60 minutes after awakening, *B = .043, p = .003, CI 95% [.014, .072]*. These results imply that as PHQ-9 score rises one point, at 45 minutes after awakening and considering all covariates, the z-score of the log AOC will rise with .025. At the time point of 60 minutes after awakening when the PHQ-9 score rises one point, the z-score of the log AOC will rise with .031. An increase in the AOC ratio, as is happening at time points 45 minutes and 60 minutes after awakening when the PHQ-9 score increases, implies that relatively more sAA is excreted compared to salivary cortisol.

The remaining time points have no significant unstandardized regression coefficients. Namely at the time point awakening a significance of *B = .023, p = .113, CI 95% [-.005, .051]* is found, and at 30 minutes after awakening a significance of *B = .021, p = .129, CI 95% [-.006, .049]* is seen. The evening time points demonstrate a significance level of *B = .028, p = .060, CI 95% [-.001, .058]* at 10 PM and at 11 PM *B = .016, p =.276, CI 95% [-.013, .044]*. The AUC value has an insignificant unstandardized regression coefficient of *B = .026, p = .085, CI 95% [-.004, .055]*.

Table 3 – Association of PHQ-9 score and AOC per time point and per model.

	B	**CI 95%**	**Significance**
Awakening	.023	[-.005, .051]	.113
30 min	.021	[-.006, .049]	.129
45 min	.042	[.014, .071]	.004*
60 min	.043	[.014, .072]	.003*
10 PM	.028	[-.001, .058]	.060
11 PM	.016	[-.013, .044]	.276
AUC	.026	[-.004, .055]	.085

** $p < .05$*

5. Conclusion

This study had the goal of investigating the relationship between the ratio of baseline sAA to baseline salivary cortisol and MDD or MDD symptoms. A previous performed study by Ali et al. (2012) demonstrated that the ratio of reactive AOC is influenced by the presence or absence of MDD[1]. Based on these results, this study therefore looked into the, so far not researched, topic of the effect of MDD and MDD symptoms on the ratio of baseline AOC. Results of this study show that at time points 45 minutes after awakening and 60 minutes after awakening there is a significant positive relationship between PHQ-9 score and AOC values, after adjusting for age, gender, stress, level of education, smoking, alcohol intake and medication use (beta-blocker, corticosteroid and antidepressant). However, there was no significant difference found between the MDD group and AOC score at any of the time points.

Firstly the diurnal pattern of sAA and cortisol were analyzed separately. The pattern of the diurnal sAA and salivary cortisol levels in this sample population is similar to the pattern found in other studies[16,26]. Despite the similarity in the diurnal pattern, the averages of sAA do show differences. The study by Veen et al. (2013) found mean sAA levels of 230 U/l for MDD subjects and 214 U/l in control subjects. The averages found in this study range between 65 U/l and 165 U/l. Yet the average sAA levels found in studies varies significantly as a study by Rohleder et al. (2006) found sAA averages ranging between 60 U/l and 220 U/l[26].

Following the analysis of the diurnal pattern, analyses on the relationship between MDD and AOC were performed. Based on the statistical analyses, there is an indication that the use of MDD grouping, as performed in this study, does not lead to significant differences in AOC values among the three groups. In this study the formation of the three MDD groups (no MDD, subthreshold MDD and MDD) was formed based on cut-off scores of the PHQ-9 score and a MDD diagnosis using the Composites International Depression Interview. Nonetheless figure 4 illustrates that particularly at time points awakening and 10 and 11 PM, the MDD group has a relatively large difference compared to the other groups. The subthreshold MDD group likewise demonstrates relatively large differences in their mean average z-score of the log AOC at all time

points except 30 minutes after awakening. Addressing some of the weaknesses of this study as described later, may clarify this upon further investigations.

Analyses were also performed on the relationship between the PHQ-9 score, as a measure of MDD symptom severity, and AOC levels. At time points 45 minutes after awakening and 60 minutes after awakening a significant positive association was found. The other morning time points, as well as 10 PM, 11 PM and the AUC values did not form a significant association. These results indicate that for the morning time points (45 and 60 minutes after awakening) an increase in PHQ-9 score significantly alters the AOC values. This provides some indication that a higher PHQ-9 score, as an indication of worse MDD symptom severity, leads to altered higher AOC values. Likewise it can be concluded that the other time points may have significant associations with AOC values, when the power of the sample population is greater. The insignificant time points, particularly time point 10PM and the AUC levels, are close to significant values. Therefore for all time points, a greater power of the analytical sample may demonstrate that PHQ-9 score is associated with AOC levels, also at the time points that in this study do not demonstrate significance.

This study is the first to explore the relationship between MDD diagnosis as well as MDD symptom severity and baseline AOC values. The current study builds on the results found in the study by Ali et al. in 2012, where a strong association (*r(37) = .390 and p = .017*) was found between the ratio of reactive sAA to salivary cortisol and MDD[1]. In that study, 37 subjects underwent the Trier Social Stress Test. 20 of the subjects had received low care in their early life and 17 subjects received high care in their early years. The sample population had an age range of 18 to 35 years with a mean age of 26 years (SD = 6). The study collected salivary samples at 10 minute intervals throughout the testing, to measure sAA and salivary cortisol. Afterwards the subjects completed standardized, self-report questionnaires which were used to assess their self-esteem, quality of early-care and the subject's perception of their own chronic stress and depressive symptoms. To assess their depressive symptoms the Beck depression inventory-II was used. The measurements used in this study included the AOC ratio as well as the cortisol over sAA (COA ratio). These were calculated by dividing the AUC levels of both biomarkers after

which the ratio was z-transformed[1]. The larger sample size and multiple ways of assessing depression in the current study increase the power of the findings in this study. The current study likewise differs from the study by Ali et al. in the sample population, as this study has an average age that is higher and contains only subjects with symptomatic atherosclerotic disease. Nevertheless this does not detract from the power of this study as it implies that merely different subsets of the population have been studied.

All in all, findings of this research indicate that depressive symptom expression, as indicated by the PHQ-9 score, is associated with the AOC ratio. The MDD grouping as performed in this study is not associated with the AOC ratio with the current analytic population. In light of other research this is a promising lead for further investigations on the use of the AOC ratio. Currently research on using a ratio as a marker for MDD is very minimal. Yfanti et al. (2014) have successfully used this marker in relation to anxiety[27]. Other studies looking at MDD do mention the promising results found by Ali et al. (2012) but no studies thus far have continued their work[22,28,29]. This research should thus be seen as a primary study into the effects of having MDD, subthreshold MDD or no MDD as well as different PHQ-9 scores on the baseline ratio of AOC. Further studies can explore and expand on the information found within the current study.

Several strengths can be attributed to this study. Firstly the overall size of the sample population (n=614) adds support to the conclusions. Additionally the study has great depth of knowledge on many important subject information providing sufficient important covariates to consider in the statistical analyses. Furthermore the salivary samplings of the subjects took place at several time points adding to the research by providing multiple time points that were included in the analysis. Finally the use of not one but at least two measurements of depression (the PHQ-9 assessment and the Composites International Depression Interview) gives more depth of knowledge on the patients MDD status.

Several factors should be taken into account when considering this research. Among others this includes the fact that this study was performed on a sample which, although large in total, contained relatively few subjects within the subthreshold MDD and MDD group. 485 subjects

fell within the no MDD group which is a large and relatively representative sample, yet within the subthreshold group a mere 79 subjects were placed and only 44 in the MDD group. Although this sample size is larger than in the previous study by Ali et al. (2012), it becomes clear that the distribution among the groups is very unequal[1]. More significance may be found upon using larger samples of subthreshold and MDD groups. Likewise significance may also be found for other time points for a linear regression of PHQ-9 scores if the groups of subthreshold and MDD were larger in size.

Limitations of this study also include that all subjects were recruited initially because of their disease status. All subjects had symptomatic atherosclerotic disease and therefore the results of this study can only be regarded for subjects with symptomatic atherosclerotic disease and is not representative of all people. The disease status of the subjects may have an influence on their stress response systems such as sAA and cortisol[30,31.32].

Finally a note of caution should be made on the large male dominance of this study. 81% of the total sample population was male and therefore only 19% female. Results of this study are therefore not necessarily valid for females in the general population. Additionally the time points taken in this study, although numerous, could have been repeated over multiple days to ensure that the collected data was more representative and less influenced by events on the data collection day. Future studies could focus on gathering more samples spread out across several days.

As previously mentioned, this study should be seen as a primary study on the association between MDD diagnosis and symptoms and the AOC ratio. Ali et al. (2012) were a pioneer in studying the reactive AOC ratio in relationship to MDD and despite the promising finds in their research, little studies have thus far been conducted to continue Ali et al.'s work[1]. This study provides such a continuation. A positive significant association has been found between the PHQ-9 score and AOC at several time points after awakening, yet this study has found no significant difference in AOC levels among the three MDD groups. Further research will be needed to examine more closely the effect of MDD on AOC in a population more representative

of the general population (i.e. in subjects without symptomatic atherosclerotic disease) and in a sample that contains more subjects with (subthreshold) MDD. This increase in the (subthreshold) MDD group may prove of importance in increasing the significance of the PHQ-9 linear regression and likewise perhaps providing more subtleties in the MDD grouping – thereby increasing the strength of the ANCOVA. Together with work by Ali et al. (2012) this study provides promising evidence that, with more elaborate research, AOC may be associated with MDD and can ultimately provide useful in diagnostics, prevention, and treatment of MDD.

6. References

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