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RESEARCH ARTICLE

The relationship between the diagnostic value of salivary cortisol levels and behavioral symptoms in patients with Alzheimer's disease

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ABSTRACT

Objective: Dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis and elevated cortisol levels are frequently observed in Alzheimer's disease (AD) patients. This study aimed to explore the potential use of saliva cortisol levels as an early marker for the initial clinical stages of AD and to investigate the correlation between saliva cortisol levels and behavioral symptoms in AD patients.

Method: The sample included 81 participants of similar ages: AD patients (n=30), individuals with subjective memory loss (SML) (n=31), and a healthy control group (n=20). Saliva samples were collected at 08:00 and 16:00, and cortisol levels were compared across these groups. The Mini-Mental State Examination (MMSE), adjusted for educational level, was administered to all participants. The SML and healthy groups completed the Subjective Memory Complaints Questionnaire (SMCQ) and the Geriatric Depression Scale (GDS). Caregivers of AD patients were interviewed using the Neuropsychiatric Inventory (NPI) to assess the relationship between cortisol levels and behavioral symptoms in AD patients.

Results: Evening cortisol levels in the AD group were significantly higher than those in the SML group ($p<0.001$). However, when examining salivary cortisol levels, no significant differences were detected between the SML group and the healthy controls. A negative correlation was found between salivary cortisol levels and MMSE scores in AD patients. Additionally, a positive correlation was observed between evening cortisol levels and several items in the NPI among AD patients.

Conclusion: Although no significant changes in salivary cortisol levels were found in the SML group, which does not support its use as an early indicator of AD, the results suggest that the HPA axis may be a cause of behavioral problems, particularly in patients with severe AD.

Keywords: Alzheimer's disease, behavioral symptoms, cortisol, memory

INTRODUCTION

Alzheimer's disease (AD) is a progressive dementia syndrome typically characterized by initial disturbances in recent memory, which eventually impair other cognitive functions (1). With the number of people affected by dementia increasing, there is a growing emphasis on the early diagnosis of AD and studies focusing on preventive treatments. Moreover, the neuropsychiatric symptoms observed in AD impose significant financial and emotional burdens on both the patient and the caregiver, necessitating

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primary investigation. Self-reported memory complaints, without verifiable cognitive impairment through neuropsychological testing, are hallmarks of subjective memory loss (SML), which may represent a very early stage of AD (2).

Numerous studies have demonstrated that pathophysiological processes leading to dementia may begin up to a decade before cognitive symptoms manifest, possibly at the SML stage (3). Understanding the preclinical stages of dementia has significantly increased the importance of monitoring individuals with memory problems for the development of AD, emphasizing the need for new studies to enable early diagnosis. In addition, numerous studies have explored the relationship between dysfunction of the hypothalamic-pituitary-adrenal (HPA) axis and the pathogenesis of AD (4–6). These studies suggest that cortisol measurement could serve as a useful biomarker for early AD diagnosis, aiding in understanding the disease's pathophysiology and shedding light on its clinical symptoms. Given that cortisol levels are also associated with psychiatric diseases and symptoms, understanding the relationship between cortisol levels and the behavioral symptoms in AD could provide insights into this particularly problematic aspect of the disease (4). Adrenocortical activation plays a critical role in the body's adaptive response to physical and psychogenic stressors. Glucocorticoids limit the activity of the HPA axis through negative feedback mechanisms, mediated by glucocorticoid receptors located in the hippocampus, hypothalamus, and anterior pituitary (7).

The primary aim of this study is to determine whether the dysregulation of cortisol observed in AD can serve as a biomarker for early diagnosis among individuals with memory complaints who have not yet been diagnosed with AD. Considering the glucocorticoid cascade hypothesis as one of the mechanisms behind behavioral changes in the progression of AD, a secondary aim is to investigate the potential relationship between difficult-to-treat behavioral symptoms of AD and salivary cortisol levels.

METHODS

Participants

At the onset of the study, saliva samples were collected from 89 individuals. Eight of these individuals were subsequently excluded from the study for the following reasons: four had AD, three exhibited subjective memory loss (SML), and one was healthy. Despite providing samples both in the morning and evening, the saliva quantity and quality from these eight individuals were inadequate for analysis. As a result, the study proceeded with 81 participants divided into three groups: 30 in the AD group, 31 in the SML group, and 20 in the healthy group. The study spanned from June 2020 to March 2021. Approval was granted by the Ethics Committee of Kartal Dr. Lutfi Kirdar City Hospital, Istanbul (IRB Approval Date: 27.02.2019 and No.: 2019/514/148/11).

Exclusion criteria applicable to all three groups included a history of stroke, severe neurological or physical conditions unrelated to the study (such as epilepsy or carcinoma), any acute systemic disease in the preceding month, alcohol abuse, uncorrected visual or hearing impairments, motor deficiencies that could hamper cognitive testing, endocrine disorders (including Cushing's syndrome, Cushing's disease, and Addison's disease), and the use of medications like steroid. Inclusion criteria for the AD group were based on patients diagnosed with a probable AD, following the National Institute on Aging and Alzheimer's Association (NIA-AA) 2011 criteria, who were receiving care at the dementia outpatient clinic of the Neurology Department at Kartal Dr. Lutfi Kirdar City Hospital and participated during the study period. Patients with other types of dementia in addition to AD or those for whom the specific type of dementia could not be accurately identified were not included in the study. For the SML group, inclusion criteria were set as follows: participants must be over 60 years of age, have experienced amnesia that led them to seek medical help within the last three months, and the amnesia must not impair their activities of daily living. Additionally, participants in the SML group are required to score 23 or higher on the Mini Mental State Examination (MMSE) and answer 'yes' to three or more questions on the Subjective Memory Complaints Questionnaire (SMCQ).

The control group was composed of individuals over 60 years of age, considered healthy, with no amnesia complaints in the past three months when compared to their peers. Control participants were also required to score 23 or higher on the MMSE, answer 'yes' to fewer than three questions on the SMCQ, and score below 14 on the Geriatric Depression Scale (GDS) to exclude the presence of major depression. Participants with serious uncontrolled medical illnesses were excluded from the study.

Data Collection Instruments

All participants underwent the MMSE, which was administered in versions appropriate for either educated or uneducated individuals, depending on their level of education. The MMSE is a brief, reliable test used to assess cognitive function. A threshold score of 23/24 on the MMSE has been shown to possess high sensitivity and specificity for diagnosing mild dementia among the Turkish elderly population (8). Both the GDS and SMCQ were administered to participants in the control and SML groups. The SMCQ (9) consists of 14 yes/no questions aimed at evaluating memory function. The total score for the questionnaire is derived by tallying the 'yes' responses (10). The validity and reliability of the SMCQ were evaluated in the Turkish population. A cut-off point of 5.5 predicted dementia diagnosis with 77% accuracy. The average cut-off point for the control group was determined to be 3.2, leading us to set the SMCQ score threshold at three or less (11). Yesavage et al. (12) developed the GDS, which consists of 30 yes/no questions. The total score ranges from 0 to 30. It is reported that a score above 14 can identify older adults with depression, exhibiting a sensitivity of 90% and a specificity of 97% (13). All participants were provided with a sociodemographic data collection form.

The Neuropsychiatric Inventory (NPI) interview was conducted with individuals caring for patients with AD or their relatives. The interview begins with scanning questions about the presence of symptoms in 12 behavioral areas: 1. delirium, 2. hallucinations, 3. agitation/aggressiveness, 4. depression/dysphoria, 5. anxiety, 6. elation/euphoria, 7. apathy/abulia, 8. disinhibition, 9. irritability/lability, 10. abnormal motor behaviors, 11. sleep/night behaviors, and 12. appetite and eating changes. If a symptom's presence is confirmed by the patients' relatives, further detailed questions about that area are posed. Scores for each item are calculated by multiplying the frequency of symptoms (rated from 1 for 'occasionally' to 4 for 'very frequently, daily') by the severity (rated from 1 for 'slight' to 3 for 'severe'). The maximum possible score is 144. The Turkish adaptation and the validity and reliability study of the NPI have been conducted (14, 15). Verbal and written consent were obtained from the patients and their caregivers or legal representatives (if applicable).

Salivary Cortisol Analysis

In this study, saliva samples were utilized to measure cortisol levels. The choice of saliva over serum cortisol for the analysis was due to the invasive nature of serum collection, which requires vein puncture at least twice a day. Such a procedure could elevate cortisol levels by increasing agitation in AD patients who may already exhibit behavioral problems. Therefore, we opted to use salivary cortisol as it is a reliable indicator of cortisol levels and its collection is non-invasive. Unlike plasma cortisol, which is linked to proteins, saliva cortisol is not. Therefore, the biologically active fraction of cortisol is generally accepted as a better indicator. Saliva cortisol is also recognized for its ecological validity (16).

Two separate saliva samples were collected from the patients at 08:00 and 16:00 using Salivette® tubes (Sarstedt, Nümbrecht, Germany). The patients were instructed not to eat, drink, brush their teeth, or smoke for 30 minutes before providing the samples. The swab in the Salivette tube was chewed for at least one minute, and the tube was then transported to the biochemistry laboratory within 30 minutes. In the laboratory, the saliva samples were centrifuged at 10,000 rpm for 10 minutes. The samples were subsequently stored at -20 °C for up to 10 months until analysis. Electrochemiluminescence was examined using the immunological method in a Cobas (Roche Diagnostics, Penzberg, Germany) apparatus.

Statistical Analysis

The Statistical Package for the Social Sciences (SPSS) 17.0 (IBM, USA) was utilized for all statistical analyses. Descriptive statistics of continuous variables were presented as mean, standard deviation, median, and for categorical variables, frequency. The Kolmogorov-Smirnov test was employed to assess normality. Categorical variables were analysed using Chi Square test. The one-way Analysis of Variance (ANOVA) test was utilized for group comparisons of normally distributed variables, The Mann-Whitney U test was used to assess non-normally distributed variables between two groups, while the Kruskal-Wallis test was used to assess variables between more than two groups. For post hoc evaluation, pairwise comparisons were conducted using the Bonferroni test.

The relationship between NPI data and variables such as age, MMSE scores, and cortisol levels was assessed using Spearman's correlation analysis. A p-value of less than 0.05 was considered statistically significant for all tests.

Table 1: Comparison of sociodemographic and disease-related data of groups

	Control (n=20)		SML (n=31)		AD (n=30)		p
	n/ Mean±SD	% (min–max)	n/ Mean±SD	% (min–max)	n/ Mean±SD	% (min–max)	
Age†	65.30±4.79[a]	(59–75)	67.65±7.05[a]	(57–84)	74.70±8.31[b]	(60–90)	<**0.001**
Gender‡							0.355
Female	13	(65.00)	23	(74.19)	17	(56.67)	
Male	7	(35.00)	8	(25.81)	13	(43.33)	
Education‡							**0.002**
<5 years	9[a]	(45.00)	23[b]	(74.19)	27[b]	(90.00)	
>5 years	11[a]	(55.00)	8[b]	(25.81)	3[b]	(10.00)	
HT‡	8	(40.00)	16	(51.61)	20	(66.67)	0.166
DM‡	6	(30.00)	13	(41.94)	10	(33.33)	0.644
CAD‡	2	(10.00)	6	(19.35)	10	(33.33)	0.134
Antidepressant use‡	2[a]	(10.00)	7[a]	(22.58)	21[b]	(70.00)	<**0.001**
Antipsychotic use	–	–	–	–	9	(30.00)	
Early AD (MMSE≥15)					14	(46.67)	
Moderate-to-severe AD (MMSE<15)					16	(53.33)	
CI	–	–	–	–	6	(20.00)	
M	–	–	–	–	5	(16.66)	
M+CI	–	–	–	–	19	(63.33)	
AD illness duration (years)					3.73±2.23		

SD: Standard deviation; AD: Alzheimer's disease; CAD: Coronary artery disease; CI: Cholinesterase inhibitors; DM: Diabetes mellitus; HT: Essential hypertension; M: Memantine; SML: Subjective memory loss group. †: Kruskal Wallis Test; ‡: Chi Square Test. Values not sharing identical superscript letters denote statistically significant differences, as revealed by post-hoc analyses. P<0.05 statistically significant (bold values).

RESULTS

Demographic Data and Scale Data

The baseline demographic and clinical characteristics are detailed in Table 1. The AD group was significantly older than both the SML and control groups. A significant difference in education levels was observed among the three groups. The average duration of the disease was calculated to be 3.73±2.23 years. Notably, 44 individuals in the study had hypertension (HT), 29 had diabetes mellitus (DM), and 18 had coronary artery disease (CAD). All individuals within the AD group were under medication. Patients with MMSE scores below 15 were classified as moderate-late stage, while those with scores of 15 and above were categorized as early stage in our analysis (17).

In the diagnostic comparison, MMSE scores were evaluated across the three groups. GDS and SMCQ scores were compared between the control and SML groups (Table 2).

Saliva Cortisol Levels

The morning cortisol level, evening cortisol levels, and variation in cortisol levels across all three groups were compared. The evening cortisol levels in the AD group were higher than those in the SML group (p<0.001). No significant correlation was found between the control group and the SML group or between the control group and the AD group (Table 3). To identify individuals in the early stages of AD, those with MMSE scores below 15 were excluded. Subsequently, an additional early-stage AD group consisting of 14 individuals was formed and compared with all SML patients and the control group. No significant differences were observed in cortisol levels.

The correlation between cortisol levels and age, the GDS, MMSE, and SMCQ scores was examined in the control group, the SML group, and the AD group. In the control group, a negative correlation was observed between MMSE scores and evening cortisol levels (Table 4).

NPI in the AD Group and Related Evaluations

In the AD group, correlations between age, morning cortisol level, evening cortisol level, MMSE scores, and NPI scores were examined. The total NPI score is presented in Table 2. Subitem scores of the NPI

Table 2: Scale data of groups

	Control (n=20)			SML (n=31)			AD (n=30)			p
	Mean	**SD**	**Median**	**Mean**	**SD**	**Median**	**Mean**	**SD**	**Median**	
MMSE*	28.55[a]	1.76	29.00	27.16[a]	1.86	28.00	14.60[b]	6.14	13.50	<**0.001**
GDS**	5.15	3.41	4.50	12.35	6.45	12.00				<**0.001**
SMCQ**	0.90	0.91	1.00	6.35	2.76	6.00				<**0.001**
NPI delirium							4.37	4.44	4.00	
NPI hallucination							2.57	4.01	0.00	
NPI agitation							8.20	3.77	9.00	
NPI aggression							3.90	4.36	1.50	
NPI dysphoria							7.30	3.50	9.00	
NPI anxiety							5.43	3.78	4.00	
NPI elation/euphoria							2.87	2.91	2.00	
NPI apathy/abulia							7.70	3.68	9.00	
NPI disinhibition							5.80	4.07	4.00	
NPI irritability/lability							6.20	4.41	7.50	
NPI abnormal motor behavior							5.83	4.04	4.00	
NPI sleep disorders							5.13	4.17	4.00	
NPI appetite/eating disorders							5.13	3.61	4.00	
NPI abnormal vocalizations							3.87	3.99	4.00	
NPI total							73.37	33.27	75.00	

SD: Standard deviation; AD: Alzheimer's disease; GDS: Geriatric Depression Scale; MMSE: Mini-mental state examination; NPI: Neuropsychiatric inventory; SMCQ: Subjective Memory Complaints Questionnaire; SML: Subjective memory loss. †: Kruskal Wallis Test; ‡: Mann Whitney U Test. Values not sharing identical superscript letters denote statistically significant differences, as revealed by post-hoc analyses. P<0.05 statistically significant (bold values).

Table 3: Cortisol levels of groups

	Control			SML			AD			p
	Mean	**SD**	**Median**	**Mean**	**SD**	**Median**	**Mean**	**SD**	**Median**	
Morning cortisol (nmol/L)	9.37	5.56	8.06	9.21	5.44	6.95	11.34	5.78	10.59	0.170
Evening cortisol (nmol/L)	3.58[a,b]	2.61	3.10	2.88[a]	1.68	2.25	5.70[b]	3.55	4.39	**0.001**
Cortisol difference	-5.79	6.03	-4.48	-6.33	5.10	-5.37	-5.64	6.07	-5.03	0.911

SD: Standard deviation; AD: Alzheimer's disease; SML: Subjective memory loss. Results of the Kruskal-Wallis Test. Values not sharing identical superscript letters denote statistically significant differences, as revealed by post-hoc analyses. P<0.05 statistically significant (bold values).

were provided as supportive data. Additionally, we analyzed the correlations between the items of the NPI and other parameters such as age, MMSE scores, and cortisol levels. A positive correlation was found between age and scores for NPI delirium and NPI sleep disorders. Similarly, a positive correlation was observed between the evening cortisol level and scores for NPI hallucinations, NPI agitation, NPI apathy/abulia, NPI irritability/lability, NPI sleep disorders, NPI appetite and eating disorders, and NPI total. A negative correlation was noted between MMSE scores and scores for NPI delirium, NPI hallucination, NPI agitation, NPI aggression, NPI irritability/lability, NPI abnormal motor behavior, NPI sleep disorders, and NPI total (Table 4).

DISCUSSION

In our study, the evening cortisol levels, as assessed from saliva samples in the AD group, were significantly higher than those in the SML group. However, the difference in evening cortisol levels between the control and AD groups did not reach statistical significance. We had anticipated a discernible difference in cortisol levels between the AD group and the control group, and we expected the cortisol levels in the SML group to be closer to those of the AD group due to the potential presence of preclinical AD in some patients within this group. However, the outcomes did not align with these predictions. Contrary to our initial hypothesis, the results did not support the notion that cortisol

Table 4: Correlation between diagnosis groups' cortisol levels and scale data

	Morning cortisol		Evening cortisol	
	r	p	r	p
Control				
Age	-0.122	0.610	0.021	0.929
GDS	-0.289	0.217	0.226	0.338
MMSE	0.121	0.611	-0.539	**0.014**
SMCQ	0.183	0.440	-0.008	0.972
SML				
Age	0.134	0.474	-0.049	0.793
GDS	-0.184	0.321	-0.125	0.502
MMSE	0.015	0.936	-0.196	0.291
SMCQ	0.010	0.958	0.123	0.511
Alzheimer				
Age	-0.336	0.070	0.160	0.399
MMSE	-0.374	**0.042**	-0.541	**0.002**
NPI deliriums	0.056	0.769	0.300	0.107
NPI hallucinations	0.149	0.433	0.586	**0.001**
NPI agitation	0.258	0.169	0.378	**0.039**
NPI aggression	0.000	0.999	0.320	0.085
NPI dysphoria	0.303	0.104	0.109	0.567
NPI anxiety	0.147	0.440	0.115	0.546
NPI elation/euphoria	-0.064	0.737	0.012	0.949
NPI apathy/abulia	0.082	0.668	0.398	**0.029**
NPI disinhibition	-0.143	0.451	0.110	0.564
NPI irritability/lability	0.073	0.700	0.372	**0.043**
NPIabnormal motor disorder	0.124	0.514	0.317	0.088
NPI sleep disorders	-0.074	0.698	0.414	**0.023**
NPI appetite/eating disorders	0.005	0.978	0.380	**0.039**
NPIabnormal vocalizations	-0.031	0.872	0.358	0.052
NPI total	0.108	0.570	0.512	**0.004**

GDS: Geriatric Depression Scale; MMSE: Mini-mental state examination; NPI: Neuropsychiatric inventory; SMCQ: Subjective Memory Complaints Questionnaire; SML: Subjective memory loss. r: Spearman Correlation Coefficient; p<0.05 statistically significant (bold values).

levels could be elevated in the early stages of AD, such as during the SML period, and thus serve as an early indicator. Nonetheless, the significant discrepancy in evening cortisol levels between the SML group and AD patients suggests a disturbance in the circadian rhythm of cortisol release. This finding highlights the importance of conducting a more comprehensive study on the circadian pattern of cortisol secretion, examining the entire daily cortisol rhythm rather than merely comparing morning and evening levels.

Dysregulation of the HPA axis can lead to a higher risk of elevated cortisol levels, dementia, and AD, especially in older individuals (5, 6, 18). Research indicates that tau hyperphosphorylation mediated by chronic stress and glucocorticoids, triggers neuropathological events in the pathology of AD (19). This phenomenon is associated with clinically observed lower morning cortisol levels, higher evening cortisol levels, a flattened diurnal cortisol rhythm, and an increased risk of cognitive decline in apolipoprotein E4 (APOE-E4) carriers without dementia compared to non-carriers (20). However, the utility of cortisol as a tool for diagnosing AD and differentiating related memory issues from Mild Cognitive Impairment (MCI) or SML in the early stages of the disease is not certain. In a review examining the relationship between high cortisol levels and the risk of dementia and AD, elevated cortisol levels

have been associated with cognitive decline and increased risk of AD in cognitively healthy individuals. Higher cerebrospinal fluid (CSF) cortisol levels have been observed in individuals with MCI due to AD and those with dementia, compared to cognitively healthy control groups. Elevated CSF cortisol may be linked to a more rapid cognitive decline in MCI due to AD (5, 18). A study investigating the predictive role of stress and cortisol on cognitive performance in older adults indicated that capillary cortisol levels predicted cognitive flexibility. It has been discussed that cortisol levels measured in plasma or saliva samples reflect concentrations within a very recent time window (21).

However, the literature presents conflicting results related to our hypothesis. For instance, a clinical study argued that increased CSF cortisol levels in AD had no prognostic value for AD and suggested that HPA axis dysfunction is a result, rather than a cause, of the progression of AD (22). Another study found no relationship between salivary cortisol levels and the conversion rate to dementia in the MCI group (23). Moreover, some preclinical studies support the argument that the increase in glucocorticoid levels is a consequence of amyloid toxicity. For example, a study conducted with an AD rat model proposed that high glucocorticoid levels primarily resulted from amyloid toxicity (24). To explore this further, patients in the AD group with a MMSE score less than 15 were excluded, and an additional early-stage AD group was formed. Saliva cortisol levels were then compared again among the early-stage AD, SML, and control groups. The evening cortisol levels of the early-stage AD group—those with MMSE scores above 15—were higher than those in the control group. However, this difference was not statistically significant. In contrast, the significantly higher evening cortisol levels in the AD group, compared to the SML group, support the hypothesis of HPA axis dysfunction in AD. Furthermore, a study measuring cortisol concentrations in cerebrospinal fluid revealed that these levels were higher in subjects with AD than in both control and MCI subjects. There was no significant increase in cortisol levels in MCI subjects compared to the control group, suggesting that an increase in cerebrospinal fluid cortisol is not an early event in AD development (25). This evidence supports the argument that high cortisol levels in AD may manifest at more advanced stages of the disease rather than in its early phases. Additionally, the negative correlation between MMSE scores and cortisol levels (both morning and evening) indicates that the increase in cortisol levels in AD patients is associated with the severity of the disease.

When examining the clinical characteristics of the SML group in more detail, it was determined that their GDS scores were higher than those of the control group. A study investigating the relationship between depression and cortisol levels in the aged population found that older adults with depressive symptoms exhibited characteristic cortisol circadian patterns, with cortisol levels at 22:00 higher than those in healthy young and older controls (26). However, in the present study, despite high depression scores in the SML group, no significant difference in cortisol levels was observed when compared with the control group. Furthermore, another study reported that cortisol samples collected between 02:00 and 07:30, and between 20:00 and 01:30 from older participants, were higher than those from younger participants (27). The absence of a significant difference in cortisol levels between the control and the SML groups in the current study could be due to the fact that evening cortisol levels were measured no later than 16:00, and night-time cortisol levels were not evaluated. Another consideration may be the statistically smaller number of participants in the control group compared to the SML group. O'Brien et al. (28) discovered that cortisol levels in an aged population with depression differed from those in the control group, yet no correlation was identified between cortisol levels and either cognitive performance or a reduction in hippocampal volume. The mechanisms behind depression-related hypercortisolism and hypocortisolism observed in AD may differ (29).

The association between AD, particularly in behavioral symptoms, and disruptions in the HPA axis, has been previously discussed (30), but few studies have analyzed these symptoms using the NPI and compared this data with that of an aged population with amnesia complaints and a healthy aged population without memory complaints. In a study exploring this issue, higher CSF cortisol was linked with greater NPI severity scores at baseline, even after adjusting for covariates, including AD pathology status. Higher CSF cortisol levels were associated with elevated baseline scores of depression/dysphoria, anxiety, and apathy/indifference. CSF cortisol might serve as a useful biomarker for predicting the progression of neuropsychiatric symptoms over time. Furthermore, interventions targeting the HPA axis could be effective in mitigating the severity of these symptoms and preventing their development or exacerbation (31). In the current study, a significant positive correlation was observed in the AD patient group between evening cortisol levels and the NPI sub-categories of hallucinations, agitation, apathy/abulia, irritability/

lability, sleep disorders, and appetite and eating disorders, as well as the NPI total score. It is believed that the glucocorticoid cascade hypothesis, which posits a role for elevated cortisol levels in behavioral changes, is the primary mechanism underlying for behavioral alterations as Alzheimer's disease progresses. (32, 33). A study with AD patients indicated that plasma cortisol levels reflect the behavioral disorders present in AD rather than the severity of depression (34), suggesting that elevated cortisol levels in AD patients are more closely associated with AD itself than with concurrent depression. Although a link between behavioral symptoms in AD patients and high cortisol levels has been noted, a detailed investigation into which specific behavioral symptoms correlate with this relationship is lacking. The sundown syndrome, prevalent among individuals with dementia, is characterized by the emergence or worsening of neuropsychiatric symptoms such as agitation, confusion, anxiety, and aggressiveness in late afternoon, evening, or night (35). One study showed that after aerobic exercise, dementia patients experienced reduced cortisol levels and a 50% decrease in NPI scores. The changes in NPI were significantly correlated with reductions in cortisol levels, indicating that exercise-induced cortisol reduction may point to a dysregulation in the HPA axis underlying the sundown syndrome (36).

The present study demonstrated a significant positive correlation between evening cortisol levels and symptoms such as apathy, sleep disorders, and eating disorders, which are behavioral indicators of AD. This finding leads to the conclusion that elevated evening cortisol levels are associated with a variety of behavioral symptoms in AD, extending beyond the sundown phenomenon. The observed relationship between behavioral symptoms and cortisol levels may offer a new perspective on treatment approaches, considering the pathogenesis of behavioral symptoms, which are exceedingly difficult to treat, especially in AD. In this context, literature on molecules like carbenoxolone and mifepristone, which affect the HPA axis in the treatment of AD's behavioral symptoms, is noteworthy (37).

However, several limitations in this study need to be discussed. Firstly, the control group's statistically younger average age compared to the patient group might have influenced the results. Secondly, the higher prevalence of depression in the SML group could have impacted cortisol levels. Identifying SML patients without concurrent depression poses a challenge, particularly in older populations where depression is a significant risk factor for SML. Despite the SML group exhibiting higher depression scores compared to the control group in our study, no significant difference in cortisol levels was observed. Given the literature that suggests a link between increased cortisol levels in the evening and depression, our study's exclusion of cortisol measurements after 16:00 might have impacted our ability to replicate these findings. Since the participants in this study were outpatients, obtaining salivary cortisol levels four times a day was logistically challenging. Consequently, we were only able to measure cortisol levels twice a day. Our study's limited sample size, along with the smaller number of participants in the control group compared to the SML group, could explain the absence of a significant statistical difference in cortisol levels between these groups. Future studies, possibly longitudinal ones that track changes in salivary cortisol levels over time in individuals with SML progressing to AD, could provide valuable insights into the temporal relationship between cortisol levels and the progression of AD. It has been demonstrated in other research that chronic antidepressant use reduces HPA axis activity (38). In our study, the use of antidepressants in the AD group may have influenced cortisol levels, resulting in lower observed cortisol levels. This aspect can be considered among the limitations of our study.

CONCLUSION

In conclusion, our study found that cortisol levels were correlated with the severity of the disease rather than serving as an early biomarker for AD. Specifically, increased evening saliva cortisol levels in the AD patient group were associated with numerous AD-specific behavioral disorders, as determined by the NPI. It is believed that the increase in cortisol levels observed in the advanced stages of AD results from dysregulation of the HPA axis. Based on our current understanding and the findings of this study, we recommend further research into the circadian patterns of cortisol secretion in AD patients. This could involve the use of wearable technology for continuous monitoring. Additionally, integrating saliva cortisol level analysis with other emerging biomarkers and conducting longitudinal studies to track cortisol levels over time in individuals at risk for AD could greatly aid in early detection efforts. Exploring the mechanistic links between cortisol and AD pathology could also reveal new therapeutic targets. Adopting this multifaceted approach would not only deepen our understanding of cortisol's role in AD but also enhance the potential of saliva cortisol as a biomarker for the early detection and monitoring of Alzheimer's Disease progression.

Contribution Categories		Author Initials
Category 1	Concept/Design	B.O.B., R.B.
	Data acquisition	O.C.M., R.B.
	Data analysis/Interpretation	B.O.B., R.B.
Category 2	Drafting manuscript	R.B., O.C.M.
	Critical revision of manuscript	B.O.B.
Category 3	Final approval and accountability	B.O.B., R.B., O.C.M.
Other	Technical or material support	O.C.M.
	Supervision	B.O.B.

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REFERENCES

1. Kumar A, Sidhu J, Goyal A, Tsao JW, Doerr C. Alzheimer Disease (Nursing). In: StatPearls. Treasure Island (FL): StatPearls Publishing, 2023.
2. Jessen F, Wiese B, Bachmann C, Eifflaender-Gorfer S, Haller F, Kölsch H, et al. Prediction of dementia by subjective memory impairment effects of severity and temporal association with cognitive impairment. Arch Gen Psychiatry 2010; 67:414-422.
3. Jessen F, Amariglio RE, Van Boxtel M, Breteler M, Ceccaldi M, Chételat G, et al. A conceptual framework for research on subjective cognitive decline in preclinical Alzheimer's disease. Alzheimers Dement 2014; 10:844-852. [CrossRef]
4. Ahmad MH, Fatima M, Mondal AC. Role of hypothalamic-pituitary-adrenal axis, hypothalamic-pituitary-gonadal axis and insulin signaling in the pathophysiology of Alzheimer's disease. Neuropsychobiology 2019; 77:197-205. [CrossRef]
5. Ouanes S, Popp J. High cortisol and the risk of dementia and alzheimer's disease: A review of the literature. Front Aging Neurosci 2019; 11:43. [CrossRef]
6. Popp J, Wolfsgruber S, Heuser I, Peters O, Hüll M, Schröder J, et al. Cerebrospinal fluid cortisol and clinical disease progression in MCI and dementia of Alzheimer's type. Neurobiol Aging 2015; 36:601-607. [CrossRef]
7. Caruso A, Nicoletti F, Mango D, Saidi A, Orlando R, Scaccianoce S. Stress as risk factor for Alzheimer's disease. Pharmacol Res 2018; 132:130-134. [CrossRef]
8. Gungen C, Ertan T, Eker E, Yasar R, Engin F. Reliability and validity of the standardized mini mental state examination in the diagnosis of mild dementia in Turkish population. Turk Psikiyatri Derg 2002; 13:273-281. [Turkish]
9. Youn JC, Kim KW, Lee DY, Jhoo JH, Lee SB, Park JH, et al. Development of the subjective memory complaints questionnaire. Dement Geriatr Cogn Disord 2009; 27:310-317. [CrossRef]
10. Duman B, Ozel Kizil ET, Baran Z, Kirici S, Turan E. Discrepancy between subjective memory complaints and objective memory deficits in elderly patients with depression compared to mild cognitive impairment. Turk Psikiyatri Derg 2016; 27:1-7. [Turkish]
11. Ozel Kizil ET, Duman B, Altintas O, Kirici S, Bastug G, Baran Z, et al. Investigation of the psychometric properties of the Turkish form of subjective memory complaints questionnaire. Turk J Geriatrics 2013; 16:150-154. [Turkish]
12. Yesavage JA, Brink TL, Rose TL, Lum O, Huang V, Adey M, et al. Development and validation of a geriatric depression screening scale: A preliminary report. J Psychiatr Res 1982-1983; 17:37-49.
13. Sagduyu A. Yaşlılar için depresyon ölçeği: Hamilton depresyon ölçeği ile karşılaştırmalı güvenilirlik ve geçerlilik çalışması. Turk Psikiyatri Derg1997; 8:3-8. [Turkish]
14. Akca-Kalem S, Hanagasi H, Cummings JL, Gurvit H. Validation study of the Turkish translation of the Neuropsychiatric Inventory (NPI). 21st International Conference of Alzheimer's Disease International, Sept 28-Oct 1 2005, Istanbul. Abstract Book P47, 58. [Turkish]
15. Gurvit IH, Baran B. Scales in demantia and cognitive disorders. Arch Neuropsychiatry 2007; 44:58-65. [Turkish]
16. Kudielka BM, Gierens A, Hellhammer DH, Wust S, Schlotz W. Salivary cortisol in ambulatory assessment-some dos, some don'ts, and some open questions. Psychosom Med 2012; 74:418-431. [CrossRef]
17. Folstein MF, Folstein SE, McHugh PR. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res 1975; 12:189-198. [CrossRef]
18. Ennis GE, An Y, Resnick SM, Ferrucci L, O'Brien RJ, Moffat SD. Long-term cortisol measures predict Alzheimer disease risk. Neurology 2017; 88:371-378. [CrossRef]
19. Sotiropoulos I, Sousa N. Tau as the converging protein between chronic stress and Alzheimer's disease synaptic pathology. Neurodegener Dis 2016; 16:22-25. [CrossRef]
20. Gerritsen L, Comijs HC, Deeg DJH, Penninx BWJH, Geerlings MI. Salivary cortisol, APOE-ε4 allele and cognitive decline in a prospective study of older persons. Neurobiol Aging 2011; 32:1615-1625. [CrossRef]
21. Saez-Sanz N, Peralta-Ramirez I, Gonzalez-Perez R, Vazquez-Justo E, Caracuel A. Resilience, stress, and cortisol predict cognitive performance in older adults. Healthcare (Basel) 2023; 11:1072.
22. Gil-Bea FJ, Aisa B, Solomon A, Solas M, Del Carmen Mugueta M, Winblad B, et al. HPA axis dysregulation associated to apolipoprotein E4 genotype in Alzheimer's disease. J Alzheimers Dis 2010; 22:829-838. [CrossRef]
23. Sussams R, Schlotz W, Clough Z, Amin J, Simpson S, Abbott A, et al. Psychological stress, cognitive decline and the development of dementia in amnestic mild cognitive impairment. Sci Rep 2020; 10:3618. [CrossRef]

24. Brureau A, Zussy C, Delair B, Ogier C, Ixart G, Maurice T, et al. Deregulation of hypothalamic-pituitary-adrenal axis functions in an Alzheimer's disease rat model. Neurobiol Aging 2013; 34:1426-1439. [CrossRef]

25. Popp J, Schaper K, Kölsch H, Cvetanovska G, Rommel F, Klingmüller D, et al. CSF cortisol in Alzheimer's disease and mild cognitive impairment. Neurobiol Aging 2009; 30:498-500.

26. Balardin JB, Vedana G, Luz C, Bromberg E. Subjective mild depressive symptoms are associated with abnormal diurnal cycle of salivary cortisol in older adults. J Geriatr Psychiatry Neurol 2011; 24:19-22. [CrossRef]

27. Deuschle M, Gotthardt U, Schweiger U, Weber B, Korner A, Schmider J, et al. With aging in humans the activity of the hypothalamus-pituitary-adrenal system increases and its diurnal amplitude flattens. Life Sci 1997; 61:2239-2246. [CrossRef]

28. O'Brien JT, Lloyd A, McKeith I, Gholkar A, Ferrier N. A longitudinal study of hippocampal volume, cortisol levels, and cognition in older depressed subjects. Am J Psychiatry 2004; 161:2081-2090. [CrossRef]

29. Hoogendijk WJG, Meynen G, Endert E, Hofman MA, Swaab DF. Increased cerebrospinal fluid cortisol level in Alzheimer's disease is not related to depression. Neurobiol Aging 2006; 27:780.e1-780.e2. [CrossRef]

30. Kirby M, Lawlor BA. Biologic markers and neurochemical correlates of agitation and psychosis in dementia. J Geriatr Psychiatry Neurol 1995; 8(Suppl 1):S2-S7. [CrossRef]

31. Ouanes S, Rabl M, Clark C, Kirschbaum C, Popp J. Persisting neuropsychiatric symptoms, Alzheimer's disease, and cerebrospinal fluid cortisol and dehydroepiandrosterone sulfate. Alzheimers Res Ther 2022; 14:190. [CrossRef]

32. Esmaeili MH, Bahari B, Salari AA. ATP-sensitive potassium-channel inhibitor glibenclamide attenuates HPA axis hyperactivity, depression- and anxiety-related symptoms in a rat model of Alzheimer's disease. Brain Res Bull 2018; 137:265-276.

33. Canet G, Chevallier N, Zussy C, Desrumaux C, Givalois L. Central role of glucocorticoid receptors in Alzheimer's disease and depression. Front Neurosci 2018; 12:739. [CrossRef]

34. Zvěřová M, Fišar Z, Jirák R, Kitzlerová E, Hroudová J, Raboch J. Plasma cortisol in Alzheimer's disease with or without depressive symptoms. Med Sci Monit 2013; 19:681-689. [CrossRef]

35. Khachiyants N, Trinkle D, Son SJ, Kim KY. Sundown syndrome in persons with dementia: An update. Psychiatry Investig 2011; 8:275-287. [CrossRef]

36. Venturelli M, Sollima A, Cè E, Limonta E, Bisconti AV, Brasioli A, et al. Effectiveness of exercise- and cognitive-based treatments on salivary cortisol levels and sundowning syndrome symptoms in patients with Alzheimer's disease. J Alzheimers Dis 2016; 53:1631-1640. [CrossRef]

37. Pomara N, Greenberg WM, Branford MD, Doraiswamy PM. Therapeutic implications of HPA axis abnormalities in Alzheimer's disease: review and update. Psychopharmacol Bull 2003; 37:120-134.

38. Manthey L, Leeds C, Giltay EJ, van Veen T, Vreeburg SA, Penninx BW, et al. Antidepressant use and salivary cortisol in depressive and anxiety disorders. Eur Neuropsychopharmacol 2011; 21:691-699. [CrossRef]