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Serotonin and Antioxidant Levels as Biomarkers for Type 2 Diabetes Mellitus and Alzheimer's Disease: A Comparative Analysis

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Abstract
This study investigated the relationship between Type 2 diabetes mellitus (DM) or (T2DM) and Alzheimer's disease (AD) by examining specific biological markers. Participants were divided into three groups: a group with DM, a group with AD, and a healthy control group. All participants received treatment at designated healthcare facilities between July 2023 and February 2024. Both patient groups (DM and AD) and the control group were age-matched and balanced for gender. Within the T2DM and AD patient groups, further classification was done based on age: Patients younger than 60 years old (n=40) Patients 60 years old or older (n=40). Established laboratory techniques (ELISA and spectrophotometry) were used to measure the concentrations of relevant biomarkers, including serotonin, glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), malondialdehyde (MDA), vitamin C, vitamin E, and vitamin D. Serotonin levels were greater in DM patients (451.1 ± 30.7 ng/ml) compared to the control group (311.6 ± 53.8 ng/ml), and lower in AD (161.1 ± 32.7 ng/ml). GSH levels in AD and DM patients were 1.523±0.017 µmol/l and 1.923±0.019 µmol/l, respectively, lower than in the control group (3.277±0.0126 µmol/l). Statistical analysis revealed significant differences (p-value approximately 0.05) in the average biomarker levels between the AD, DM, and control groups. Furthermore, comparisons within the patient groups based on age and sex also showed significant differences (p-value less than 0.001).

Keywords: Diabetes mellitus, Alzheimer's disease, serotonin, vitamin D

1. Introduction

Alzheimer's disease (AD) was first identified in patients exhibiting memory loss, disorientation, and hallucinations [1]. The presenile form of AD, affecting individuals under 60 years old, makes up about 5% of all cases and is thought to be largely determined by genetics [2]. Characterized by progressive neurodegeneration, AD leads to cognitive decline, memory impairment, speech difficulties, and personality changes [3]. Mounting evidence highlights the critical role of neuroinflammation and oxidative stress in AD pathogenesis. These factors contribute to the formation of nitric oxide (NO) [4]. Diabetes mellitus (DM) is a chronic condition characterized by high blood sugar levels (hyperglycemia) resulting from impaired insulin secretion or its action

in target tissues [5]. Interestingly, AD shares some etiological factors with DM. Both are degenerative diseases involving neuronal and β-cell loss [7]. Brain structures like the frontal cortex and hippocampus contain numerous terminals from 5-HT neurons, which play a vital role in various functions [8]. Glutathione, another key player, is involved in protein and DNA synthesis, cysteine transport, and protecting cells from oxidative stress [9]. Vitamin E may play a role in preventing or mitigating the detrimental effects of β-amyloid on neurons [10]. Several sources of oxidative stress are implicated in the development of both AD and DM. Notably, DM acts as a potent catalyst for the generation of reactive oxygen species (ROS) [11]. Vitamin D is crucial for mineral metabolism, particularly calcium [12]. Recent studies suggest a link between low serum vitamin D levels and cognitive impairment or dementia [13]. Interestingly, research shows an increased risk of cognitive decline only in participants with lower levels of 25(OH)D, a specific form of vitamin D [14]. We used vitamin D in the study because vitamin D may promote the clearance of amyloid plaques by stimulating macrophages to engulf them (phagocytosis), thereby reducing amyloid-induced cytotoxicity, apoptosis, and inflammation [15]. Furthermore, vitamin D may play a role in mitigating the development of insulin resistance in DM [16]. A study demonstrated that vitamin D reduces insulin resistance and the risk of DM by suppressing inflammatory responses [17]. Supplementation with vitamin D may alleviate symptoms of heart problems in certain type 2 diabetic patients. [42]. Insulin resistance and oxidative stress are major contributing factors in both DM and cognitive dysfunction associated with vitamin D deficiency [18]. Oxidative stress is a critical determinant in the development of AD as well as insulin resistance [19]. AD is characterized by a gradual onset and slow progression. It is a sporadic disorder with episodic memory loss as a hallmark symptom [20]. The accumulation of oxidative stress, potentially occurring randomly at any time, is believed to contribute to the onset of AD [21]. ROS act as messengers in the differentiation of various cell types [22]. However, excessive ROS can harm living cells by damaging carbohydrates, DNA, and lipids [23]. Several antioxidants are present in the body to counteract the damaging effects of ROS. These antioxidants include enzymes like SOD (superoxide dismutase) and CAT (catalase), as well as non-enzymatic molecules like glutathione (GSH). Additionally, dietary sources of antioxidants such as vitamins C, E, and D contribute to the body's overall antioxidant defense system [24, 25]. Serotonin concentration has emerged as a topic of significant interest in the context of various diseases, including chronic kidney disease. Extensive research has established a strong correlation between serum serotonin levels and the risk of developing certain illnesses. [51]. The purpose of this study is to look at the relationship between serotonin and antioxidant levels, as well as the development and progression of type 2 diabetic mellitus (T2DM) and Alzheimer's disease. The study will compare these levels in people with type 2 diabetes and Alzheimer's disease to healthy controls of various ages.

2. Subjects and Methods

This study investigated the relationship between various biological factors and both diabetes mellitus (T2DM) and Alzheimer's disease (AD).

Participants:

The study recruited a total of 240 participants, divided into three main groups:

- T2DM patients: This group consisted of 80 individuals diagnosed with type 2 diabetes mellitus. Their ages ranged from 50 to 80 years old with an average age to be specified.
- Alzheimer's patients: This group also consisted of 80 individuals diagnosed with Alzheimer's disease. Their ages also ranged from 50 to 80 years old with an average age to be specified.
- Control group: This group comprised 80 healthy individuals with ages ranging from 50 to 80 years old. Again, specify the average age for this group.

Subgroup Classification:

Within the T2DM and AD patient groups, further classification was done based on age:

- Patients younger than 60 years old (n=40)
- Patients 60 years old or older (n=40)

Additionally, both patient groups were divided by gender:

- Male patients (n=40)
- Female patients (n=40)

Patient Recruitment:

Patients were recruited from Alseder and Ibn Rushd hospitals in Najaf and Baghdad between July 2023 and February 2024.

Biochemical Assays:

The following biochemical assays were performed to measure various biological factors in the blood serum of participants:

- Serotonin: Serum serotonin concentrations were determined using commercially available ELISA kits (E-EL-H2187) obtained from Elabscience Biotechnology Co. Ltd.
- GSH (Glutathione): Serum GSH levels were measured using Elaman's reagent (DTNB) as described by [26]. Briefly, samples, blanks, and standards were prepared according to the protocol. The absorbance was read at 412 nm using a spectrophotometer.
- SOD (Superoxide Dismutase) Activity: SOD activity was determined using the nitroblue tetrazolium (NBT) method as described by [27]. The reaction mixture, sodium cyanide, serum, and working buffer solution were added to separate cuvettes for samples, blanks, and standards. The absorbance was measured at 560 nm.
- Catalase Activity: Serum catalase activity was measured using a method described by [28]. Briefly, diluted serum was prepared and added to cuvettes with either hydrogen peroxide or phosphate buffer solution. The change in absorbance at 240 nm over time was used to calculate catalase activity.
- MDA (Malondialdehyde): Serum malondialdehyde levels were determined using a method involving trichloroacetic acid (TCA), thiobarbituric acid (TBA), and measurement of absorbance at 532 nm.
- Vitamin C: Serum vitamin C concentrations were measured using ELISA kits (LS-F-25060) obtained from LifeSpan Biosciences Co.
- Vitamin E: Serum vitamin E concentrations were measured using ELISA kits (LS-F-4970) obtained from LifeSpan Biosciences Co.
- Vitamin D: Serum vitamin D concentrations were measured using ELISA kits (AB213966) obtained from LifeSpan Biosciences Co.

Statistical analysis

SPSS version 17 was used for the statistical analysis, and the t-test, ANOVA test, mean, and standard error were utilized to compare the data. A p value of less than 0.05 was deemed to be statistically significant. Person's correlation significant was used to assess the correlation between all variables.

3. Results

The goal of this study was to assess the levels of various variables (Serotonin, GSH, SOD, CAT, MDA, vitamin C, vitamin E, and vitamin D) in patients with diabetes mellitus, Alzheimer's patients, and the control group. The concentrations of these variables were determined using the assay (ELISA) technique and a spectrophotometer instrument. The concentrations of these variables in patients and controls are presented in Table 1. Serotonin levels were greater in DM patients (451.1 ± 30.7 ng/ml) compared to the control group (311.6 ± 53.8 ng/ml), and lower in

AD (161.1 ± 32.7 ng/ml). GSH levels in AD and DM patients were 1.523±0.017 µmol/l and 1.923±0.019 µmol/l, respectively, lower than in the control group (3.277±0.0126 µmol/l). AD and DM had higher mean SOD levels (1.543 ± 0.027 and 1.317 ± 0.032 U/ml, respectively) compared to the control group (0.983 ± 0.033 U/ml). The CAT value in AD and DM patients (1.72±0.765 U/l, 1.22±0.776 U/l) was lower than the control group (2.41±0.777 U/l). However, MDA levels in AD and DM patients (14.12 ± 0.882 µmol/l and 11.317 ± 0.733 µmol/l) were greater than in the control group (8.214 ± 0.643 µmol/l). Vitamin C levels were lower in AD and DM patients (2.66 ± 0.291 µg/ml and 4.97 ± 0.400 µg/ml, respectively) than in the control group (6.90 ± 0.425 µg/ml). Vitamin E levels were lower in AD and DM patients (5.83 ± 0.770 µg/ml, 8.93 ± 0.634 µg/ml) than in the control group (19.33 ± 0.888 µg/ml). Vitamin D levels in AD and DM patients were lower (10.83±0.484 µg/ml, 12.93 ±0.634 µg/ml) than in the control group (19.12±0.668 µg/ml). All factors were significantly different between AD patients, DM patients, and the control group ($p < 0.05$).

Table 1. Mean (± SEM) of Serotonin, Antioxidant Levels, and Vitamin Status in Diabetes Mellitus, Alzheimer's Disease, and Control Groups

Variables	Paired Differences **N=80**				P Value
	Mean	±SEM	95% Confidence Interval of the Difference		
			Upper	Lower	
Serotonin in control (ng/ml)	311.6	53.8	323.8	287.3	< 0.05
Serotonin in DM Patinas(ng/ml)	451.1	30.7	482.7	425.6	
Serotonin in AD patients (ng/ml)	161.1	32.7	183.7	143.6	
GSH in control (µmol/l)	3.277	0.0126	3.311	3.164	
GSH in DM Patinas (µmol/l)	1.923	0.019	2.378	1.511	
GSH in AD patients (µmol/l)	1.523	0.017	1.678	1.424	
SOD in control (U/ml)	0.983	0.033	1.117	0.994	
SOD in DM Patinas(U/ml)	1.317	0.032	1.373	1.271	
SOD in AD patients (U/ml)	1.543	0.027	1.673	1.480	
CAT in control (U/l)	2.41	0.777	2.78	2.33	
CAT in DM Patinas (U/l)	1.22	0.776	1.34	1.18	
CAT in AD patients (U/l)	1.72	0.765	1.91	1.58	
MDA in control (µmol/l)	8.214	0.643	9.43	7.14	
MDA in DM Patinas (µmol/l)	11.317	0.733	13.33	9.54	
MDA in AD patients (µmol/l)	14.12	0.882	15.45	12.88	
Vitamin C in	6.90	0.425	7.32	6.31	

control(µg/ml)					
Vitamin C in DM Patinas(µg/ml)	4.97	0.400	5.18	4.57	
Vitamin C in AD patients (µg/ml)	2.66	0.291	3.28	2.11	
Vitamin E in control(µg/ml)	19.33	0.888	20.36	19.05	
Vitamin E in DM Patinas(µg/ml)	8.93	0.634	9.34	8.57	
Vitamin E in AD patients (µg/ml)	5.83	0.770	6.44	4.58	
Vitamin D in control(ng/ml)	19.12	0.668	20.14	18.05	
Vitamin D in DM Patinas(µg/ml)	12.93	0.634	13.34	12.57	
Vitamin D in AD patients (µg/ml)	10.83	0.484	11.44	9.58	

In the table (2) and table (3) classified the alzheimers patients and diabetes mellitus patients into two groups according to age, Patient's age less than 60 years (n=40) and patient older than 60 years (n=40)). The mean value of serotonin was shown in DM patients less than 60 years (418.1± 29.6 ng/ml) lower than more 60 years (486.3± 33.0 ng/ml), in AD patients the mean value of serotonin was shown in patients less than 60 years (174.2± 30.6 ng/ml) higher than more 60 years (149.3± 33.0 ng/ml). The mean value of GSH was shown in DM patients less than 60 years (2.088± 0.015µmol/l) higher than more 60 years (1.776± 0.026µmol/l), in AD patients the mean value of GSH was shown in patients less than 60 years (1.680± 0.017µmol/l) higher than more 60 years (1.374± 0.030µmol/l).Regarding to SOD the mean value was shown in DM patients less than 60 years (1.211± 0.042U/ml) lower than more 60 years (1.409± 0.029 U/ml), in AD patients the mean value of SOD was shown in patients less than 60 years (1.313± 0.037 U/ml) lower than more 60 years (1.688± 0.041 U/ml). The value of CAT in DM patients less than 60 years (1.41± 0.784U/l) higher than more 60 years (1.01± 0.786 U/l), in AD patients the mean value of CAT was shown in patients less than 60 years (1.94± 0.896 U/l) higher than more 60 years (1.50± 0.933 U/l).But in the value of MDA in DM patients less than 60 years (10.444± 0.396 µmol/l) lower than more 60 years (12.292± 0.431 µmol/l), in AD patients the mean value of MDA was shown in patients less than 60 years (12.90± 0.345 µmol/l) lower than more 60 years (15.34± 0.313 µmol/l). In the value of vitamin C was detected in DM patients less than 60 years (7.01±.662 µg/ml) higher than more 60 years (5.93± 0.654µg/ml), in AD patients the mean value of vitamin C was shown in patients less than 60 years (2.90± 0.642µg/ml) higher than more 60 years (2.11± 0.623µg/ml) . In the value of vitamin E was detected in DM patients less than 60 years (9.77±0.432µg/ml) higher than more 60 years (8.03± 0.796µg/ml), in AD patients the mean value of vitamin E was shown in patients less than 60 years (6.12± 0.498µg/ml) higher than more 60 years (4.44± 0.691µg/ml). In the value of vitamin D was detected in DM patients less than 60 years (14.03±0.876µg/ml) higher than more 60 years (11.71± 0.503µg/ml), in AD patients the mean value of vitamin D was shown in patients less than 60 years (12.01± 0.833µg/ml) higher than more 60 years (9.97± 0.498µg/ml). There all above variables was a significant difference between in AD patients, DM patients in different age groups ($p<0.001$).

Table 2. Age-Stratified Mean Concentrations (older than 60y) of Serotonin, Antioxidants, and Vitamins in Diabetes Mellitus and Alzheimer's Disease

Age	Variables	Mean N=40	±SEM	P value
More than 60y	Serotonin in DM Patinas	486.3	33.0	< 0.001
	Serotonin in AD Patinas	149.3	31.7	
	GSH in DM Patinas	1.776	0.026	
	GSH in AD Patinas	1.374	0.030	
	SOD in DM Patinas	1.409	0.029	
	SOD in AD Patinas	1.688	0.041	
	CAT in DM Patinas	1.01	0.786	
	CAT in AD Patinas	1.50	0.933	
	MDA in DM Patinas	12.292	0.431	
	MDA in AD Patinas	15.34	0.313	
	vitamin C in DM Patinas	5.93	0.654	
	vitamin C in AD Patinas	2.11	0.623	
	vitamin E in DM Patinas	8.03	0.796	
	vitamin E in AD Patinas	4.44	0.691	
	vitamin D in DM Patinas	11.71	0.503	
	vitamin D in AD Patinas	9.97	0.498	

Table 3. Age-Stratified Mean Concentrations (Less than 60y) of Serotonin, Antioxidants, and Vitamins in Diabetes Mellitus and Alzheimer's Disease

Age	Variables	Mean N=40	±SEM	P value
Less than 60 y	Serotonin in DM Patinas	418.1	29.6	< 0.001
	Serotonin in AD Patinas	174.2	30.6	
	GSH in DM Patinas	2.088	0.015	
	GSH in AD Patinas	1.680	0.017	
	SOD in DM Patinas	1.211	0.042	
	SOD in AD Patinas	1.313	0.037	
	CAT in DM Patinas	1.41	0.784	
	CAT in AD Patinas	1.94	0.896	
	MDA in DM Patinas	10.444	0.396	
	MDA in AD Patinas	12.90	0.345	
	vitamin C in DM Patinas	7.01	0.662	
	vitamin C in AD Patinas	2.90	0.642	
	vitamin E in DM Patinas	9.77	0.432	
	vitamin E in AD Patinas	6.12	0.498	
	vitamin D in DM Patinas	14.03	0.876	
	vitamin D in AD Patinas	12.01	0.833	

In the table (4) and table (5) classified the Alzheimer's patients and diabetes mellitus patients into two groups according to gender, men (n=40) and women (n=40)). The mean value of serotonin was shown in DM patients in men (403.2± 25.4 ng/ml) lower than in women (499.5± 31.2 ng/ml), in AD patients the mean value of serotonin was shown in patients in men (114.3± 32.5 ng/ml) lower than in women (157.8± 33.7 ng/ml). The mean value of GSH was shown in DM patients in men (1.866± 0.017µmol/l) lower than in women (2.556± 0.025µmol/l), in AD patients the mean

value of GSH was shown in patients in men (1.888± 0.017µmol/l) lower than in women (2.464± 0.030µmol/l).Regarding to SOD the mean value was shown in DM patients in men (1.223± 0.052U/ml) lower than in women (1.608± 0.043 U/ml), in AD patients the mean value of SOD was shown in patients in men (1.423± 0.043 U/ml) lower than in women (1.905± 0.052 U/ml). The value of CAT was detected in DM patients in men (1.12± 0.684U/l) lower than in women (1.502± 0.586 U/l), in AD patients the mean value of CAT was shown in patients in men (1.24± 0.476 U/l) lower than in women (1.660± 0.565 U/l).But in the value of MDA was detected in DM patients in men (12.434± 0.245 µmol/l) higher than in women (9.898± 0.431 µmol/l), in AD patients the mean value of MDA was shown in patients in men (14.545± 0.645 µmol/l) higher than in women (11.999± 0.313 µmol/l). In the value of vitamin C was detected in DM patients in men (3.998±0.872 µg/ml) lower than in women (6.767± 0.654µg/ml), in AD patients the mean value of vitamin C was shown in patients in men (2.55± 0.397µg/ml) lower than in women (3.575± 0.754µg/ml). In the value of vitamin E was detected in DM patients in men (7.38±0.562µg/ml) lower than in women (9.44± 0.282µg/ml), in AD patients the mean value of vitamin E was shown in patients in men (4.88± 0.644µg/ml) lower than in women (7.37± 0.551µg/ml). In the value of vitamin D was detected in DM patients in men (11.99±0.347µg/ml) lower than in women (15.07± 0.433µg/ml), in AD patients the mean value of vitamin D was shown in patients in men (10.001± 0.992µg/ml) lower than in women (12.989± 0.666µg/ml). There all above variables was a significant difference between in AD patients, DM patients in different sex groups ($p<0.001$).

Table 4. Sex-Disaggregated Mean Concentrations (Females) of Serotonin, Antioxidants, and Vitamins in Diabetes Mellitus and Alzheimer's Disease.

Gender	Variables	Mean N=40	±SEM	P value
Females	Serotonin in DM Patinas	499.5	31.2	< 0.001
	Serotonin in AD Patinas	157.8	33.7	
	GSH in DM Patinas	2.556	0.025	
	GSH in AD Patinas	2.464	0.030	
	SOD in DM Patinas	1.608	0.043	
	SOD in AD Patinas	1.905	0.052	
	CAT in DM Patinas	1.502	0.586	
	CAT in AD Patinas	1.660	0.565	
	MDA in DM Patinas	9.898	0.431	
	MDA in AD Patinas	11.999	0.313	
	vitamin C in DM Patinas	6.767	0.654	
	vitamin C in AD Patinas	3.575	0.754	
	vitamin E in DM Patinas	9.44	0.282	
	vitamin E in AD Patinas	7.37	0.551	
	vitamin D in DM Patinas	15.07	0.433	
	vitamin D in AD Patinas	12.989	0.666	

Table 5. Sex-Disaggregated Mean Concentrations (Males) of Serotonin, Antioxidants, and Vitamins in Diabetes Mellitus and Alzheimer's Disease.

Gender	Variables	Mean N=40	±SEM	P value
Males	Serotonin in DM Patinas	403.2	25.4	< 0.001
	Serotonin in AD Patinas	114.3	32.5	
	GSH in DM Patinas	1.866	0.017	

	GSH in AD Patinas	1.888	0.017	
	SOD in DM Patinas	1.223	0.052	
	SOD in AD Patinas	1.423	0.037	
	CAT in DM Patinas	1.12	0.684	
	CAT in AD Patinas	1.24	0.476	
	MDA in DM Patinas	12.434	0.245	
	MDA in AD Patinas	14.545	0.654	
	vitamin C in DM Patinas	3.998	0.872	
	vitamin C in ADPatinas	2.55	0.379	
	vitamin E in DM Patinas	7.38	0.562	
	vitamin E in AD Patinas	4.88	0.644	
	vitamin D in DM Patinas	11.99	0.347	
	vitamin D in AD Patinas	10.001	0.929	

4. Discussion

Our findings demonstrate that serotonin levels are affected differently in patients with type 2 diabetes mellitus (DM) and Alzheimer's disease (AD) compared to healthy controls. Specifically, DM patients exhibited higher serotonin levels, while AD patients had lower levels. Interestingly, within the DM group, serotonin concentration was higher in individuals under 60 years old compared to older patients. Additionally, female patients, regardless of having DM or AD, showed higher serotonin levels compared to males.

Previous studies suggest that both DM and AD are associated with increased free radical production and oxidative stress. This elevated oxidative stress in DM and AD is likely due to prolonged exposure to high blood sugar levels (glycaemia) and a disruption in the body's natural antioxidant balance [29]. In AD, oxidative stress contributes to the disease process by impairing the function of neuronal mitochondria, oxidizing essential molecules (macromolecules), and generating reactive oxygen species (ROS) through the interaction of metal ions with amyloid beta (Aβ) plaques. Additionally, oxidative stress may promote the production of hyperphosphorylated tau protein (p-tau) and Aβ [30].

In diabetes, increased free radicals lead to lipid peroxidation within cellular structures, which is believed to play a significant role in the development of atherosclerosis and microvascular complications [31]. Moreover, the activation of platelets enhances the release of serotonin (5-HT), resulting in decreased platelet 5-HT content and elevated plasma 5-HT levels in patients with both DM and AD [32]. The age-related nature and high prevalence of both DM and AD suggest a potential link between these conditions. Notably, some studies indicate that type 2 diabetes may accelerate the progression of AD [33]. Therefore, identifying DM patients at high risk for developing AD would be valuable for implementing early intervention strategies to potentially prevent or slow the progression of AD. Our findings, in conjunction with other studies [34], support the hypothesis that serotonin loss may contribute to cognitive decline in the early stages of AD.

Both DM and AD are associated with high oxidative stress and increased activity of the polyol pathway, leading to the depletion of glutathione (GSH). Our results, consistent with previous research [35], demonstrate lower GSH concentrations in DM and AD patients compared to the control group. Superoxide dismutase (SOD) activity was found to be higher in DM and AD patients compared to the control group, which aligns with other studies [36]. This observation may be attributed to an adaptive response of the SOD enzyme to chronic oxidative stress in DM and AD patients [37]. Our findings further suggest that the high SOD activity in these patients points towards elevated levels of superoxide anion (O2.-), a potent indicator of significant oxidative stress. Increased substrate availability (O2.-) stimulates SOD synthesis in DM and AD

patients, potentially offering some protection against free radical damage [38]. The natural antioxidant approach offers a promising tool to target the diverse molecular pathways contributing to Alzheimer's disease, opening up new avenues for treating this complex disorder. [44, 45] The lower CAT activity observed in DM and AD patients, compared to the control group, represents a marker of oxidative stress and possible impairment of peroxisome function [39]. Decreased vitamin C levels in DM and AD patients, compared to healthy controls, may be due to reduced absorption caused by hyperglycemia. The potential link between glucose and vitamin C uptake into specific cells and tissues is an area of ongoing research. Vitamin C depletion could further contribute to increased oxidative stress [40]. Vitamin E, another antioxidant, functions as a scavenger of reactive oxygen species (ROS), thereby reducing oxidative stress in DM and AD [40]. The potential benefits of antioxidants suggest their use as a future strategy to prevent cognitive dysfunction associated with diabetes mellitus. Therefore, incorporating both established and novel antioxidants into new treatment approaches is warranted. [41]. Several studies have highlighted the potential of 2,4-thiazolidinedione (TZD), an antioxidant, as an effective agent against diabetes and oxidative stress, attributed to its unique molecular structure. [43]. In-vitro study showed that the ethanolic extract of Citrus maxima fruit peels and Cucurbita pepo ethanolic extract displayed significant free radical scavenging activity that increased with increasing concentration in AD animals. [46,47,48]. Certain synthetic compounds that mimic antioxidants have demonstrated effectiveness in alleviating Alzheimer's disease symptoms [49], while numerous plant extracts have shown promising results in animal studies, suggesting their therapeutic potential [50]. Overall, our findings suggest that altered levels of various biological factors, including serotonin, antioxidants, and oxidative stress markers, are associated with both DM and AD. Further research is needed to elucidate the precise mechanisms underlying these associations and to explore their potential as therapeutic targets for these debilitating diseases.

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