Brain, Behavior, & Immunity - Health 14 (2021) 100259

Contents lists available at ScienceDirect

Brain, Behavior, & Immunity - Health

journal homepage: www.editorialmanager.com/bbih/default.aspx

Full Length Article

Increased telomerase activity in major depressive disorder with melancholic features: Possible role of pro-inflammatory cytokines and the brain-derived neurotrophic factor

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ARTICLE INFO

Keywords:
Major depression disorder
Pro-inflammatory cytokines
Brain-derived neurotrophic factor
Telomerase activity

ABSTRACT

The biological mechanisms responsible for depression symptoms are not yet understood. For this reason, it is important to reveal the etiopathogenetic mechanisms in this disease. This study aims to compare the levels of pro-inflammatory cytokines, Brain-Derived Neurotrophic Factor (BDNF), and telomerase activity in patients with major depressive disorder (MDD) and healthy controls. Plasma BDNF, interleukin-6 (IL-6), IL-1beta, and Tumor Necrosis Factor-alpha (TNF-alpha) levels, and telomerase activity were measured in 39 patients with major depression and 39 healthy controls matched with patients in terms of age, gender, and education year. Plasma concentration of BDNF, IL-6 levels, and telomerase activity was significantly different between patients with MDD and healthy controls. Correlation analysis showed a positive trend between plasma BDNF levels and plasma IL-6 levels in patients with MDD with melancholic features. Furthermore, the path analysis results showed that the telomerase activity was indirectly affected by gender, IL-1β, IL-6, BDNF, and BMI, via the severity of depression and anxiety and MDD status as the mediators. Further studies are needed to examine the molecular mechanism of the telomerase activity and the role of BDNF and pro-inflammatory cytokines in the telomerase activation in MDD.

1. Introduction

Major Depressive Disorder (MDD) is a common psychiatric disorder. However, the etiopathogenetic mechanisms underlying depressive symptoms in MDD are not yet understood. In recent years, molecular and cellular theories related to the development of MDD have gained importance (e.g., Duman et al., 1997; Wolkowitz et al., 2008). It is known that dysregulation of telomere dynamics and changes in the levels of inflammatory cytokines and neuroprotective mediators such as Brain-Derived Neurotrophic Factor (BDNF) in circulation have been reported in MDD (Reviewed in Gururajan et al., 2016). However, due to the lack of prospective studies in untreated patients, the interplay between these factors has not been clarified yet.

Telomerase is a member of the enzymes family that is responsible for the replication of telomere DNA and is made up of two major distinct subunits: human telomerase reverse transcriptase (hTERT) and human telomerase RNA component (hTR) (Blackburn, 2005; Leão et al., 2018). The telomerase enzyme synthesizes telomeric repeats using RNA (hTR) as a template and the catalytic subunit (hTERT) plays a major role in this process (Blackburn, 2005; Leão et al., 2018). Telomerase enzyme prevents telomeric DNA from shortening and repairing telomeres by adding telomeric DNA to short telomeres associated with a long and healthy life. Telomerase is engaged in the pathology of aging and cell growth, all of which are dependent on telomere protection. The telomeres' length is shortened over time as the outer part cannot be replicated with each cell division or exposed to genotoxic effects such as inflammation and oxidative stress (Coluzzi et al., 2014; Von Zglinicki, 2002; Wolkowitz et al., 2008). There are limited studies on telomerase activity in MDD (Reviewed in Muneer and Minhas, 2019). However, evidence has emerged that provides inconsistent results about telomerase activity in

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https://doi.org/10.1016/j.bbih.2021.100259
Received 29 December 2020; Received in revised form 11 April 2021; Accepted 13 April 2021
Available online 17 April 2021

MDD (Simon et al., 2015; Damjanovic et al., 2007; Chen et al., 2014; Wolkowitz et al., 2012). Besides, the Reverse Telomerase Transcriptase enzyme's mRNA expression shows a positive relationship with the severity of depression and anxiety in the combined sample (MDD subjects plus controls) (Teyssier et al., 2012).

Early telomere shortening can be seen in patients with MDD due to these ongoing inflammatory processes, and an increase in telomerase activity can be expected as a compensatory response to this situation. Some studies have concluded that the increase in pro-inflammatory cytokine levels in depressive patients without medication and during depressive episodes is negatively related to telomere length (Damjanovic et al., 2007; Wolkowitz et al., 2010; Wolkowitz et al., 2011). Lin, Epel, and Blackburn (2012) suggest that an excessive increase in inflammatory processes shortens cellular life and causes destruction, and this situation is regulated by a balancing system such as increased telomerase activity. Studies have found that levels of interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), and interleukin-1 (IL-1) are significantly higher in MDD patients than in healthy people (Dowlati et al., 2010; Howren et al., 2009; Irwin and Miller, 2007). Miller (2010) confirmed these findings in his study and reported that MDD patients showed "signs of inflammation, " which is confirmed by the increase of inflammatory cytokines such as TNF-α, IL-1, and IL-6 in both their blood and cerebrospinal fluid. However, there are inconsistent results about the levels of some cytokines, such as IL-1β and TNF- α, in MDD (Haapakoski et al., 2015; Y. Liu, Ho and Mak, 2012; Strawbridge et al., 2017). The increase of IL-1β and TNF- α levels in MDD patients, in comparison to non-depressive controls, were detected only in researches involving patients with MDD above the age of 40 and who are European (Haapakoski et al., 2015; Y. Liu et al., 2012). Besides, the concentrations of TNF-a and IL-1b appeared to be changed in serum samples and plasma samples (Y. Liu et al., 2012).

Pro-inflammatory cytokines have also been shown to affect neuronal functions - through neuron growth/development and synaptic plasticity - and their excessive increases can lead to impairment in various brain areas, especially the hippocampus (Barrientos et al., 2003; Calabrese et al., 2014; Goshen et al., 2007; Khairova, MacHado-Vieira, Du and Manji, 2009; Lapchak et al., 1993; Yirmiya and Goshen, 2011; You et al., 2011). However, one study revealed that BDNF level in MDD patients was positively correlated with IL-6 level, and the authors suggested that IL-6 could only be a strong predictor of BDNF in depression with melancholic features (Patas et al., 2014). Abnormal changes in inflammatory mechanisms and the BDNF level may trigger depressive symptoms, but very few studies directly show a relationship between BDNF level and depressive symptoms in depressive patients. It has been shown that BDNF is one of the reasons for the higher telomerase activity in hippocampal neurons in the early stages of post mitosis (Fu et al., 2002). Besides, higher telomerase levels caused by BDNF in neuronal cells provide more immune to apoptosis (Niu and Yip, 2011). However, inhibiting hippocampal telomerase triggered by chronic stress reduces neurogenesis and thus causes depression-like phenotype and eliminates the behavioral effects of antidepressant drugs (Zhou et al., 2011). On the contrary, overexpression of TERT in the hippocampus stimulates neurogenesis and reveals antidepressant-like behaviors (Zhou et al., 2011). It suggests that the link between telomere mechanism and depression-like behavior extends to the brain, and this link is regulated by telomerase activity. Wolkowitz et al. (2015) reported that telomerase activity is essential for neurogenesis in MDD patients who have not yet started medication. However, the fundamental mechanisms underlying this relationship between BDNF and telomerase activity (D. Lin et al., 2015); and how BDNF, together with inflammatory mechanisms, affect telomerase activity changes in MDD are unknown.

In this study, the interaction of pro-inflammatory cytokines (IL-1β, TNF-α, and IL-6) with BDNF and the "telomere maintenance system/ telomerase activity" were examined in patients with MDD and healthy individuals to determine the effect of pro-inflammatory cytokines and BDNF on telomerase activity levels and the possible relationship between them and clinical variables in patients with MDD.

2. Methods and materials

2.1. Participants

The method followed in the study were applied after obtaining approval from the Noninvasive Research Ethics Board of Dokuz Eylül University. Participants included in the study consisted of randomly selected people between the ages of 18–65, with at least primary-school graduation. An informed consent form was presented to all participants before they were included in the study, and written consent was obtained.

Forty patients with MDD and 39 healthy volunteers were included in the study. Before being included in the study, all subjects were requested to report alcohol intake, smoking, regular physical activity, and all subjects were checked for routine blood screening (such as specific endocrine and hemogram values). All subjects (MDD and healthy controls) were asked to report whether they have consumed alcohol (yes/no) and drinking frequency (Wild et al., 2001), and the number of cigarettes they smoked in a day. Individuals were not included in this study who reported that regular cigarette usage was above the one pack of cigarettes per day and drinking frequency was once a day and more than once a day. World Health Organization presented the recommendations that were used to define as regular physical activity (yes/no) (World Health Organization, 2010).

The data of 39 people with MDD and 39 healthy control subjects were included in the analysis. Those whose tests in routine blood screening were within normal limits were included in the study. The presence of a known decompensated systemic medical disease, diabetes mellitus, a rheumatological disease, active infection or severe neurological disease were exclusion criteria for all participants. Only depressed subjects who were not on medicines–being any psychotropic medicine free for at least 15 days-were enrolled. If any consulting depressed individuals were on psychotropic drugs, whether prescribed or not, we excluded them. We did not stop any patients' ongoing medical treatments. After study evaluation procedures, we referred them to usual treatment facilities. The subjects were not included in the study if they had schizophrenia, bipolar disorder, psychotic disorder, conditions affecting cognitive functions (such as delirium, dementia, and epilepsy), a diagnosis of alcohol or substance abuse, general medical condition. The MDD diagnosis was confirmed using the Structured Clinical Interview for the DSM-IV Axis I Disorders (SCID-I) (First et al., 1997) conducted by a certified psychiatrist, and those who had a score of at least 18 on the Hamilton Depression Rating Scale (21-item version-HDRS; Hamilton, 1960) were included in the study. MDD patients with melancholic features were included in the study to reduce confounding factors, and those with only atypical and psychotic MDD symptoms were excluded. Patients with melancholic features may represent a severe and distinct subtype of depression with different aetiopathogenetic mechanisms compared to depression patients with atypical and/or psychotic features that may represent different subtypes. Thus we excluded patients with atypical and psychotic MDD symptoms were excluded. One of the patients in the MDD group showed both atypical and melancholic characteristics.

The healthy control subjects were selected from the staff working at Dokuz Eylül University Medical Faculty. The healthy control group has also been screened using SCID, HDRS, and Hamilton Anxiety Rating Scale (HARS). The healthy control consisted of individuals who did not have any previous or present DSM-IV Axis I diagnosis and did not meet the exclusion criteria. The control group was similar to the MDD group with respect to demographic characteristics such as age, gender, and education level.

2.2. Measures

2.2.1. Demographics and healthy lifestyles

Age, gender, education, marital status, and occupation were collected via self-report. The smoking amount and alcohol intake were also

collected via self-report. Waist circumference (cm) of all subjects was measured, and Body Mass Index (BMI) (kg/m^2) was calculated at a clinic evaluation.

2.2.2. Structural clinical interview for the DSM-IV Axis I disorders (SCID-I)

SCID-I, which was developed by First et al. (1997), was a diagnostic tool used to classify DSM-IV Axis I Disorders. Özkürkçügil, Aydemir, Yıldız, Esen Danacı, and Köroğlu (1999) translated it into Turkish and examined it for its validity and reliability.

2.2.3. Hamilton Depression Rating Scale (HDRS-21)

Hamilton developed HDRS in 1967 (Hamilton, 1967). It is a 21-item scale that investigates depressive symptoms, evaluated by a clinician, and is applied to all subjects. In the 21-item version of the scale, the highest score is 67. The implementation of the scale took approximately 15 min. The study's reliability and validity in the Turkish language were conducted in 1996 by Akdemir et al. The scale was used to evaluate the depression severity. The Cronbach alpha value of the scale for this study is 0.94.

2.2.4. Hamilton Anxiety Rating Scale (HARS-13)

This scale, developed by Hamilton (1959), was used to measure the subjects' anxiety severity. The highest score can be obtained from this 13-item version of the scale is 72. Yazıcı, Demir, Tanrıverdi, Karaağaoğlu and Yolaç (1998) conducted a Turkish reliability and validity study of the scale. The Cronbach alpha value of the scale for this study was 0.89.

2.3. Blood sampling and testing

People who met the inclusion criteria and whose routine blood screening values were within normal limits were given an appointment for blood donation for the next day. After one fasting night except for water, the subjects gave blood between 8:00 and 10:00 in the morning. The blood sample was taken into Vacuumed Mononuclear Cell Preparation Tubes (CPT Vacutainer tubes; 2 × 8 ml). The blood was taken into citrated, ficolled CPT Vacutainer tubes and then kept upright at room temperature for 2 h. The tubes were then turned upside down 3-5 times and centrifuged at 2600 rpm for 20 min at room temperature. Plasma obtained after centrifugation was divided into DNase-RNase free tubes and stored to −80 °C. PBMCs (Peripheral Blood Mononuclear Cells) were taken into 15 ml falcones and then washed with phosphate-buffered saline (PBS) and centrifuged, and the washing process with PBS was repeated two times. Then the cell pellet was stored in liquid nitrogen and lifted to −80°.

IL-6, IL-1β, and TNF-α plasma concentrations were measured similarly and three times (triplicate) by ELISA (Enzyme-Linked Immunosorbent Assay) according to the instructions of the manufacturers (Boster Biotechnology Company, Wuhan, China). Cytokine concentrations were calculated using standard curves. According to the manufacturer's instructions, the BDNF plasma level was measured by the ELISA method (CYT306; Millipore Co., Billerica, MA, USA). Telomerase activity was measured on the PBMCs and by a real-time polymerase chain reaction (qPCR) system using the TRAPeze RT Telomerase Detection Kit (Millipore S7710).

2.4. Statistical analysis

Except for some variables (IL-6, IL-1β, TNF-α, and telomerase activity), all other variables are within acceptable ranges for skewness and kurtosis, providing normal distribution. Logarithmic transformation (base10) was applied for IL-6, IL-1β, TNF-α, and telomerase activity, which were not suitable for normal distribution, and all analyzes in the study were performed with these values. Descriptive statistics for variables are given as mean (±Standard Deviation). Descriptive statistics for categorical variables are given as percentages. A Chi-square test was used to compare categorical variables between groups. An independent sample *t*-test was used for comparing continuous variables between groups. The relationship between continuous variables was examined using Pearson correlation coefficients. The reliability of the scales was evaluated with the Cronbach alpha coefficient. *P*-value is smaller than 0.05 (two-tailed) was considered statistically significant. Statistical analysis was made using the SPSS v.21 program (IBM Corp., Armonk, New York, USA).

Path analysis was used to understand whether there is a causal relationship between demographics, clinical features, and biomarkers of MDD and estimate the magnitude and significance of causal connections between demographics, clinical features, and biomarkers of MDD. Path analysis, using IBM SPSS AMOS 22 program (Arbuckle, 2013); IBM SPSS Statistics, New York, USA), was used to examine the indirect or direct effects of the severity of depression and anxiety and biomarkers of depression (IL-6, IL-1β, TNF-α, BNDF) on telomerase activity in MDD and healthy controls. All variables are included in the model as the observed variable. Many fit indices were used to decide the model fit: 1) x^2 goodness of fit test shows the goodness of fit if the p-value is greater than 0.05, 2) Comparative Fit Index (CFI), and Goodness of Fit Index (GFI) should be greater than 0.90, 3) Standard Root Mean Residual (SRMR), and Root Mean Square Error of Approximation (RMSEA) fit index should be lower than 0.05 (Schermelleh-Engel et al., 2003; Steiger, 1990). Standardized beta (β) value was used for path coefficients showing direct and indirect paths.

3. Results

3.1. Descriptive statistics

Demographic data of MDD patients and healthy controls are presented in Table 1. There were no significant between-group variations in demographic characteristics (all $p > 0.05$). The BMI (t (78) = −2.61, p = 0.011) and waist circumference (t (78) = −2.72, p = 0.008) of the healthy controls were significantly higher than the MDD. Apart from regular physical activity in healthy life data, there was no difference between MDD and healthy controls in other data. The healthy controls did sports regularly compared to the MDD group (χ^2 (1, 78) = 6.303, p = 0.012).

3.2. Group comparisons on biochemical measures

Among the possible biomarkers of MDD, IL-6 (t (78) = 2.41, p = 0.02) and telomerase activity (t (78) = 3.43, p = 0.001) were significantly higher in the MDD group than the healthy controls. BDNF was significantly lower in MDD than healthy controls (t (78) = −3.57, p = 0.001). However, IL-1β (t (78) = 0.67, p = 0.504) and TNF-α (t (61.28) = 0.93, p = 0.93) were not significantly different between MDD and healthy controls.

3.3. Associations between biochemical measures and demographic variables

Firstly, biochemical measures were evaluated by bivariate analysis in all subjects (Table 2). Telomerase activity was significantly associated with depression severity (r = 0.37, p = 0.001) and anxiety severity (r = 0.37, p = 0.001). Depression severity was significantly associated with anxiety severity (r = 0.93, $p < 0.001$), BDNF (r = −0.38, p = 0.001) and IL-6 (r = 0.29, p = 0.01). Also, anxiety severity was significantly associated with BDNF (r = −0.34, p = 0.002) and IL-6 (r = 0.32, p = 0.004). IL-6 was significantly associated with the level of IL-1β (r = 0.26, p = 0.023). IL-1β level showed a positive correlation with age (r = 0.25, p = 0.03).

97.4% of the MDD group consisted of patients with melancholic characteristics. Although a 0.05 level of significance was not found, BDNF was positively associated with IL-6 (r = 0.30, p = 0.06) at a trend level in the MDD group. Anxiety severity was significantly associated with depression severity (r = 0.46, p = 0.003) and gender (r = 0.41, p =

Table 1
Characteristics of the participants included in the study.

	All subjects (N = 78)	MDD (n = 39)	Healthy control (n = 39)	*p* values
Demographics				
Age (year ± SD)	33.05 ± 10.45	33.31 ± 10.60	32.79 ± 10.42	0.83
Sex (% Female)	71.8%	71.8%	71.8%	1.00
Education (year ± SD)	11.99 ± 3.62	11.31 ± 3.71	12.66 ± 3.44	0.10
Marital status (% Married)	53.8%	64.1%	43.6%	0.07
Occupation (% Active)	75.6%	69.2%	82.1%	0.19
Healthy lifestyle				
Waist circumference (cm ± SD)	80.80 ± 16.25	76.0 ± 15.82	85.62 ± 15.41	0.008**
BMI (kg/m^2 ± SD)	23.36 ± 4.76	22.01 ± 4.06	24.73 ± 5.07	0.011**
Smoking (%)	30.8%	35.9%	25.6%	0.33
Smoking amount (per day)	3.93 ± 8.33	5.63 ± 10.62	2.23 ± 4.68	0.07
Alcohol intake (%)	16.7%	17.9%	15.4%	0.76
Clinical data				
Depression Severity	14.31 ± 14.69	28.72 ± 3.32	0.09 ± 0.42	0.001***
Anxiety Severity	13.18 ± 14.16	25.96 ± 8.34	0.40 ± 1.24	0.001***
Melancholic (%)	%48.7	%97.4	N/A	ND
Melancholic + Atypical (%)	%1.3	%2.6	N/A	ND
Biomarkers				
(Ln) IL-6 (pg/ml ± SD)	0.74 ± 0.29	0.82 ± 0.30	0.66 ± 0.26	0.02*
(Ln) IL-1β (pg/ml± SD)	0.91 ± 0.29	0.93 ± 0.28	0.88 ± 0.29	0.504
(Ln) TNF-α (pg/ml ± SD)	1.78 ± 0.06	1.78 ± 0.08	1.78 ± 0.05	0.93
BDNF (pg/ml ± SD)	60.83 ± 32.95	48.43 ± 29.16	73.23 ± 32.16	0.001***
(Ln) Telomerase activity (unit/10.000 cells ± SD)	0.97 ± 0.34	1.09 ± 0.34	0.85 ± 0.29	0.001***

Note. Mean ± Standard Deviation (SD) or percentage values are presented. Natural log (Ln) transform is applied for IL-6, IL-1 β, TNF-α, and Telomerase activity that is not normally distributed. MDD = Major Depression Disorder. BMI = Body Mass Index. BDNF = Brain-Derived Neurotrophic Factor. (Ln) = Natural log (Ln) transform is applied. IL-6 = interleukin-6. IL-1β = interleukin-1β. TNF-α = Tumor Necrosis Factor-alpha. BDNF = Brain-Derived Neurotrophic Factor. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

0.009) in the MDD group. In the MDD group, IL-1β level was positively correlated with IL-6 ($r = 0.36, p = 0.025$) and TNF-α levels ($r = 0.36, p = 0.024$). There was no significant relationship in the severity of depression, BMI, and telomerase activity with other variables (all $p > 0.05$).

3.4. Path analyses

Our major concern in the current study was to demonstrate the factors that caused the different telomerase activity levels between MDD and healthy controls. Thus, we used path analysis. Variables used in the path analysis were those that have significant associations with clinical features or biomarkers of MDD in bivariate analysis. We determined the pathways between variables based on the models presented biological pathways in depressed mood (Lung et al., 2007; Manoliu et al., 2018; Webb et al., 2020). For example, Lung et al. (2007) found that MDD status (MDD vs. healthy controls) directly explains the 31% of the telomere length variance in path analysis. Moreover, potential biological pathways linking depressed mood are illustrated in some reviews used in our current path models (Manoliu et al., 2018; Webb et al., 2020). The severity of depression and anxiety, BMI, gender, BDNF, IL-6, and IL-1β levels, hypothesized to be directly or indirectly effective on telomerase activity, were added to the model. BMI and waist circumference show similar relations with other variables in the current bivariate analysis. Thus, only BMI was included in the model. The TNF-α level was not significantly associated with any variable in the bivariate analysis of this study. Moreover, there were not any significant relations between age and clinical features or biomarkers of MDD. Thus, TNF-α and age was not added to the models.

Model 1 was tested using path analysis to explain the factors that directly or indirectly affect all subjects' telomerase activity. In this model, BDNF, IL-1β, gender, and BMI are exogenous variables; MDD status, telomerase activity, the severity of depression and anxiety, and IL-6 were tested as endogenous variables.

Model 1 (see Fig. 1) showed overall good fit index, $\chi 2\ (18) = 15.47; p = 0.63; \chi 2/df = 0.86$; GFI = 0.96; CFI = 1.00; RMSEA = 0.00; SRMR = 0.04. Model 1 contained pathways that were significant and nonsignificant. IL-β level had a direct effect on IL-6 ($\beta = 0.26, p = 0.02$). There was a statistically significant relationship between BMI and gender (1: Men; 2: Women) ($\beta = 0.30, p = 0.012$). Gender ($\beta = 0.11, p = 0.006$), BMI ($\beta = -0.10$, $p = 0.017$) and anxiety severity ($\beta = 0.88$, $p = 0.001$) had a significant direct effect on depression severity. R^2 for the severity of depression was 0.88. It means that direct effect of gender, BMI and the severity of anxiety on the severity of depression explained 88% of the severity of depression variance. Also, there was an indirect effect of IL-1β ($\beta = 0.08, p = 0.014$), BDNF ($\beta = -0.272, p = 0.007$) and IL-6 ($\beta = 0.293$, $p = 0.008$) on depression severity. However, BMI had a nonsignificant indirect effect on the severity of depression ($\beta = -0.19, p = 0.086$).

IL-6 ($\beta = 0.33, p = 0.001$), BDNF ($\beta = -0.31, p = 0.002$) and BMI ($\beta = -0.22$, $p = 0.029$) had a significant direct effect on the severity of anxiety. R^2 for anxiety severity was 0.27. It means that the direct effect of IL-6, BDNF, and BMI on the severity of anxiety explained 27% of the severity of anxiety variance. IL-1β ($\beta = 0.086, p = 0.013$) had significant indirect effect on anxiety severity.

The severity of depression had a significant direct effect on MDD status (1: MDD; 2: Control) ($\beta = 1.03, p = 0.001$). However, the severity of anxiety had no significant direct effect ($\beta = 0.05$, $p = 0.29$). The R^2 value for the MDD status was 0.97. It means that the direct effect of the severity of depression on MDD status explained 97% of the variance of MDD status. Gender ($\beta = -0.116$, $p = 0.03$), BMI ($\beta = -0.911$, $p = 0.008$), anxiety severity ($\beta = -0.032, p = 0.004$), IL-1β ($\beta = -0.03, p = 0.013$), IL-6 ($\beta = -0.305, p = 0.007$) and BDNF ($\beta = 0.346, p = 0.003$) levels had significant indirect effects on the MDD status.

MDD status had a significant direct effect on the level of telomerase activity ($\beta = -0.37$, p = 0.001) and the direct effect of the MDD status explained 13% of the variances of telomerase activity. Gender ($\beta = 0.043$, $p = 0.022$), BMI ($\beta = -0.107$, $p = 0.004$), IL-6 ($\beta = 0.112$, $p = 0.005$), BDNF ($\beta = -0.127, p = 0.002$), depression ($\beta = 0.38, p = 0.004$), and anxiety severity ($\beta = 0.316$, $p = 0.006$) had significant indirect effects on telomerase activity.

Model 2 was conducted by deleting the non-significant paths (paths: from BDNF to depression severity; from IL-6 to depression severity; from anxiety severity to MDD status) and correlations. Model 2 showed good fit with the data, $\chi 2\ (26) = 24.447; p = 0.55; \chi 2/df = 0.94$; GFI = 0.94; CFI = 1.00; RMSEA = 0.00; SRMR = 0.06. All paths found in the final model showed significant paths (Fig. 2). 13% of the variance in telomerase activity was explained by the variables in Model 2. As in Model 1, MDD status had a significant direct effect on the level of telomerase activity ($\beta = -0.37, p = 0.001$). The severity of depression had a significant direct effect on the MDD status ($\beta = -0.99$, p = 0.001). Gender ($\beta = 0.11$, p = 0.009), BMI ($\beta = -0.10$, p = 0.017) and the severity of anxiety ($\beta = 0.91$, p = 0.001) had a significant direct effect on the severity of depression. IL-6 ($\beta = 0.34$, p = 0.001), BDNF ($\beta = -0.31$, p = 0.002) and BMI ($\beta = -0.22$, p = 0.027) had a significant direct effect on the severity of anxiety. IL-1β had a significant direct effect on the IL-6 ($\beta = 0.26$, p = 0.02). Besides, all indirect paths for the Model 2 were presented in Table 3 in which the standardized indirect effects and two tailed significance values were included.

Table 2
Bivariate correlation between variables of interest (N = 78).

	Age	Gender	Waist circumference	BMI	Depression Severity	Anxiety Severity	BDNF	(Ln) IL-1β	(Ln) IL-6	(Ln) TNF-α	(Ln) Telomerase
MDD status (1: MDD; 2: Control)	-0.025	0.000	0.298*	0.287*	-0.987**	-0.908**	0.379**	-0.077	-0.266*	-0.011	-0.366**
Age	--	0.195	0.362**	0.496**	0.013	0.025	0.147	0.246*	0.180	-0.007	-0.133
Gender (1:Men; 2: Women)		--	0.420**	0.297**	0.025	0.113	-0.064	0.002	0.099	0.085	0.112
Waist circumference			--	0.858**	-0.309**	-0.260*	0.145	0.124	0.059	-0.103	-0.063
BMI				--	-0.292**	-0.242*	0.133	0.176	0.045	-0.111	-0.112
Depression Severity					--	0.927**	-0.383**	0.069	0.289*	0.017	0.368**
Anxiety Severity						--	-0.338**	0.037	0.324**	0.056	0.373**
BDNF							--	-0.044	-0.002	-0.016	-0.042
(Ln) IL-1β								--	0.257*	0.104	0.156
(Ln) IL-6									--	0.122	-0.034
(Ln) TNF-α										--	-0.159
(Ln) Telomerase											--

Pearson's r correlation =	Small ± 0.2	Medium ± 0.5	Large ± 0.8

Note. Subjects were coded as 1 for Major Depression Disorder (MDD) group and 2 for healthy controls. Gender was coded as 1 for men and 2 for women. (Ln) = Natural log (Ln) transform is applied. BMI = Body Mass Index. BDNF = Brain Derived Neurotrophic Factor. IL-1β = interleukin-1β. IL-6 = interleukin-6. TNF-α = Tumor Necrosis Factor alpha. $^{*}p < 0.05$, $^{**}p < 0.01$.

4. Discussion

The molecular and biochemical basis of MDD and biomarkers used in early diagnosis and follow-up of MDD is unknown. As summarized in the introduction, several studies have shown that pro-inflammatory cytokines (Dowlati et al., 2010; Howren et al., 2009; Irwin and Miller, 2007), BDNF (e.g., Banerjee et al., 2013; Karege et al., 2005) and telomerase activity (Chen et al., 2014; Wolkowitz et al., 2012) may be the candidate biomarkers of MDD. Although the role of interplay between inflammatory cytokines, BDNF, and telomeres in MDD has been explored in a large number of independent studies, the interaction between altered telomerase activity, inflammation, and BDNF have been rarely studied in the same experimental setting. Therefore, the current study was designed to determine the interplay between pro-inflammatory cytokines, BDNF, telomerase activity, and their correlations with clinical features, demographics, and healthy lifestyles.

This study provided the first clues about the importance of simultaneous determination of plasma concentration of IL-6, BDNF, and PBMC telomerase activity to distinguish healthy and depressed people. We observed that IL-6 and BDNF levels were significantly different between healthy controls and MDD; PBMC telomerase activity was increased in patients with MDD compared to healthy controls. The most significant clinically meaningful result was that plasma IL-1β, IL-6, and BDNF levels indirectly predicted telomerase activity.

Most of the immunological studies showed that cytokine levels increase in depression patients (Dowlati et al., 2010; Howren et al., 2009; Irwin and Miller, 2007; Miller, 2010). IL-1β, IL-6, and TNF-α plasma levels increase during the depression episode, and most cytokines decrease with effective anti-depressant treatment (Dahl et al., 2014), but sometimes may not decrease to normal levels (Hannestad et al., 2011). Our study found that only IL-6 plasma levels in depressed patients with melancholic features differed from healthy controls. The IL-6 elevation in depressed patients reflects the activated inflammatory processes. However, the current study did not show any trace of difference in plasma IL-1β and TNF-α levels between healthy controls and MDD. Some studies showed that TNF-α and IL-1β plasma levels were not different between people with MDD and healthy controls (Brambilla and Maggioni, 1998; Brambilla et al., 2004; Kagaya et al., 2001). These studies showed similar cytokine concentration levels in plasma samples from MDD subjects and healthy controls (Brambilla and Maggioni, 1998; Brambilla et al., 2004; Kagaya et al., 2001), as shown in the current study. However, the shallow levels of cytokine concentration in plasma make it challenging to measure. It has been suggested that cytokine levels in serum give more sensitive results compared to cytokine levels in plasma (Y. Liu et al., 2012). Besides, the inconsistent results may be due to different subtypes of MDD. For example, a higher IL-6 response was correlated with the presence of the most severe melancholic features (Primo de Carvalho Alves & Sica da Rocha, 2020). It seems important to select a single subtype of MDD and measure cytokine levels in serum to reduce confounding factors. However, our findings of path analysis showed that IL-1β directly affected IL-6 concentration in the combined sample. It was known that human monocytes stimulated by IL-1 cause an increase in the expression of IL-6 mRNA (Bauer et al., 1989). For this reason, IL-1β may trigger IL-6 concentration, and in this way, IL-1β may indirectly cause higher levels in the severity of anxiety and depression and indirectly contribute to the hyperactivity in telomerase via the occurrence of MDD.

The BDNF level is significantly lower in patients with depression than in healthy people, consistent with the literature (Banerjee et al., 2013; Karege et al., 2002, 2005). This finding supports that the decrease in plasma BDNF level may have a role in forming depression symptoms. It has been suggested that BDNF level may have a potential role as a biological marker of depression or a determinant of the effectiveness of anti-depressant treatments (Sen et al., 2008). Thus there is an increase in BDNF level in patients with MDD who respond to treatment compared to those who do not, so BDNF may be a successful predictor in evaluating the response to treatment (Polyakova et al., 2015). In the current study, plasma BDNF level is positively correlated with plasma IL-6 in the depression group. It can be assumed that BDNF, which regulates neurogenesis and is responsible for the survival and proliferation of neurons, is inhibited in the hippocampus and other regions of the brain with the excessive increase of pro-inflammatory cytokines. BDNF levels in serum and plasma are highly correlated with BDNF levels in cerebrospinal fluid (Pillai et al., 2010); thus, decreased peripheral levels of BDNF are mirrored in the brain in acutely depressed subjects. IL-6 is released from activated microglia that plays an important role in brain inflammatory response in depression (Ting et al., 2020), and the connection of increased IL-6 level with activated microglial cells affect neuronal signaling (Lima Giacobbo et al., 2019). An in vitro study on human monocytes showed that IL-6 stimulation increased BDNF release (Schulte-Herbrüggen et al., 2005). The available information may point to a bidirectional interaction between the immune system and neuroplasticity regulation, although BDNF even appears to be an immunomodulatory (Muneer, 2016; Patas et al., 2014). Besides, depression is not a clinically homogenous disorder, and these variations can contribute to differences in biological markers. Our findings indicate that immunological dysregulation affects the BDNF level, and even immunological

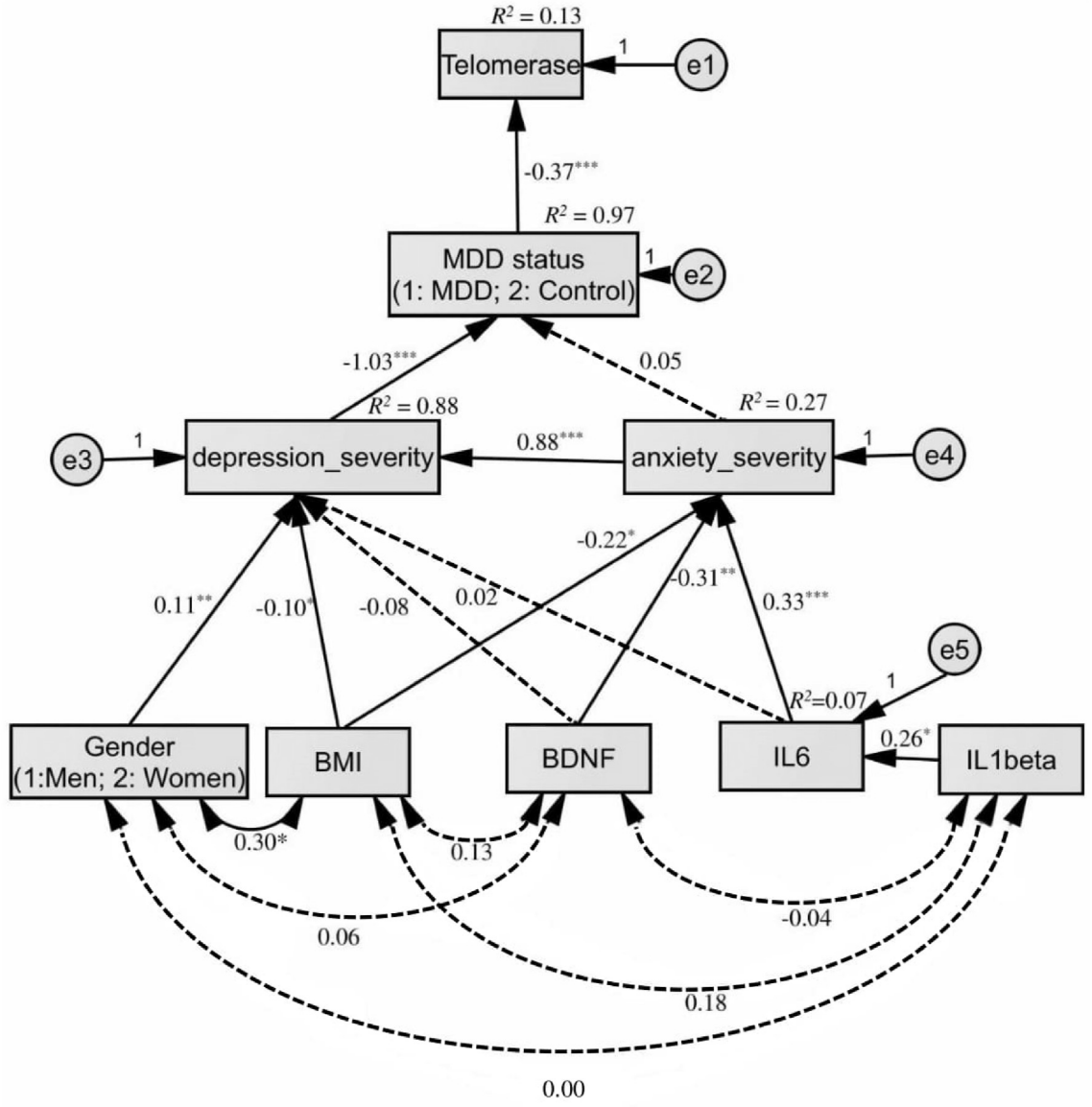

Fig. 1. Model 1 shows the hypothesized pathways. Dashed lines indicate non-significant paths. e icons mean the error term of unobserved variables. Group was coded as 1 for Major Depression Disorder (MDD) group and 2 for healthy controls. Gender was coded as 1 for women and 2 for men. BMI = Body Mass Index. BDNF = Brain-Derived Neurotrophic Factor. IL-1β = interleukin-1β. IL-6 = interleukin-6. TNF-α = Tumor Necrosis Factor-alpha. *p < 0.05, **p < 0.01, ***p < 0.001.

processes may regulate BDNF release in melancholic type depression, as consistent with Patas et al.'s (2014) findings. IL-6 may only be a strong predictor of BDNF in depression with melancholic features, and this positive relationship between IL-6 and BDNF may be a biomarker specific to the "melancholic subtype of MDD" (Patas et al., 2014). The correlation of IL-6 level with BDNF in the melancholic subtype of MDD can be clarified by future studies, whether the melancholic subtype of MDD distinguishes from depression with atypical or psychotic characteristics.

The current study consolidates previous findings that the PBMC level of telomerase activity is significantly higher in MDD patients than in healthy controls (Damjanovic et al., 2007; Lee et al., 2013; Chen et al., 2014; Wolkowitz et al., 2012). Other studies have shown telomere shortening in MDD patients and increased telomerase activity as feedback (Chen et al., 2014; Wolkowitz et al., 2012, 2015). It is known that the increase in telomerase activity prevents the aging of cells and causes them to proliferate (Bodnar et al., 1998). As a result of depression affecting telomeres at the molecular level, an increase in telomerase activity may trigger a compensatory system (Shalev et al., 2014). The telomere length is shorter in patients with depression than in healthy people (Hartmann et al., 2010; Hoen et al., 2011; Z. Liu et al., 2020; Lung et al., 2007; Simon et al., 2006; Verhoeven et al., 2014; Wikgren et al., 2012). The increase in telomerase activity indicates a stabilizing mechanism to overcome the increased telomere erosion (shortening of telomeres) due to depression (Damjanovic et al., 2007; Lee et al., 2013; Wolkowitz et al., 2012). The examination of telomerase activity in patients with MDD seems essential for monitoring an active physiopathological process. Contrary to our assumption, there is no direct statistical correlation between telomerase activity, pro-inflammatory cytokines, and BDNF. However, this finding does not indicate that these biomarkers do not have intervening factors/influences. Path analysis showed that the primary mechanism explaining the hyperactivity of telomerase in MDD indirectly links with alterations in the levels of pro-inflammatory cytokines (IL-1β and IL-6), BDNF, BMI, clinical features, and gender. Previous research showed that depressive status predicts telomere length as examined by the pathways (J. J. Liu et al., 2017). We extended this finding by the current path analysis that telomerase activity indirectly associate with pro-inflammatory cytokines (IL-1β and IL-6), BDNF, clinical features, BMI, and gender via MDD status (MDD vs. healthy controls).

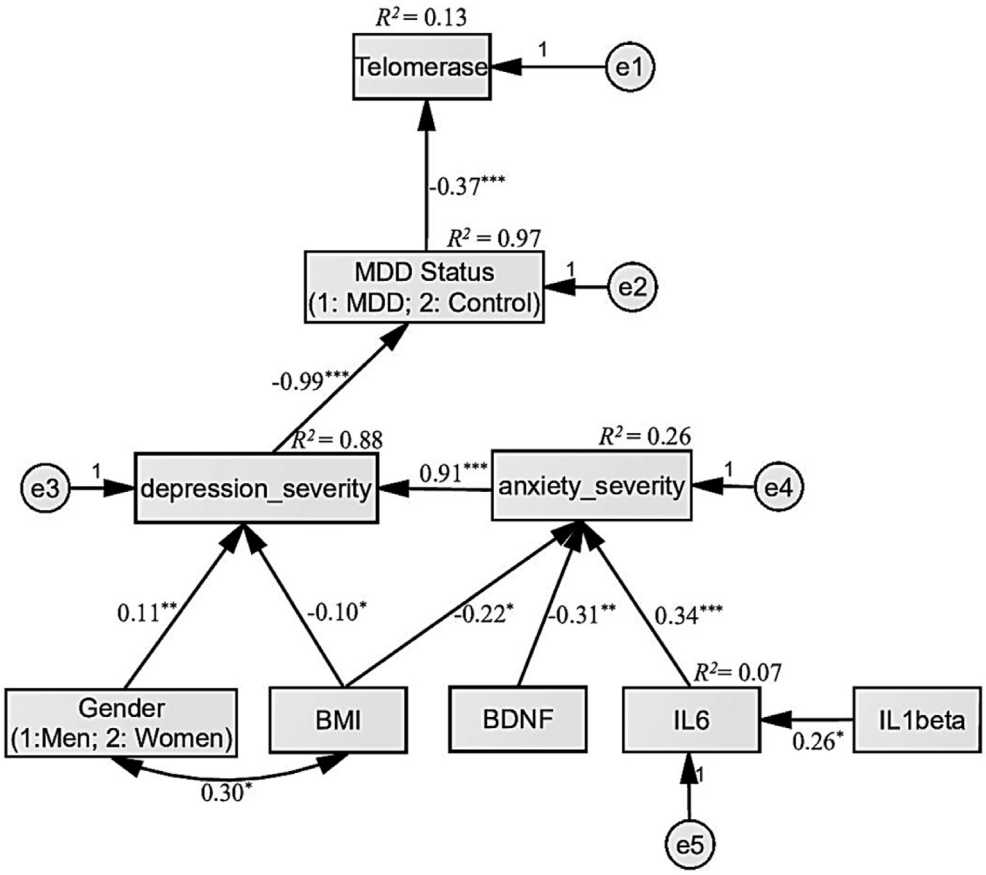

Fig. 2. Model 2 shows the hypothesized pathways. e icons mean the error term of unobserved variables. Group was coded as 1 for Major Depression Disorder (MDD) group and 2 for healthy controls. Gender was coded as 1 for men and 2 for women. BMI = Body Mass Index. BDNF = Brain-Derived Neurotrophic Factor. IL-1β = interleukin-1β. IL-6 = interleukin-6. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Table 3
Indirect paths in Model 2.

Variables	IL-1β		Gender		BMI		BDNF		IL-6		Anxiety severity		Depression severity	
	β	*p*	*β*	*p*	*β*	*p*	*β*	*p*	*β*	*p*	*β*	*p*	*β*	*p*
IL6	…	…	…	…	…	…	…	…	…	…	…	…		…
anxiety severity	0.086	0.01**	…	…	…	…	…	…	…	…	…	…	…	…
depression severity	0.079	0.01**	…	…	−0.198	0.077	−0.283	0.021*	0.306	0.018*	…	…	…	…
MDD status	−0.078	0.01**	−0.109	0.045*	0.297	0.011*	0.28	0.024**	−0.302	0.016*	−0.9	0.085	…	…
Telomerase	0.028	0.01**	0.04	0.038*	−0.109	0.008**	−0.102	0.015*	0.11	0.01**	0.329	0.014*	0.36	0.014*

Note. MDD status was coded as 1 for Major Depression Disorder (MDD) group and 2 for healthy controls. Gender was coded as 1 for men and 2 for women. BMI = Body Mass Index. BDNF = Brain-Derived Neurotrophic Factor. IL-1β = interleukin-1β. IL-6 = interleukin-6. *$p < 0.05$, **$p < 0.01$.

The more severe anxiety symptoms by dysregulation of biological systems (such as inflammation and neuron growth/development) are directly responsible for increasing depressive severity levels. More severe depressive symptoms by the increased anxiety severity predict the occurrence of MDD and indirectly responsible for hyperactivation of telomerase in our sample.

Path analysis of the current study revealed that the severity of anxiety and depression are two important mediators to explain the effect of MDD status on the hyperactivity of telomerase. Firstly, anxiety symptomatology is an important mediator between dysregulation of biological systems and depressive severity. In this way, anxiety symptomatology indirectly causes the occurrence of MDD and hyperactivity in telomerase levels. In our sample, the higher the level of IL-6 and the lower the levels of BDNF and BMI, the more anxiety symptomatology is reported. Studies showed that considerably higher severity of anxiety is often characterized by melancholic depression (Day and Williams, 2012), and anxious participants tended to have significantly higher IL-6 concentrations (O'Donovan et al., 2010) and lower BDNF levels (Suliman et al., 2013), as also confirmed by the current path analysis. This finding showed that anxiety symptomatology was an important mediator to explain the relationship between the severity of depression, inflammatory markers, BDNF, and BMI. This study highlighted that only anxious people with melancholic depression might exhibit higher IL-6 concentration, lower BDNF, and BMI. In future works, it is recommended to measure anxiety symptomatology in which to explain the inconsistent results in the inflammatory markers of depression.

Secondly, the severity of depression is a mediator between the severity of anxiety, BMI, gender, and MDD status. It means that women with more severe anxiety symptoms and lower BMI level are related to higher depression severity. This way, the severity of depression indirectly predicts the telomerase activity levels with regard to the MDD status. Loss of appetite or lower BMI than healthy controls is one of the vegetative deficiencies in melancholic depression (Lamers et al., 2013; Parker et al., 2010) and a strong predictor of depressive severity (Berlin and

Lavergne, 2003). We confirmed these findings and extended that lower BMI is directly related to anxiety severity and indirectly affects telomerase activity via MDD status. The women classified as anxious and lowered BMI had higher levels of depressive severity.

One of the strengths of this study is that the MDD group consisted of newly diagnosed MDD with melancholic features, their symptoms were severe, and they had not received any treatment yet. Another important feature is that all kinds of comorbid diagnoses were carefully excluded during the forming of MDD and healthy groups, and thus homogeneous groups were obtained. However, the relatively low number of participants in the groups and 71.8% of participants are females are the most important limitation of our study. Males and females are biologically different and may answer differently to psychiatry disorders. Considering the low number of males, this could be a confounding factor for the analysis. Future studies should focus on only male or female subjects to see the more robust effects of MDD on the analyzed targets. Another important limitation is that targeted markers, especially pro-inflammatory cytokines, were studied in plasma instead of serum. In addition to measuring telomere length could provide useful insight into the potential role of telomere length in biomarkers of MDD. Furthermore, the pathways in this study explained the only 13% variance in hyperactivity of telomerase. Other variables might be the answers to telomerase's hyperactivity in MDD and should therefore be considered for future studies. Other immunopathological mechanisms, such as NLRP3 inflammatory responses (Shao et al., 2018), may also affect telomere length and telomerase activity. On the other hand, many different processes, such as hypercortisolemia or insulin resistance, which play a role in developing depression, may mediate telomere/telomerase dysregulations (Reviewed in Manoliu et al., 2018). Stress-related cortisol changes can have major effects on telomerase activity, and in this context, the underlying mechanisms are just beginning to be elucidated (Muneer and Minhas, 2019).

Our findings are preliminary and need to be repeated. Identifying biomarkers in MDD is undoubtedly important in the disease's treatment process or even treatment alternatives and understanding the basic mechanisms of the MDD. Future studies should focus on the telomere/telomerase system in larger samples by correlating it with biochemical or molecular markers, even with new data obtained by neuroimaging and neurocognitive tests.

5. Conclusion

The current study showed that the plasma concentration of IL-6, BDNF, and PBMC telomerase activity enhances value for distinguishing between healthy and depressed people. IL-1β and TNF-α levels were not significantly different between depressed and healthy people. However, path analysis showed that IL-1β indirectly affects telomerase activity by directly affecting the level of IL-6. Similarly, BDNF, BMI, and gender indirectly affect telomerase activity. The severity of depression and anxiety had a mediating role between-group difference in telomerase activity and IL-1β, IL-6, BDNF, BMI, and gender. It seems that changes in the levels of these biomarkers are significant contributors to depression and anxiety symptoms. The elevated level of telomerase activity may show a compensatory response to the varying level of biomarkers in MDD. Although it is clear that more studies are needed, current results presented the potential molecular and biochemical pathways in MDD have implications for improving diagnostic reliability, understanding clinical features, using appropriate intervention methods, and effective therapy.

Author contribution

P Bürhan-Çavuşoğlu: Protocol development, Data analysis, Data collection or management, Manuscript writing, and editing.

E İşcan: Protocol development, Data management, Manuscript editing.

A Güneş: Protocol development, Data management.

N Atabey: Protocol/project development, Data management, Manuscript writing, and editing.

T Alkın: Protocol/project development, Manuscript writing, and editing.

Declaration of competing interest

There are no conflicts of interest for the writers of this manuscript to declare.

Acknowledgments

This work was supported by Dokuz Eylül University Scientific Research Projects Unit (Project Number: B.08.6.YÖK.2.DE.0.06.0.01.00/930) Turkey.

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