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Depressive Symptoms are Associated with low Serotonin Levels in Plasma but are not 5–HTTLPR Genotype Dependent in Older Adults

Pedro Enrique Trujillo-Hernández[1], Aide Sáenz-Galindo[1], Odila Saucedo-Cárdenas[2], María de los Ángeles Villarreal-Reyna[1], Mauricio Andrés Salinas-Santander[1], Ana Laura Carrillo-Cervantes[1], Reyna Torres-Obregón[1] and Sandra Cecilia Esparza-González[1]

[1] *Universidad Autónoma de Coahuila (Mexico)*
[2] *Universidad Autónoma de Nuevo León (Mexico)*

Abstract. Depressive symptoms are diagnosed by physicians using scales but their pathophysiology is unclear. Low serotonin (5–HT) levels play an important role in depression, and the 5–HT transporter (5–HTT) is an important regulator of plasma serotonin levels and reuptake. Additionally, the 5–HTT gene-linked polymorphic region (5–HTTLPR) is associated with depression. The aim was to clarify the roles of plasma serotonin levels in plasma and the 5HTTPLR polymorphism in depressive symptoms in older adults. A total of 84 older adult participants were evaluated. Depressive symptoms were measured using the Center for Epidemiologic Studies Depression Scale of 20 items (CESD–20). The plasma serotonin levels were determined by ELISA, and the 5–HTTLPR genotype was analyzed by PCR. Depressive symptoms were present in 39.3% (N = 33) of the participants. The median plasma serotonin level was 204.34 ng/mL (SD = 93.88). A significant correlation was found between the CESD–20 scale and plasma serotonin levels (r = –.256; p = .019). Low serotonin levels were associated with the presence of depressive symptoms (p = .001). The 5–HTTLPR analysis showed that of the 84 older adults, 35.7% had the SS genotype, 10.7% had the LL genotype, and 53.6% were heterozygous. The 5–HTTLPR polymorphism was not associated with depressive symptoms (p = .587) and plasma serotonin levels (p = 0.391). *Depressive symptoms* correlate with low serotonin levels in plasma, but not with the 5–HTTLPR polymorphism in older Mexican adults.

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Depression is defined as a mental disorder with principal comorbidities, such as anxiety, altered mood and sleep, persistent feelings of sadness, hopelessness and disinterest in life projects, anhedonia, metabolic problems that affect the weight and energy of the patient, and increased negative thoughts and ideas towards life. According to DSM–5 criteria, a diagnosis of depression can be made if the patient shows 5 or more symptoms for 2 weeks and at least one of the following symptoms: Depressed mood or loss of interest or pleasure (American Psychiatric Association [APA], 2015).

This disorder can become chronic or recurrent and alter the performance of daily activities, and in severe cases, could lead to suicide (Jayasingam et al., 2020). Depression is currently considered one of the top 10 causes of psychiatric disability and is predicted to be the second leading cause of disability worldwide by 2020 after heart disease (World Health Organization [WHO], 2017).

Contrary to beliefs, depression in the elderly is not a natural part of aging. Identifying this condition is important and providing timely and appropriate treatment will help restore health. However, if depression remains untreated, it could lead to cognitive and social deterioration and increased disability (Burke et al., 2019). Since symptoms of depression are considered a normal part of aging, only 15% of older adults receive appropriate treatment (Christensen et al., 2019).

Correspondence concerning this article should be addressed to Cecilia Esparza González. Universidad Autónoma de Coahuila. 25280 Saltillo (Mexico).

E-mail: sceciliaesparza@gmail.com

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Several methods can assess and identify depressive symptoms (measurement scales). These scales (pencil and paper instruments) allow health professionals to identify whether a patient has symptoms of depression. Among the different scales to identify symptoms of depression, the CESD–20 is a research tool that has been validated in different populations, including Mexico (Gonzalez et al., 2017; Malakouti et al., 2015; van Dam & Earleywine, 2011).

These scales are associated with biological and social factors (e.g., deficiency of neurotransmitters and loss of a spouse, friends, or work) (Alghadir et al., 2016; Paykel et al., 1996) that influence the development of depression; however, the pathophysiology of the disease is not clear (Jesulola et al., 2018).

Depression is associated with a decrease in serotonin levels in both the brain and blood (Bamalan & Al Khalili, 2020; Cha & Hong, 2015). Patients with recurrent episodes of depressive disorder and without medication show lower plasma serotonin levels (Aleksovski et al., 2018). Increased plasma serotonin levels showed improvement in a murine model of depression using an antidepressant compared with animals that did not receive the antidepressant (Park et al., 2018). Similar to these findings, elevated serotonin concentrations in plasma improve depression symptoms in patients receiving selective serotonin reuptake inhibitors (SSRI) (Pehrson et al., 2015). Researchers have determined that low levels of serotonin inhibit the response to treatment with SSRI in patients with depression (Holck et al., 2019).

The serotonin transporter (SERT) is a protein that is found in the platelet plasma membrane. It regulates circulating serotonin levels by inducing the accumulation of serotonin within platelets (Amador & McDonald, 2018). The SERT protein expressed in platelets is identical to that found in neurons, displaying similar structural and functional properties in both tissues (Mammadova-Bach et al., 2018).

Other studies have shown that the serotonin transporter gene (SCL6A4) has a polymorphic region in its promoter sequence (5–HTTLPR) that has two forms, one long (L) and one short (S); the S allelic form is associated with depression (Gao et al., 2014; Grzesiak et al., 2017).

Few studies have investigated the presence of polymorphisms according to the concentration of peripheral serotonin and 5–HTTLPR polymorphisms were identified in autistic children, where a high concentration of serotonin was reported in plasma but no association was found between the concentration and genotype (S/S, S/L, or L/L) (Meguid et al., 2015). The pathophysiology of depression is complex, and an endless number of factors are involved. Thus, our objective was to determine the association between biological and genetic factors and depressive symptoms using the CESD–20 Depression Scale.

Method

Study Participants

Eighty-four elderly adults from the Family Care and Integration Center (CAIF) of Saltillo, Coahuila, Mexico, were randomly selected from the list provided by the administration of the Integral Family Development (DIF) System of the State of Coahuila. The size of the sample was determined considering the total number of elderly who attended the CAIF on the day of data collection; non-probabilistic convenience sampling was used. The protocol met the requirements of the Ethics Committee of the University Hospital and the ethical recommendations contained in the Declaration of Helsinki.

Individuals over 60 years of age who provided written informed consent, knew how to read and write, and obtained a score < 6 in the Pfeffer Functional Activities Questionnaire (FAQ), which measures functional changes related to cognitive impairment, were included. Older adults taking antidepressants and smokers were excluded from the study. People on antidepressant treatment were eliminated because the drugs have been shown to alter the concentration of serotonin in plasma (Holck et al., 2019).

All participants were informed about the study protocol, blood sampling, and the scale used (CESD–20). For the collection of sociodemographic data, a Personal Data Card was used that consisted of 11 questions about the participant's age, marital status, occupation, religion, sex, the occurrence of falls in the last year, household composition, health status, and whether he or she takes any medication.

Assessment of Depressive Symptoms

The Center for Epidemiologic Studies Depression Scale 20–item version (CESD–20) (Radloff, 1977) in Spanish was used to measure depressive symptoms. Each participant was interviewed by the nursing staff. The items assess various components of depression, such as depressed mood, feelings of worthlessness, hopelessness, and loss of appetite. Each item is related to the frequency with which the symptoms occurred in the week before its application.

The participant is asked how many days in the last week he or she has had these symptoms. The response options are 0 days, 1 to 2 days, 3 to 4 days, and 5 to 7 days, or all days, coded with values from 0 to 3. The possible range is from 0 to 60; that is, the higher the score, the greater the presence of depressive symptoms. It has four reverse-worded items (4, 8, 12, and 16) that

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refer to conditions of positive affect. The cutoff point was 16 points as an indicator of clinically significant depressive symptoms (Radloff & Locke, 1986)

The psychometric properties of the CESD–20 according to Radolff (1977) are the following: a) Internal consistency of .85 for the general population and .90 for psychiatric patients; b) the test-retest reliability is .54 for the general population and .53 for psychiatric patients; c) the non-response percentage is low, so its acceptability is high. The scale is generalizable since the analysis by subgroups contains alpha coefficients greater than .80. In the Mexican population, it has a Cronbach´s alpha of .74 to .85 that explains 50.6% of the variance (Aguilera-Guzmán et al., 2004; Escobar Bravo et al., 2013). To evaluate the structure of the CESD–20 in the studied sample, internal consistency values were estimated, Cronbach's alpha (α) to establish the reliability of the scale, obtaining a result of .72.

Plasma Serotonin Levels

Peripheral blood samples were collected and sent to a certified laboratory for clinical analysis. Plasma serotonin levels were analyzed by ELISA (Serotonin ELISA; Immuno-Biological Laboratories, Minneapolis, MN). The detection range was 0/15–2500 ng/mL, and the sensitivity 6.2 ng/mL. According to the literature, normal serotonin levels oscillate between 101 and 283 ng/mL (Bamalan & Al Khalili, 2020). A concentration of 101 ng/mL was used to categorize 2 groups according to the serotonin levels, under and upper groups.

Analysis of the 5–HTTLPR Polymorphism

The phenol-chloroform technique was used to extract DNA (Ghaheri et al., 2016). Polymorphisms were determined by polymerase chain reaction (PCR), following a previously described protocol. The forward primer was 5'– GGCGTTGCCGCTCTGAATGC –3 and the reverse primer 5'– GAGGGACTGAGCTGGACAACCAC –3' (McCauley et al., 2004). The cycle conditions included an initial denaturation of 94 °C for 5 min, followed by 40 cycles of 95 °C for 1 min, 63 °C for 1 min, 72 °C for 1 min, and a final extension at 72 °C for 10 min. The PCR products were analyzed on 3% agarose gel containing ethidium bromide. The L and S alleles corresponded to 528 and 484 base pair fragments, respectively (Collier et al., 1996).

Statistical Analysis

Descriptive analysis was performed (measures of central tendency and frequencies) and qualitative values as percentages. The chi-square (χ^2) test measured categorical variables, and when the number was lower than 5, Fisher´s exact test was used. Spearman´s correlation was performed to correlate the CESD–20 score and the plasma serotonin concentration. Analyses of the allele and genotypic frequencies were performed using the Hardy-Weinberg formula (genotype frequency = p2 + 2pq + q2 and allelic frequency = p + q = 1). The Cochran-Armitage trend test was used to associate genotypes and the CESD–20 or serotonin concentration (Runde, 2013). The Mann-Whitney *U* test was used to compare quantitative variables.

Odds ratios (ORs) were determined to estimate the presence of 5– HTTLPR polymorphisms, and if the serotonin concentration is associated with the CESD–20 scale. A p-value $\leq$ .05 was considered statistically significant. All of the statistical analyses were performed using SPSS software version 21 (IBM, Corp., Armonk, NY).

Results

Sample Characteristics

The mean age was 68.68 (SD = 6.2) years. Most of the participants were women (75%) and the median serotonin level was 204.34 (SD = 93.88) ng/mL. Regarding these variables, 54.7% of the participants were not in a relationship and 78.6% lived alone; only 11.9% reported working, and 78.6% had some type of illness (40.1% had diabetes, 37.4% hypertension, and 1.1% Parkinson). Table 1 shows some sociodemographic data that are considered risk factors to develop depressive symptoms, such as living alone, being unemployed, and comorbidities.

Detection of Depression using the CESD–20 Scale

According to the CESD–20, 39.3% (M = 31. 6; SD = 10.1) and 60.7% (M = 8; SD = 4.5) of the participants had and did not have depressive symptoms, respectively. The overall mean was 15.6 (SD = 12.9) (Table 1). Regarding gender, more women than men showed depressive symptoms; a difference that approached statistical significance (p = .053). Social variables were also analyzed (having a partner, living accompanied, and occupation). The variables were compared by proportions using Fisher´s exact test ($N < 5$) or χ^2. There was no association between depressive symptoms and remaining unemployed (p = .0804). Additionally, variables related to functionality—falls during the year, the use of a support device, and comorbidities— were analyzed and the statistical analysis showed an association between comorbidities and depressive symptoms according to the CEDS–20 (p = .0312). Thus, serotonin levels were significantly lower in participants with depressive symptoms (CESD–20) than in those without depressive symptoms (p = .001).

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Table 1. *Demographic Characteristics of All Participants according to CESD-20 Screening*

Variable	Participants *n* (%) (*n* = 84)	CESD–20		
		No Depression (*n* = 51)	Depression (*n* = 33)	*p*
Age	68.68 ± 6.2	68.59 ± 5.8	68.82 ± 6.8	.870
Gender				
Male	21 (25%)	9 (17.6%)	12 (36.4%)	.053
Female	63 (75%)	42 (82.4%)	21 (63.4%)	
Plasma Serotonin Levels (ng/mL)[a]	204.34 ± 93.88	231.09 ± 95.79	162.95 ± 74.97	**.001***
< 101 ng/ml		2 (3.9%)	7 (21.2%)	**.0251***
> 101 ng/ml		49 (89.1%)	26 (78.8%)	
Marital Status				
With Couple	38 (45.3%)	23 (45.1%)	15 (45.5%)	1.000
Single	46 (54.7%)	28 (54.9%)	18 (54.5%)	
Living				
Alone	66 (78.6%)	42 (82.4%)	24 (72.7%)	.294
With Someone	18 (21.4%)	9 (17.6%)	9 (27.3%)	
Occupation				
Unemployed	74 (88.1%)	42 (82.4%)	32 (97.0%)	.0804
Employed	10 (11.9%)	9 (17.6%)	1 (3.0%)	
Illness				
YES	66 (78.6%)	36 (70.6%)	30 (91.0%)	**.0312***
NO	18 (21.4%)	15 (29.4%)	3 (9.0%)	
Falls in the Year				
YES	35 (41.7%)	21 (41.2%)	14 (42.4%)	.910
NO	49 (58.3%)	30 (58.8%)	19 (57.6%)	
Use a Support Device				
YES	12 (14.3%)	5 (9.8%)	7 (21.2%)	.144
NO	72 (85.7%)	46 (90.2%)	26 (78.8%)	

Note. χ^2 and Fisher Test; *$p < 0.05$ significative difference; [a]Spearman Coefficient -.256 $p = 0.019$

A nonparametric correlation analysis was performed using Spearman´s coefficient. A significant correlation was found between the CESD–20 scale and the plasma serotonin levels of the participants (r = –.256; p = .019). Based on previous studies, the normal peripheral levels of serotonin are 101 to 283 ng/ml. Thus, the population was divided into two groups according to the serotonin level, levels lower than 101.0 ng/ml, and normal levels (above 101.1 ng/ml). The CESD–20 scores were associated with the plasma serotonin concentration (p = .0251) (Table 1)

Analysis of the Population according to Genotype

The presence of the 5–HTTLPR polymorphism was classified as wild-type homozygous LL, heterozygous LS, and homozygous SS. Analysis of the 5–HTTLPR polymorphism showed proportions of 35.7% for the SS, 10.7% for the LL, and 53.6% for the LS polymorphism; however, 89.3% of the Mexican sample had the S allele. No significant difference was found between the allelic variants; thus, the sample was in Hardy-Weinberg equilibrium (Table 2). Frequency analyzes revealed that the presence of polymorphisms was not associated with the CESD–20 score and serotonin levels (p = .587; p = .391, respectively).

Discussion

The objective of this study was to determine the association between biological and genetic factors and depressive symptoms using the CESD–20 scale. In our current sample, the internal consistency of the CESD–20 was analyzed using Cronbach's alpha, obtaining acceptable values (.72); similar values have been reported in the Mexican population (Bojorquez & Snyder, 2009).

In this study, 39.3% of the participants showed depressive symptoms and there was a higher incidence of depressive symptoms in women (63.4%) than in men (36.4%). These data corroborate previous reports in which the prevalence of depressive symptoms was higher in women, especially in elderly women (Carayanni et al., 2012; Girgus et al., 2017). Although the results were not statistically significant, there is a trend related to gender and depressive symptoms in the study population (p = .053). The present study was limited by the small number of participants, and like other research, the small sample did not permit

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Table 2. *Polymorphism Frequency according to the Measurement Instruments*

	CESD-20			SEROTONIN			
Genotype	Non-Depressive Symptoms[b] ($n = 51$)	Depressive Symptoms[c] ($n = 33$)	p	Greater than 101.0 ng/mL[d] ($n = 75$)	Less than 101.0 ng/mL[e] ($n = 9$)	p
(S/S)	19 (37.3%)	14 (42.4%)	0.587[a]	31 (41.3%)	2 (22.2%)	.391[a]
(S/L)	26 (51%)	16 (48.5%)		36 (48%)	6 (66.7%)	
(L/L)	6 (11.7%)	3 (9.1%)		8 (10.7%)	1 (11.1%)	

Note. [a] Cochran-Armitage trend test. $p < .05$ significative difference.
[b] $\chi^2 = 0.27$, $p = .60$; [c] $\chi^2 = 0.4$, $p = .518$; [d] $\chi^2 = 1.1$; $p = .29$; [e] $\chi^2 = 0.26$, $p = .60$.

controlling for additional covariates such as gender (Lewis et al., 2019).

Concerning social variables, such as marital status, living alone, or being unemployed, we did not find an association between these variables and the presence of depressive symptoms. The findings corroborated those in the literature that both marital status and living alone in the elderly are not risk conditions for their health (McLaren, 2018; Paykel et al., 1996); in contrast, social isolation represents a risk factor for developing depression (Evans et al., 2019). However, we found that unemployment was not associated with depressive symptoms. This could be related to the daily activity this group of older adults receives at the CAIF (Sarkar et al., 2017). Previous literature also demonstrated an association between the presence of comorbidities and depression (Panagioti et al., 2016). In addition, pathologies such as diabetes have been shown to affect serotonin synthesis in the brain as well as decreased tryptophan in plasma, with this condition being a risk factor for developing depression since it also suggests antidepressant-induced diabetes mellitus (Manjarrez-Gutiérrez & Hernández-Rodríguez, 2016; Nguyen et al.,2018; Prabhakar et al., 2015).

In this study, low serotonin plasma levels correlated with high scores in the CESD–20, indicating that low serotonin levels are associated with depressive symptoms; although the sample size was small, Spearman´s coefficient was close to the intermediate value and showed statistical significance (Amor Andrés et al., 2015). These results were consistent with the correlation between a decreased concentration of plasma serotonin and a mood disorder (Paul-Savoie et al., 2011; Tong et al., 2015). Similarly, low serotonin levels in plasma were associated with nonresponses to treatment using selective serotonin reuptake inhibitors (SSRI) (Saldanha et al., 2009).

Additionally, women with postpartum depression showed low serotonin levels in plasma in a case-control study (Xie et al., 2018). Furthermore, it was recently established that low serotonin levels in plasma were associated with patients with a low response to antidepressant treatment, presenting depressive symptoms (Holck et al., 2019). Additionally, platelets with low serotonin levels are a reliable biomarker in suicide risk assessment (Giurgiuca et al., 2016). Thus, lower levels of serotonin in plasma suggest an increased presence of depressive symptoms according to the CESD–20 score.

Analysis of the 5–HTTLPR polymorphism revealed that 89.2% of the sample harbored the S allele, a finding that, in the literature, was associated with developing depressive symptoms (Hu et al., 2019). This prevalence of the allele S is consistent with a recent report in which the S allele showed a prevalence of 78.9% in Mexican adolescents (Sarmiento-Hernández et al., 2019). In

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contrast, in this study, no significant association was observed between the 5–HTTLPR polymorphism and depressive symptoms, as measured by the CESD–20 score. However, our result was consistent with reports showing no association between the 5–HTTLPR polymorphism and major depression in the Colombian population (Perez-Olmos et al., 2016). Additionally, other authors have reported the importance of stress as a priority factor in developing depressive symptoms and not the presence of the S allele; furthermore, the lack of association could be due to the age of the study group because age is a factor in the association between the S allele and depression, with a significant association in patients younger than 37 years (Brummett et al., 2008).

The null association between these two variables can also be due to the scale used to determine the depressive symptoms; differences have been reported in evaluations depending on the scale used (Gao et al., 2014; Rief et al., 2018; Roppolo et al., 2015).

Additionally, plasma serotonin levels were not associated with the presence of the 5–HTTLPR polymorphism; findings that are consistent with those previously reported that the mean 5–HTT RNA expression level was not associated with the 5–HTTLPR genotype in patients or controls (Iga et al., 2016). Another report supporting the results demonstrated that plasma serotonin levels are not associated with the presence of the 5–HTTLPR polymorphism in autistic children (Meguid et al., 2015). These results are consistent with reports where the increase in plasma serotonin levels is observed when patients receive treatment and show a good response to clinical symptoms (Saldanha et al., 2009; Spreux-Varoquaux et al., 1996). Likewise, this protective effect of depressive symptoms correlates with the results obtained with the increase in 5–HTT mRNA in response to treatment using antidepressants for 32 weeks (Kao et al., 2018).

Based on these results, clinical intervention is suggested, such as exercise to increase plasma serotonin levels (Zimmer et al., 2016), thus avoiding symptoms of depression (Kvam et al., 2016). One of the limitations of this study was the number of participants and differences in the participant characteristics in each group. To identify differences in the genotype of the SLC6A4 gene, samples with a larger number of participants, as well as homogeneous populations, are needed (Culverhouse et al., 2018; Zhu, et al., 2017). Additionally, the CESD–20 score, the only screening tool used, would serve better as a supplement to a clinical diagnosis. Specifically for genotype analysis, it is more difficult to detect effects in smaller samples and it would be necessary to increase the sample to improve the statistical power of the methodology; however, with this sample size, it is possible to identify the clear association between the blood concentration of serotonin with the CESD–20 score.

Plasma serotonin levels correlate with the scores obtained using the CESD–20 scale. If an older adult has low plasma levels of serotonin, higher CESD–20 scores likely indicate states of depression. Variables such as comorbidities are influential factors that can cause the elderly to develop depression. The S allele has a high prevalence in the Mexican population participating in this study. Based on these results, we suggest investigating the prevalence of polymorphisms in larger samples to determine this risk factor to prioritize developing anti-depression programs.

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