

ORIGINAL ARTICLE

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Annexin A3 levels in patients with schizophrenia and bipolar disorder

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Abstract

Schizophrenia and bipolar disorder are disorders characterized by alterations in cellular and molecular mechanisms. Annexin A3 (ANXA3) has various effects on neural mechanisms and membrane phospholipid function and both mechanisms are thought to be associated with psychotic disorders. Our study consisted of 93 schizophrenia and bipolar disorder and 52 healthy controls. ANXA3 was analyzed by enzyme-linked immunosorbent assay (ELISA). ANXA3 levels were significantly different between the groups ($p<0.001$). ANXA3 levels of the control group were higher than ANXA3 levels of schizophrenia and bipolar disorder groups ($p<0.001$, $p<0.001$). The ANXA3 levels of the bipolar group were higher than the ANXA3 levels of the schizophrenia group ($p<0.001$). Laboratory levels of B12, folate, vitamin D, glucose, urea, creatinine, prothrombin time (PT), gamma-glutamyl transferase and lactate dehydrogenase were compared between the groups. B12 levels of the schizophrenia group were lower than B12 levels of bipolar and control groups ($p=0.05$, $p<0.001$). Creatinine levels of the schizophrenia group were higher than the creatinine levels of the healthy group ($p<0.05$). According to posthoc test results, PT levels of the schizophrenia group were higher than PT levels of the healthy group ($p<0.05$). In line with these findings, we suggest that ANXA3 protein deficiency may have an important role in the physiopathology of schizophrenia and bipolar disorder and may be used as a biomarker in the diagnosis of some psychotic disorders.

Key words: Schizophrenia, bipolar disorder, ANXA3, biomarker

Introduction

Bipolar disorder is a mental illness whose mechanisms have not yet been clearly identified. Patients usually experience successive episodes of hypomania or depression and symptom-free episodes [1]. Although the causal algorithm of this pathological condition is unknown, plasticity of central nervous system cells, monoaminergic pathway and mitochondrial stages are found in the pathogenesis process [2]. Schizophrenia is a syndrome of unknown etiology characterized by symptoms of psychosis. Generally, schizophrenia manifests as paranoid delusions and

auditory hallucinations [3]. Although the mechanisms involved in the development of schizophrenia have not yet been fully determined, its relationship with impaired neuroplasticity and inflammation has recently gained special importance.

Annexin A3 (ANXA3) is a protein known to be associated with inflammation and membrane metabolism cascades [4]. Alterations in tissue or cellular expression of annexins have been shown to be associated with various conditions such as asthma, atherosclerosis, autoimmune diseases, cancers, Parkinson's and Alzheimer's disease [5-10]. ANXA3 has important effects on

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the functions of important cells and membrane phospholipid metabolism, suggesting that changes in the expression of this molecule may be associated with psychotic disorders [11-24]. We aimed to investigate the relationship between ANXA3 levels and schizophrenia and bipolar disorder by comparing them with a healthy control group.

Material and Methods

Study setting and study population

93 patients diagnosed with schizophrenia and bipolar disorder according to the Diagnostic and Statistical Manual of Mental Disorders (DSM-V) criteria in the Psychiatry Outpatient Clinic of Sabuncuoğlu Şerefeddin Training and Research Hospital of Amasya University were examined. A healthy group of 52 patients was included to compare the disease groups. The control group was determined as age and gender matched after the patient groups were determined. The characteristics of the healthy control group included in our study were being older than 18 years, not having a known psychotic disease, not suffering from alcoholism, not having a chronic inflammatory disease, not having a history of chronic medication, and not having a malignant tumor.

ELISA analysis

A signed consent form and venous blood samples were obtained from all individuals included in the study. Serum samples were obtained by centrifugation (fixed angle, 3000-3500 rpm, 15 minutes). The serums were stored at -20 °C. These sera were thawed after sample collection for all groups. ANXA3 protein levels were analyzed by ELISA method using CLOUD CLONE-SEE786HU (Wuhan, Hubei 430056, China) commercial kit. In addition, complete blood count, routine biochemistry and hormone results of the patients were recorded on the day of sampling. These tests were analyzed with Beckman Coulter Inc. (California, USA) models LH780, AU5800 and UniCel DxI 800, respectively.

Statistical analysis

Our data were evaluated with the SPSS (Version 22, SPSS Inc, Chicago, IL, USA) program. Ratio comparisons were performed using the Chi-square test. Descriptive statistics of numerical data were reported using mean±standard deviation (SD) or median (min-max). Shapiro-Wilk, Kolmogorov-Smirnov tests and various graphical techniques (such as histograms and Q-Q plots) were used together to evaluate the assumption that numerical data had a normal distribution. Levene's test was used to assess the homogeneity of variances between three independent groups. Numerical data with normal distribution between three independent groups were compared using one-way analysis of variance (ANOVA), and numerical data without normal distribution were compared using the Kruskal-Wallis test. Tukey

or Games-Howell post-hoc tests were used depending on the assumption of homogeneity of variances to determine the groups in which the difference occurred after the ANOVA test. Dunn-Bonferroni post-hoc test was used to determine the groups in which the difference occurred after the Kruskal Wallis test. The $P<0.05$ value was accepted as the limit of significance.

Ethics considerations

Required ethics committee approval was obtained from Amasya University Clinical Research Ethics Committee (Amasya, Türkiye) (Dated: 03.03.2022; Acceptance number: 2022-03/34).

Results

There was no difference between the groups in terms of gender homogeneity ($P=0.404$). In the schizophrenia group 71.7% ($n=43$) were male and 28.3% ($n=17$) were female, in the bipolar group 62.8% ($n=27$) were male and 37.2% ($n=16$) were female, and in the control group 60% ($n=31$) were male and 40% ($n=21$) were female. There was no statistically difference in mean age between the study groups ($P=0.598$). The mean age of the schizophrenia group was 38.72 ± 8.78 (18-59), the mean age of the bipolar group was 39.86 ± 9.63 (21-58) and the mean age of the control group was 40.26 ± 6.18 (25-54).

Statistical findings for the comparison of ANXA3, White Blood Cell (WBC), Hemoglobin (HGB), Platelet (PLT), Thyroid Stimulating Hormone (TSH), Triiodothyronine (T3) and Thyroxine (T4) levels between the study groups are given in Table 1. ANXA3 levels were significantly different between the groups ($P<0.001$). ANXA3 levels of the control group were significantly higher than ANXA3 levels of schizophrenia and bipolar groups ($P<0.001$, $P<0.001$, respectively). ANXA3 levels of the bipolar group were significantly higher than ANXA3 levels of the schizophrenia group ($P<0.001$). Boxplot showing the distribution of ANXA3 levels between the groups is presented in Figure 1.

T4 results were significantly different between the groups ($P=0.008$). T4 levels of the control group were significantly higher than T4 levels of schizophrenia and bipolar groups ($P=0.026$, $P=0.015$, respectively). There was no significant difference between the T4 levels of schizophrenia and bipolar groups ($P=0.908$). WBC, HGB, PLT, TSH and T3 levels were similar between the groups ($P=0.524$, $P=0.117$, $P=0.204$, $P=0.073$, $P=0.781$, respectively).

Statistical findings for the comparison of B12, folate, vitamin D, glucose, urea, creatinine, PT, GGT and LDH blood levels between the study groups are presented in Table 2. B12 levels were significantly different between the groups ($P<0.001$). B12 levels of schizophrenia group were significantly lower than B12 levels of bipolar and control groups ($P=0.006$, $P<0.001$, respectively). There was no significant difference between the B12 levels of the bipolar and control groups ($P=1.000$).

Table 1. Comparison of ANXA3 and laboratory blood levels between research groups

	Schizophrenia (1) (n=60)	Bipolar (2) (n=43)	Control (3) (n=52)	P levels	Post-hoc P levels
					1-2: <0.001
ANXA3	13.75±3.69	23.12±4.65	30.44±5.71	<0.001 ^c	1-3: <0.001 2-3: <0.001
WBC	7.6±2.14	7.94±2.03	7.46±1.95	0.524 ^a	-
HGB	14.5 (7.8-17.9) (14.44±1.77)	14.3 (2.0-40.7) (14.56±4.89)	14.9 (11.1-17.1) (14.99±1.21)	0.117 ^d	-
TSH	224.5 (35-406) (223.2±72.46)	236 (35.6-702) (244.9±93.39)	236.5 (159-345) (238.7±40.81)	0.204 ^d	-
PLT	1.99 (0.36-7.84) (2.53±1.63)	2.46 (0.59-6.16) (2.95±1.41)	2.33 (0.78-4.04) (2.37±0.83)	0.073 ^d	-
T3	3.35±0.45	3.41±0.44	3.37±0.46	0.781 ^a	-
					1-2: 0.908
T4	1.15±0.18	1.13±0.17	1.24±0.19	0.008 ^b	1-3: 0.026 2-3: 0.015

a: One way ANOVA (mean±standard deviation), b: One way ANOVA with Tukey post-hoc test (mean±standard deviation), c: One way ANOVA with Games-Howell post-hoc test (mean±standard deviation), d: Kruskal-Wallis test (median (min-max) and (mean±SD)), e: Kruskal-Wallis test with Dunn-Bonferroni post-hoc test (median (min-max) and (mean±SD)), ANXA3: anneksin A3, WBC: white blood cell count, HGB: hemoglobin, PLT: platelet count, TSH: thyroid stimulating hormone, T3: triiodothyronine, T4: thyroxine

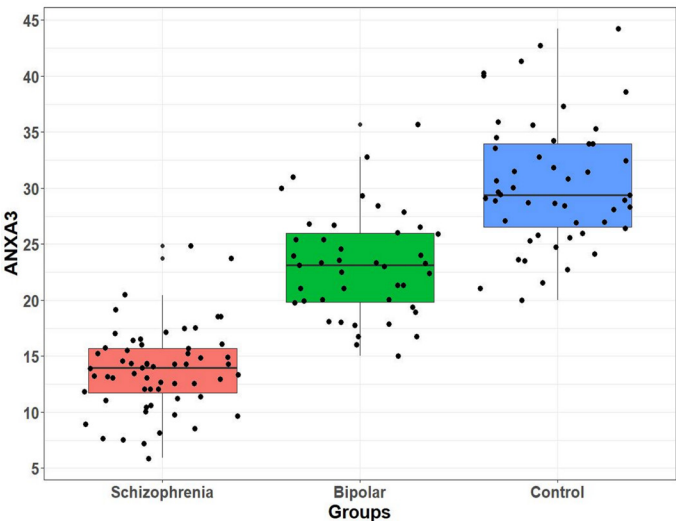


Figure 1. Box plot showing the distribution of ANXA 3 levels among research groups

Folate and vitamin D levels were significantly different between the groups (P<0.001, P<0.001, respectively). Folate and vitamin D levels of schizophrenia and bipolar groups were significantly lower than those of the control group (P<0.05, Table 2). The folate and vitamin D levels of the schizophrenia group were significantly lower than those of the bipolar group (P=0.001, P<0.001, respectively). Boxplot showing the distribution of T4, B12, folate and vitamin D levels between the groups is presented in Figure 2.

Glucose levels were significantly different between the groups (P<0.001). Glucose levels of the control group were significantly lower than the glucose levels of schizophrenia and bipolar groups (P<0.001, P<0.001, respectively). There was no difference between the glucose levels of schizophrenia and bipolar groups (P=0.679).

Creatinine levels were significantly different between the groups (P=0.004). The creatinine levels of the schizophrenia group were significantly higher than the creatinine levels of the control group (P=0.003). The creatinine levels of the other groups were similar (P=0.098, P=0.529, respectively).

PT levels were significantly different between the groups (P=0.027). PT levels of the schizophrenia group were significantly higher than PT levels of the control group (P=0.024). There was no significant difference between the PT levels of the other groups (P=1.000, P=0.235, respectively).

LDH levels were significantly different between the groups (P<0.001). LDH levels of the control group were significantly lower than LDH levels of schizophrenia and bipolar groups (P<0.001, P<0.001, respectively). There was no significant difference between the LDH levels of schizophrenia and bipolar groups (P=1.000). Boxplot showing the distribution of glucose, creatinine, PT and LDH levels between the groups is presented in Figure 3. Urea and GGT levels were not different between the groups (P=0.693, P=0.479, respectively).

Table 2. Comparison of laboratory blood levels between research groups

	Schizophrenia (1) (n=60)	Bipolar (2) (n=43)	Control (3) (n=52)	P levels	Post-hoc P levels
B12	355 (190–696) (368.3 ± 128.2)	443 (192–1716) (485.4±267.8)	451 (238–652) (447.1±94.09)	<0.001^c	1-2: 0.006 1-3: <0.001 2-3: 1.000
Folate	6.29 (2.73–15) (6.78±2.4)	8.3 (4.86–13.11) (8.29±1.83)	9.32 (5.84–16.5) (9.91±2.54)	<0.001^c	1-2: 0.001 1-3: <0.001 2-3: 0.020
Vitamin D	9.31±2.98	11.67±2.08	18.11±4.72	<0.001^c	1-2: <0.001 1-3: <0.001 2-3: <0.001
Glucose	98 (12–439) (105.1±49.43)	102 (77–309) (106.3±34.97)	87.5 (68–104) (86.74±8.49)	<0.001^c	1-2: 0.679 1-3: <0.001 2-3: <0.001
Urea	22.21±6.84	23.27±7	22.22±6.86	0.693 ^a	
Creatinine	0.81±0.16	0.74±0.16	0.71±0.15	0.004^b	1-2: 0.098 1-3: 0.003 2-3: 0.529
PT	22 (9–84) (26.05±16.9)	21 (6–86) (23.74±16.18)	17 (10–24) (16.92±3.31)	0.027^c	1-2: 1.000 1-3: 0.024 2-3: 0.235
GGT	23.5 (6–194) (31.38±27.33)	23 (7–162) (31.4±27.81)	27 (14–65) (28±10.03)	0.479 ^d	-
LDH	167 (83–282) (169.6±36.23)	169 (100–340) (178.6±49.06)	143 (70–192) (139.8±24.71)	<0.001^c	1-2: 1.000 1-3: <0.001 2-3: <0.001

a: One way ANOVA (mean±standard deviation), b: One way ANOVA with Tukey post-hoc test (mean±standard deviation), c: One way ANOVA with Games-Howell post-hoc test (mean±standard deviation), d: Kruskal-Wallis test (median (min-max) and (mean±SD)), e: Kruskal-Wallis test with Dunn-Bonferroni post-hoc test (median (min-max) and (mean±SD)), PT: prothrombin time, GGT: gamma glutamyl transferase, LDH: lactate dehydrogenase

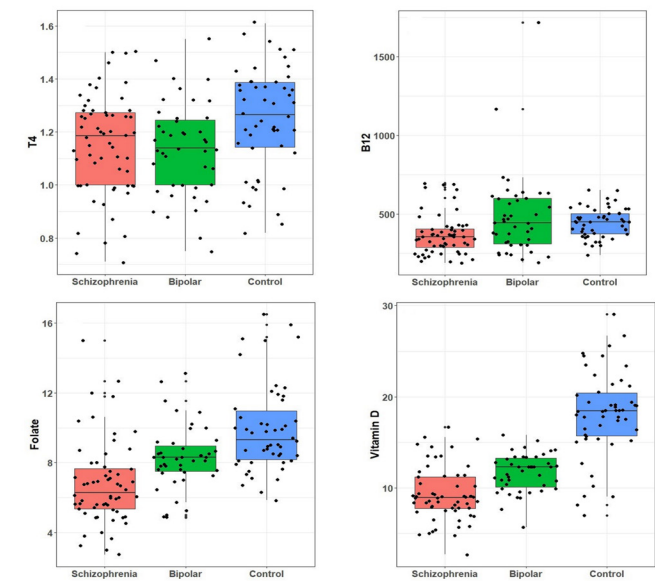


Figure 2. Box plot showing the distribution of T4, B12, Folate, and Vitamin D levels among research groups

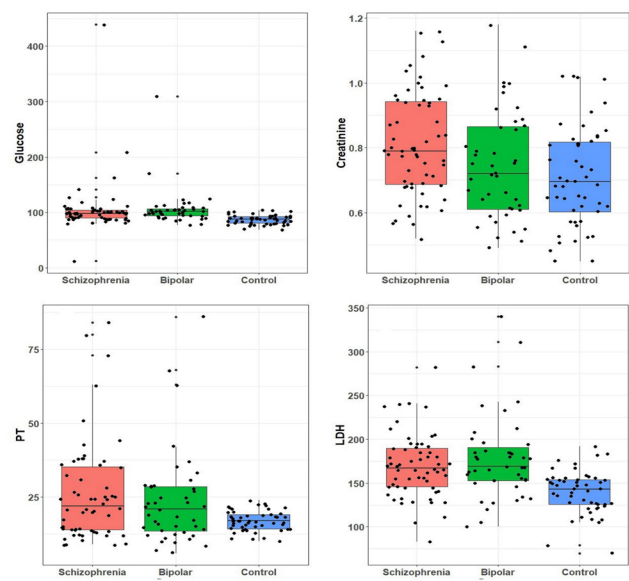


Figure 3. Box plot showing the distribution of glucose, creatinine, PT, and LDH levels among research groups

Discussion

ANXA3 molecule is known to have important effects on many conditions such as neuronal activity and membrane phospholipid metabolism [11-24]. In our study, we showed that ANXA3 levels were significantly decreased in patients with bipolar disorder and schizophrenia. We compared many parameters other than this important molecule between these groups. For example, we found that T4, vitamin D and folate were significantly lower in the two disease groups. We also found that B12, PT, LDH, creatinine and glucose were significantly higher in the schizophrenia group. There is no doubt that genetic studies are necessary to confirm the results of our study.

In a study, annexin-V and TNF- α molecules were investigated in 38 patients with schizophrenia and chronic drug users and 38 healthy control groups and it was found that serum annexin-5 levels were higher in schizophrenia patients [25]. In our study, contrary to this study, ANXA3 levels in the control group were higher than ANXA3 levels in schizophrenia and bipolar groups. In addition, ANXA3 levels of the bipolar group were higher than the ANXA3 levels of the schizophrenia group. ANXA3 is thought to have an effect on membrane phospholipid metabolism. Liu et al. investigated the functions of ANXA7 and some genes in schizophrenia patients. 476 schizophrenia families were included in the study and clustered in two different ways and it was found that the expression of ANXA7 gene was low [26]. As we have given examples above, there are many studies on annexins in the literature. However, only one study examining the relationship of ANXA3 molecule with these psychiatric cases appears in the literature. This study was conducted by Joaquim et al. in 2019 with 28 schizophrenia patients, 27 bipolar patients and 30 healthy controls [4]. In this study conducted on patients diagnosed according to DSM-IV and who have not yet received treatment, it was reported that ANXA3 levels were lower in schizophrenia patients than in bipolar disorder and healthy control groups. 20 of 28 schizophrenia patients did not have detectable ANXA3 levels. On the other hand, detectable ANXA3 levels were observed in all of the bipolar disorder and healthy control groups. Unlike our results, there was no difference between the bipolar and healthy groups in terms of ANXA3 levels. ANXA3 analysis in the study groups was performed by western blot method which is superior to ELISA method.

Studies with larger samples have been suggested to investigate the role of decreased ANXA3 levels as a possible risk factor for schizophrenia. Although there is no study conducted on these patient groups, an important study in this respect is available in the literature since it is related to the central nervous system. An important article published in 2021 investigated ANXA3 expression in microglia. In this study, the structure and localization of ANXA3-labeled microglia cells were reported to be quite similar to those labeled by the CD11b and Iba-1 markers. ANXA3 was almost never detected in non-parenchymal macrophages. Loss of ANXA3 has been shown to suppress microglia proliferation and motility. In conclusion, in this study,

it was reported that ANXA3 may have a new biomarker and physiological functions for microglia [27].

In the study by Jose et al. T4 levels were found to be higher in patients with schizophrenia in contrast to our study [28]. Saedisomeolia et al. reported lower folate levels in patients with schizophrenia in parallel with our study, but higher B12 levels in patients with schizophrenia in contrast to our results [29].

In 2019, a study conducted in Iran showed that vitamin D supplementation therapy has beneficial effects in schizophrenia [30]. This is in parallel with our results.

Limitations of study

As in every study, we know that our study has some limitations. Among the limitations that should be noted were the fact that it was a single-center study, that a large patient population could not be reached, and that patients' diseases such as diabetes mellitus, vitamin deficiencies, thyroid diseases, anemia and renal failure could not be followed up because they could directly affect the parameters we looked at in the study. Confirmation of ELISA results with genetic analyzes was another important limitation of this study.

Conclusion

According to the results of our study, we believe that ANXA3 molecule can be used as a biomarker in the diagnosis of schizophrenia and bipolar disorder. The fact that there are not many studies on this subject in the literature makes our study more valuable. Multicenter and long-term studies may be useful for generalizing all our results.

Conflict of Interests

The authors declare that there is no conflict of interest in the study.

Financial Disclosure

The authors declare that they have received no financial support for the study.

Ethical Approval

The study protocol was approved by the Amasya University Ethics Committee (Dated: 03.03.2022; Acceptance Number: 2022-03/34).

References

- Smoller JW, Finn CT. Family, twin, and adoption studies of bipolar disorder. *Am J Med Genet C Semin Med Genet.* 2003;123C:48-58.
- McIntyre RS, Berk M, Brietzke E, et al. Bipolar disorders. *Lancet.* 2020;396:1841-56.
- Hegarty JD, Baldessarini RJ, Tohen M, et al. One hundred years of schizophrenia: a meta-analysis of the outcome literature. *Am J Psychiatry.* 1994;151:1409-16.
- Joaquim HPG, Costa AC, Serpa MH, et al. Reduced Annexin A3 in schizophrenia. *Eur Arch Psychiatry Clin Neurosci.* 2020;270:489-94.
- Lee SH, Lee PH, Kim BG, et al. Annexin A5 protein as a potential biomarker for the diagnosis of asthma. *Lung.* 2018;196:681-9.
- Hedhli N, Falcone DJ, Huang B, et al. The annexin A2/S100A10 system in health and disease: emerging paradigms. *J Biomed Biotechnol.* 2012;2012:406273.

7. Weiss R, Bitton A, Ben Shimon M, et al. Annexin A2, autoimmunity, anxiety and depression. *J Autoimmun.* 2016;73:92-9.
8. Wu N, Liu S, Guo C, et al. The role of annexin A3 playing in cancers. *Clin Transl Oncol.* 2013;15:106-10.
9. Lessner G, Schmitt O, Haas SJ, et al. Differential proteome of the striatum from hemiparkinsonian rats displays vivid structural remodeling processes. *J Proteome Res.* 2010;9:4671-87.
10. Sohma H, Imai S, Takei N, et al. Evaluation of annexin A5 as a biomarker for Alzheimer's disease and dementia with lewy bodies. *Front Aging Neurosci.* 2013;5:15.
11. Gerke V, Moss SE. Annexins: from structure to function. *Physiol Rev.* 2002;82:331-71.
12. Hofmann A, Raguénès-Nicol C, Favier-Perron B, et al. The annexin A3-membrane interaction is modulated by an N-terminal tryptophan. *Biochemistry.* 2000;39:7712-21.
13. Chen WQ, Viidik A, Skalicky M, et al. Hippocampal signaling cascades are modulated in voluntary and treadmill exercise rats. *Electrophoresis.* 2007;28:4392-400.
14. Weitzdörfer R, Höger H, Shim KS, et al. Changes of hippocampal signaling protein levels during postnatal brain development in the rat. *Hippocampus.* 2008;18:807-13.
15. Geisow MJ, Walker JH, Boustead C, Taylor W. Annexins--new family of Ca²⁺-regulated-phospholipid binding protein. *Biosci Rep.* 1987;7:289-98.
16. Auer DP, Wilke M, Grabner A, et al. Reduced NAA in the thalamus and altered membrane and glial metabolism in schizophrenic patients detected by 1H-MRS and tissue segmentation. *Schizophr Res.* 2001;52:87-99.
17. Ben-Shachar D, Laifenfeld D. Mitochondria, synaptic plasticity, and schizophrenia. *Int Rev Neurobiol.* 2004;59:273-96.
18. Blüml S, Tan J, Harris K, et al. Quantitative proton-decoupled 31P MRS of the schizophrenic brain in vivo. *J Comput Assist Tomogr.* 1999;23:272-5.
19. Deicken RF, Calabrese G, Merrin EL, et al. 31phosphorus magnetic resonance spectroscopy of the frontal and parietal lobes in chronic schizophrenia. *Biol Psychiatry.* 1994;36:503-10.
20. Jensen JE, Miller J, Williamson PC, et al. Focal changes in brain energy and phospholipid metabolism in first-episode schizophrenia: 31P-MRS chemical shift imaging study at 4 Tesla. *Br J Psychiatry.* 2004;184:409-15.
21. Lutkenhoff ES, van Erp TG, Thomas MA, et al. Proton MRS in twin pairs discordant for schizophrenia. *Mol Psychiatry.* 2010;15:308-18.
22. Miller J, Drost DJ, Jensen E, et al. Progressive membrane phospholipid changes in first episode schizophrenia with high field magnetic resonance spectroscopy. *Psychiatry Res.* 2012;201:25-33.
23. Pettegrew JW, Keshavan MS, Panchalingam K, et al. Alterations in brain high-energy phosphate and membrane phospholipid metabolism in first-episode, drug-naïve schizophrenics. A pilot study of the dorsal prefrontal cortex by in vivo phosphorus 31 nuclear magnetic resonance spectroscopy. *Arch Gen Psychiatry.* 1991;48:563-8.
24. Stanley JA, Williamson PC, Drost DJ, et al. An in vivo study of the prefrontal cortex of schizophrenic patients at different stages of illness via phosphorus magnetic resonance spectroscopy. *Arch Gen Psychiatry.* 1995;52:399-406. Erratum in: *Arch Gen Psychiatry* 1995;52:799.
25. Francesconi LP, Ceresér KM, Mascarenhas R, et al. Increased annexin-V and decreased TNF- α serum levels in chronic-medicated patients with schizophrenia. *Neurosci Lett.* 2011;502:143-6.
26. Liu CM, Fann CS, Chen CY, et al. ANXA7, PPP3CB, DNAJC9, and ZMYND17 genes at chromosome 10q22 associated with the subgroup of schizophrenia with deficits in attention and executive function. *Biol Psychiatry.* 2011;70:51-8.
27. Zhang Z, Li Z, Ma Z, et al. Annexin A3 as a marker protein for microglia in the central nervous system of rats. *Neural Plast.* 2021;2021:5575090.
28. Jose J, Nandeesha H, Kattimani S, et al. Association between prolactin and thyroid hormones with severity of psychopathology and suicide risk in drug free male schizophrenia. *Clin Chim Acta.* 2015;444:78-80.
29. Saedisomeolia A, Djalali M, Moghadam AM, et al. Folate and vitamin B12 status in schizophrenic patients. *J Res Med Sci.* 2011;16:S437-41.
30. Ghaderi A, Banafshe HR, Mirhosseini N, et al. Clinical and metabolic response to vitamin D plus probiotic in schizophrenia patients. *BMC Psychiatry.* 2019;19:77.