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RESEARCH

Comparative analysis of COMT expression levels in blood and exosome-derived samples of patients with schizophrenia

Şizofreni tanılı hastalarda kan ve eksozom kaynaklı örneklerde COMT ekspresyon düzeylerinin karşılaştırmalı analizi

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Abstract

Purpose: The objective of this study was to analyse the variability of COMT expression in peripheral blood and exosome samples from individuals diagnosed with schizophrenia and to establish its correlation with the drugs employed in the treatment of schizophrenia.

Materials and Methods: 45 subjects diagnosed with chronic schizophrenia were included in the patient group and 45 healthy volunteers with no family history of chronic schizophrenia were included in the control group. RNA isolation from exosome and peripheral blood was performed for each individual in the sample group. Real-time polymerase chain reaction was used to determine COMT expression levels obtained from peripheral blood and exosome samples and their association with drugs used in antipsychotic therapy was evaluated.

Results: Differential gene expression analysis was performed using the 2-ΔΔCT method. The analysis revealed an increase in COMT gene expression levels in exosome samples. Similarly, an increase in COMT expression levels was detected in peripheral blood samples. In peripheral blood samples, 31 patients exhibited an increase and 14 patients exhibited a decrease. In the exosome samples, a decrease in COMT gene expression was observed in 25 subjects, while an increase was observed in 20 subjects. A statistically significant difference was found between COMT expression levels in peripheral blood and exosome samples.

Conclusion: Investigation of alterations in COMT expression levels in schizophrenia and its treatment may provide a valuable framework for future studies to elucidate the mechanisms of this psychiatric disorder.

Keywords: Schizophrenia, COMT, exosome, antipsychotic treatment, biomarker

Öz

Amaç: Bu çalışmanın amacı, şizofreni tanısı almış bireylerden elde edilen periferik kan ve eksozom örneklerinde COMT ekspresyonundaki değişkenliği analiz etmek ve bu ekspresyon seviyelerinin şizofreni tedavisinde kullanılan ilaçlarla olan korelasyonunu belirlemektir.

Gereç ve Yöntem: Çalışmaya, kronik şizofreni tanılı 45 hasta ile ailesinde kronik şizofreni öyküsü bulunmayan 45 sağlıklı gönüllü kontrol grubu olarak dahil edilmiştir. Tüm bireylerden periferik kan ve eksozom örnekleri alınarak RNA izolasyonu gerçekleştirilmiştir. Periferik kan ve eksozom örneklerinden elde edilen COMT ekspresyon seviyeleri, gerçek zamanlı polimeraz zincir reaksiyonu yöntemi ile belirlenmiş ve antipsikotik tedavide kullanılan ilaçlarla olan ilişkisi değerlendirilmiştir.

Bulgular: Diferensiyal gen ekspresyon analizi 2-ΔΔCT yöntemi kullanılarak gerçekleştirilmiştir. Yapılan analizde, eksozom örneklerinde COMT gen ekspresyon seviyelerinde artış tespit edilmiştir. Periferik kan örneklerinde 31 hastada COMT ifadesinde artış, 14 hastada düşüş saptanmıştır. Eksozom örneklerinde 25 hastada COMT ifadesi azalırken 20 hastada artmıştır. Periferik kan ile eksozom örneklerindeki COMT ekspresyon seviyeleri arasında istatistiksel olarak anlamlı bir farklılık saptanmıştır.

Sonuç: Şizofreni hastalarında COMT ekspresyon seviyelerindeki değişimlerin ve bu değişimlerin şizofreni tedavisinde kullanılan ilaçlarla olan ilişkilerinin incelenmesi, bu psikiyatrik bozukluğun altında yatan mekanizmaların aydınlatılmasına yönelik gelecekteki çalışmalara önemli bir çerçeve sağlayabilir.

Anahtar kelimeler: Şizofreni, COMT, eksozom, antipsikotik tedavi, biyobelirteç

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INTRODUCTION

Schizophrenia (SCZ) is a mental illness with significant public health implications, including positive, negative and cognitive symptoms[1]. SCZ is a chronic disorder with a complex etiology that involves genetic, neurochemical and environmental factors, often leading to functional impairment. There is significant interest in identifying the genetic underpinnings of the disease. However, due to the significant impact of environmental factors such as childhood trauma and stress, the results obtained so far are still far from conclusive[2]. The catechol-O-methyltransferase (COMT) gene is located on chromosome 22q11.2 and is known as the enzyme that performs the methyl group transfer of S-adenosylmethionine from catecholamines, including the neurotransmitters dopamine, epinephrine and norepinephrine. Studies indicate that there is evidence of an irregularity in the COMT gene, which codes for the enzyme that reduces dopamine. It has been reported that the results of gene studies in COMT containing polymorphisms that predispose to SCZ are compatible[3,4]. The COMT gene has a common functional polymorphism at codon 158, where a nucleotide transition (G to A) causes a change in the amino acid sequence (Val to Met) in the protein product[4-8]. The COMT enzyme is widely expressed in the human brain and appears to play a particularly important role in dopamine levels in the prefrontal cortex[9]. The COMT enzyme is known to play a role in cognitive functions related to the dopaminergic system, which is particularly active in the

prefrontal cortex[10,11]. Some studies show that increased dopamine disruption resulting from COMT hyperactivity affects attention deficit, intelligence and working memory performance[12,13]. The genetic variation of COMT, which plays a catalytic role in dopamine degradation, is believed to have long contributed to susceptibility to schizophrenia and in particular, to negative symptoms[14].

Exosomes, typically 40-150 nm diameter nano-vesicles that play a critical role in cell communication, have been implicated in the pathophysiology of neuropsychiatric diseases but their role in SCZ is unclear[15]. Exosomes have emerged as potential biomarkers for neurological diseases, including SCZ. However, the study of exosomal lipids in the context of SCZ is still limited and requires more detailed research[16]. DNA, RNA, proteins and metabolites make up the exosomal contents. Exosomes are released from donor cells into the circulation to reach neighbouring and distant cells and are delivered to recipient cells where they regulate the phenotype of the target cells[15]. Exosomes provide a wealth of information about the functional state of cells. This information is also crucial in the cells of the central nervous system. Exosomes are extracellular vesicles that are released from cells into the bloodstream, carry biomolecular cargoes such as RNA and protein and play a role in physiological and pathological regulation in the body. These molecules loaded into exosomes have the potential to be biomarkers as they have properties such as signal transduction and cell communication[17-20]. Exosomes can be secreted by nearly all cells and are commonly found in body fluids (such as blood, urine, saliva, etc.). Thanks to their advantages as natural nanocarriers, exosomes can cross the blood brain barrier (BBB) and play an important role in communication within the central nervous system (CNS). They can also participate in significant physiological processes such as nerve regeneration, notable plasticity, axon support, and neuroinflammation. In recent years, exosomes have garnered considerable interest in the fields of neurodegenerative diseases and mental disorders, particularly as potential biomarkers. Furthermore, the molecular characterization of exosomes in the SCZ process is extremely important for patient diagnosis and personalized treatment approaches[21].

Variations in the hereditary mechanism of catecholamine metabolism, its pharmacology and catechol metabolism are very important because an enzyme molecule with an important function in dopamine pathways is encoded by COMT. Candidate genes associated with dopaminergic and serotonergic pathways are promising candidates for the treatment process in SCZ. Dopaminergic and serotonergic genes are being intensively studied for antipsychotic response in SCZ. Candidate gene studies continue to confirm the COMT gene and its association with SCZ[6,22].

Antipsychotics are neuroleptic drugs used to treat SCZ and psychotic disorders. The use of antipsychotics in SCZ is limited. These treatments are effective in half of patients and their positive symptoms improve but their negative and cognitive symptoms cannot be treated. They are thought to interact with neurotransmitter receptors. These are

the traditional molecular targets for antipsychotic drugs. The most important are dopamine, serotonin, and adrenaline receptors and several G protein-coupled receptors (GPCRs). The clinical effectiveness of antipsychotic drugs in helping people with SCZ to lead relatively normal lives has been demonstrated in controlled trials. Some patients do not respond to drug therapy. For example, Clozapine is often resistant to other neuroleptics recommended in patients. There are some assumptions that polymorphisms in the family of dopamine and serotonin receptors may play a role[1]. Investigating the molecular biology of the dopaminergic system will benefit the development of pharmaceutical interventions in the treatment of SCZ.

The objective of this study was to examine the expression levels of the COMT gene, derived from both exosome samples and peripheral blood, with a view to ascertaining whether antipsychotic drugs used by individuals diagnosed with schizophrenia exert a substantial influence on these expression levels. The results of this study will be the first data to be obtained on this subject in Turkey and the Manisa region.

MATERIALS AND METHODS

Sample

The patient group consisted of 45 patients who were diagnosed with SCZ in Department of Psychiatry Faculty of Medicine Hafsa Sultan Hospital Manisa Celal Bayar University, according to Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) criteria[23]. Also 45 patients had chronic SCZ for more than six months, had no acute exacerbation and were in remission according to Andreasen's criteria. Blood samples were taken after these patients (and/or their guardians) had the "Informed Consent Form" read and approved.

The control group consisted of 45 individuals who was read and approved of the "Informed Consent Form" and who had not been diagnosed with chronic SCZ and than blood samples were taken. This study was supported by Manisa Celal Bayar University Faculty of Medicine Scientific Research Project Commission. All molecular studies in this study were conducted in Manisa Celal Bayar University Faculty of Medicine, Department of Medical Biology, Molecular Biology Research Laboratory.

The inclusion criteria for the patient group were as follows: diagnosis of schizophrenia according to DSM-5 criteria; age over eighteen years; absence of a known organic brain disease history (Parkinson, dementia, epilepsy, intracranial lesion, etc.); and absence of mental retardation. The inclusion criteria for the control group were as follows: age over eighteen years; agreement to participate in the research voluntarily; and cooperation to follow the instructions of the research. The exclusion criteria for the patient group are as follows: the presence of alcohol and substance addiction, the presence of a metabolic disease, and the absence of the mental capacity to follow the test instructions in the study. The exclusion criteria for the control group are analogous to those for the patient group, with the exception of the inclusion of the additional criterion of metabolic disease.

Procedure

Ethics Approval for this study and approval for use of patient data were obtained by the Scientific Committee of Manisa Celal Bayar University Faculty of Medicine (Date and Number of Documents: 29/09/2017-E.75947 and 31/05/2018-E.49230). Written informed consent from the patients' legal guardians was obtained to participate in this study following the national legislation and the institutional requirements.

RNA isolation

Blood samples were collected in two distinct vacuum tubes: a 5 cc vacuum EDTA tube (Cat. No. 454217, Greiner Bio-One GmbH, Kremsmünster, Austria). The tubes were utilised under aseptic conditions. For the isolation of total RNA from the exosome, the peripheral blood was subjected to centrifugation at 3000 rpm for a duration of 10 minutes on the same day that the blood sample was collected. Total RNA isolation was performed from the exosomes with using the exoRNeasy Serum/Plasma Midi Kit (Cat.no.77044, Qiagen, Hilden, Germany), cellular RNA isolation was performed from the peripheral blood with using the QIAamp RNA Blood Mini Kit (Cat.no.52304, Qiagen, Hilden, Germany) in "QIAcube Fully Automatic Isolation Device" according to the manufacturer's protocol. Following the isolation of the RNA, its purity was measured at 260/280 nm and in ng/µl by means of a spectrophotometer. The resultant solution was then stored at -20°C until further analysis.

Gene expression analysis

The complementary DNA of each RNA sample was synthesised using the RT² First Strand Kit (Cat. No. 330411, Qiagen, Hilden, Germany) according to the protocol provided by the manufacturer for the analysis of RT-PCR based gene expression changes. COMT expression levels were determined by using complementary DNA, RT² SYBR Green qPCRMastermiks (Cat.no.330500, Qiagen, Hilden, Germany) and RT² qPCR primer assay Human COMT (Cat.no.330001, Qiagen, Hilden, Germany) according to the manufacturer's protocol, in a RT-PCR. Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH) gene region primer, RT² qPCR primer assay Human GAPDH (Cat.no.330001, Qiagen, Hilden, Germany), was used in each analysis as a control to ensure the separation of the data. COMT gene primer sets and GAPDH gene primer sets were purchased as a commercial kit for use in this study. Quantitation analysis was performed using the Rotor-Gene Q series software 2.3.1 program.

Statistical analysis

The sample size was calculated using the G*Power tool (https://www.psychologie.hhu.de/arbeitsgruppen/allgemeine-psychologie-und-arbeitspsychologie/gpower). Power: 80%, significance level (α): The calculation of effect size, based on Cohen's w of 0.3, was performed on a scale of 0.05. Following the power analysis, the sample was constituted by 44 patients and 44 healthy subjects. The sample size was deemed adequate to ensure statistical power in the specified parameters.

Statistical analysis was conducted utilising the IBM SPSS version 21.0 program. The Chi-Square (x2) p-value was calculated in order to determine the prevalence of various factors and the significance of changes in COMT gene expression levels. p values less than 0.05 were considered statistically significant ($p<0.05$).

RESULTS

RT-PCR data obtained from exosomes and peripheral blood samples was used to compare the mean $2^{-\Delta CT}$ value of the patient group with the mean $2^{-\Delta CT}$ value of the control group. The relative expression of the COMT gene between patient and control was found to be 1.18 in exosomes and 1.57 in peripheral blood (Figure 1). COMT expression levels were elevated in the patient group, as demonstrated by the study results.

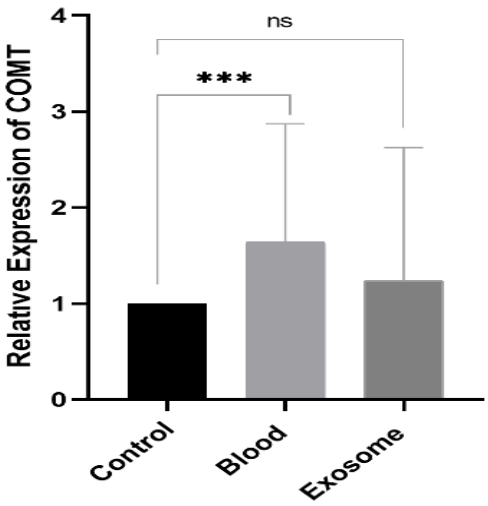

Figure 1. The relative gene expression levels of COMT in the exosome and peripheral blood of schizophrenia patients (*p< 0.01).**

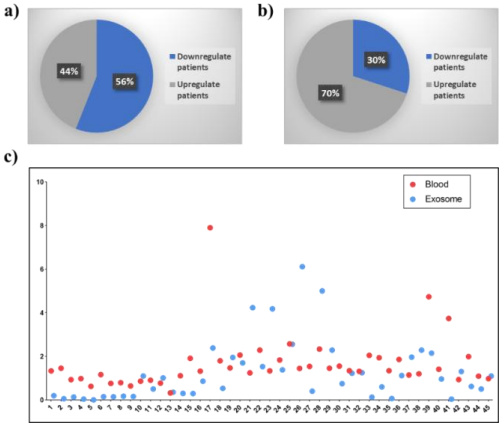

Figure 2. a) The ratio of COMT expression levels in the exosomes of patients b) The ratio of COMT expression levels in the peripheral blood samples of patients c) Relative expressions of COMT in the exosomes and peripheral blood samples of each patient.

The expression levels of COMT in the exosomes and peripheral blood samples of each patient was given in Figure 2a-c. The study cohort revealed heightened COMT expression levels in both exosome and peripheral blood samples, with a 44% elevation

observed in the respective exosome samples and a significant 70% increase detected in the peripheral blood samples. Conversely, exosome expression exhibited a 56% decrease, while peripheral blood expression demonstrated a 30% decrease. The correlation results between the antipsychotic drugs used by SCZ patients and COMT gene expression levels.

A considerable body of previous research has indicated that the aberrant expression of dopaminergic and serotoninergic genes within the brain may be a contributing factor to the development of SCZ. Moreover, experimental evidence has demonstrated that antipsychotic treatment can modulate the expression of these genes, suggesting that they may play a role in the alleviation of symptoms during treatment. Antipsychotic drugs used by SCZ patients are listed in Table 1 and COMT expressions of blood and exosome samples of SCZ patients are given in Table 2. Subsequent analysis was conducted on the relationship between the antipsychotic medications administered to patients with SCZ and their COMT expression levels in their peripheral blood and exosome samples, as illustrated in Figures 3 and 4.

Table 1. Antipsychotic drug treatment of schizophrenia patients

Patient	Gender	Age	Antipsychotic Drug Treatment
1	F	37	Risperidone** 3 mg/Day
2	F	32	Olanzapine** 20 mg/Day
3	F	49	Clozapine** 500 mg/Day Aripiprazole** 5 mg/Day
4	M	32	Clozapine** 400 mg/Day Aripiprazole** 400 mg/month
5	M	26	Clozapine** 200 mg/Day Aripiprazole** 5 mg/Day
6	M	27	Clozapine** 600 mg/Day Aripiprazole** 30 mg/Day
7	F	54	Clozapine** 200 mg/Day
8	M	40	Clozapine** 300 mg/Day
9	M	56	Aripiprazole** 30 mg/Day Risperdal** consta 50 mg/every fortnight Quetiapine 200 mg/Day
10	F	31	Olanzapine** 20 mg/Day Zuclopenthixol* 200 mg/once a month
11	F	31	Aripiprazole** 30 mg/Day
12	M	32	Clozapine** 200 mg/Day Paliperidone** 100 mg / once a month
13	M	43	Aripiprazole** 10 mg/Day
14	M	51	Olanzapine** 10 mg/Day Aripiprazole** 30 mg/Day
15	F	53	Quetiapine** 600 mg/Day Zuclopenthixol* 200 mg / every fortnight
16	F	47	Paliperidone** 6 mg/Day
17	F	44	Aripiprazole** 10 mg/Day
18	M	23	Amilsulpride** 400 mg/Day
19	M	50	Risperdal** consta 50 mg/ every fortnight
20	M	25	Olanzapine** 20 mg/Day Zuclopenthixol* 200 mg/ every fortnight
21	M	27	Olanzapine** 20 mg/Day Risperdal** consta 50 mg/every fortnight
22	M	46	Amilsulpride** 400 mg/Day Quetiapine** 100 mg/Day
23	M	53	Risperidone** 2 mg/Day Quetiapine** 50 mg/Day
24	F	56	Zuclopenthixol* 4 mg/Day

			Quetiapine** 400 mg/Day
25	M	66	Paliperidone** 150 mg/Day
26	M	54	Olanzapine** 20 mg/Day Quetiapine** 300 mg/Day
27	M	30	Clozapine** 250 mg/Day Aripiprazole** 10 mg/Day
28	F	59	Olanzapine** 20 mg/Day Zuclopenthixol* 200 mg/ every fortnight
29	M	38	Olanzapine** 20 mg/Day Quetiapine** 600 mg/Day
30	M	28	Clozapine** 300 mg/Day Aripiprazole** 5 mg/Day
31	F	28	Olanzapine** 10 mg/Day
32	F	63	Amilsulpride** 800 mg/Day
33	F	42	Olanzapine** 10 mg/Day
34	F	38	Ziprasidone** 120 mg/Day Flupentixol* 20 mg/ once a month
35	M	65	Paliperidone** 6 mg/Day
36	F	58	Olanzapine** 15 mg/Day
37	M	27	Olanzapine** 15 mg/Day
38	M	34	Risperidone** 6 mg/Day Haloperidol* 10 mg/Day
39	F	36	Olanzapine** 20 mg/Day Trifluoperazine* 2 mg/Day
40	M	43	Clozapine** 400 mg/Day Zuclopenthixol* 2 mg/Day Aripipirozal** 30 mg/Day
41	F	46	Aripiprazole** 5 mg/Day Quetiapine** 800 mg/Day
42	M	54	Olanzapine** 10 mg/Day
43	M	47	Risperidone** 4 mg/Day Zuclopenthixol* 200 mg/ every fortnight
44	F	29	Aripiprazole** 10 mg/Day
45	F	25	Aripiprazole** 15 mg/Day Olanzapine** 15 mg/Day Quetiapine** 300 mg/Day

*Typical Antipsychotics, **Atypical Antipsychotics

Table 2. COMT expressions in blood and exosome of schizophrenia patients

Patient	COMT expression (Blood)	COMT expression (Exosome)
1	1.330529	0.193713983
2	1.45599	0.057193698
3	0.927873	0.133230842
4	0.974004	0.038794609
5	0.625031	0.002923678
6	1.158293	0.149892489
7	0.75891	0.145793671
8	0.791137	0.170991913
9	0.638164	0.158439065

10	0.859756	1.095811766
11	0.902501	0.500693628
12	0.769504	1.008352455
13	0.321302	0.356506429
14	1.111109	0.299784978
15	1.907919	0.293615493
16	1.321338	0.859756486
17	7.900807	2.381713699
18	1.792533	0.532923368
19	1.466117	1.948008537
20	2.05908	1.695839929
21	1.241427	4.23393758
22	2.284692	1.528376521
23	1.330529	4.17564771
24	1.830198	1.377450046
25	2.570413	2.552657538
26	1.445932	6.113506083
27	1.539007	0.401090583
28	2.332699	5.00024921
29	1.45599	2.284692494
30	1.549712	0.743291492
31	1.339784	1.224336392
32	1.312211	1.250062303
33	2.044857	0.12869251
34	1.934553	0.599569957
35	1.339784	0.066615421
36	1.855747	1.118837101
37	1.142346	1.961558008
38	1,19914	2.284692494
39	4.730524	2.146520573
40	1.406393	0.960594864
41	3.737309	0.034482304
42	0.934327	1.303147149
43	1.98894	0.620713746
44	1.088242	0.500693628
45	0.974004	1.095811766

In the evaluation of the patient group, it was determined that 17 patients utilised a single pharmaceutical agent, while 28 patients employed multiple agents in their therapeutic regimens. Subsequent to the evaluation of COMT gene expression levels obtained from peripheral blood, it was ascertained that the expression levels of 12 patients who were administered a single pharmaceutical agent exhibited an increase, while the expression levels of 5 patients who were administered a single pharmaceutical agent exhibited a decrease. In a cohort of 28 patients undergoing treatment with multiple drugs, elevated COMT gene expression levels were observed in 19 patients, while the remaining 9 patients exhibited reduced expression levels (Figure 3).

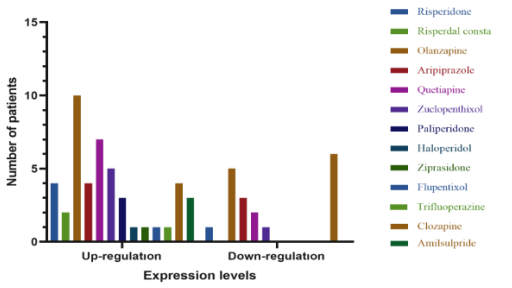

Figure 3. The relationship between COMT expression levels and antipsychotic drugs in peripheral blood samples.

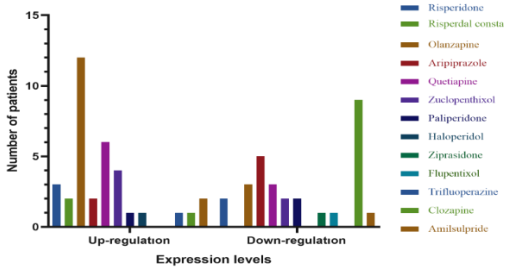

Figure 4. The relationship between COMT expression levels and antipsychotic drugs in exosome samples.

An evaluation of the COMT gene expression levels obtained from the exosome samples revealed that 7 patients exhibited increased expression levels when administered single drugs, while 10 patients demonstrated decreased expression levels when administered multiple drugs. COMT gene expression levels of 13 patients using multiple drugs were found to be upregulated, while the levels of 15 patients were downregulated (Figure 4). Briefly, in exosome samples, 25 subjects exhibited a decrease in COMT gene expression, while 20 exhibited an increase.

A significant discrepancy was identified through statistical analysis, examining COMT gene expression levels in RNA extracted from peripheral blood and those extracted from exosome samples ($p=0.007$).

In this study, no statistically significant difference was observed in the expression levels of COMT in the peripheral blood ($p=0.804$) or at the exosome level ($p=0.124$) between patients using single or multiple drugs. However, an increasing trend in the expression levels of the COMT gene was observed in patients using multiple drugs but this difference was not statistically significant.

DISCUSSION

Schizophrenia is a complex and debilitating psychiatric disorder that affects approximately 1% of the population. The dysfunction of neurotransmitter systems has thus far been identified as a pathological feature of SCZ. A plethora of candidate genetic association studies of dopaminergic and serotoninergic genes for SCZ and antipsychotic response have been conducted extensively. In the extant literature, the greatest focus has been on four candidate genes (DRD2, COMT, 5-HTR2A, and SLC6A4) from the dopaminergic and serotoninergic pathways, which have been hypothesised to be promising candidate genes for SCZ and treatment[6]. It is clear that the role of COMT in dopamine metabolism is prompting investigations into its variants. These investigations are looking into the aetiology of numerous psychiatric disorders. Among these are psychotic, affective and anxiety disorders. The majority of work focuses on SCZ and bipolar disorder[24]. Despite the prevailing consensus that SCZ is a neuropsychiatric disorder that materialises primarily in the brain, a number of studies have identified various biological markers in the peripheral blood of patients with SCZ[25]. A particular study revealed that human COMT expression occurs in lymphoblasts. The investigation established a strong correlation between gene expression in lymphocytes and that of the brain. The findings of the study indicate that peripheral blood markers also reflect the

relevant expression and functional changes of COMT in the brain[26].

The prevention of drug resistance in the treatment of SCZ is a significant clinical necessity, as evidenced by the utilisation of drugs derived from incidental clinical observations[1,27]. The underlying pathomechanism of SCZ remains to be fully elucidated, resulting in the presence of substantial limitations in the current antipsychotic drugs. A recent study revealed that SCZ patients who did not use antipsychotic drugs during an acute episode exhibited significantly elevated levels of COMT mRNA expression in their peripheral blood, as compared to individuals in the control group[6].

The primary targets of antipsychotic drugs in the treatment of SCZ are the cerebral dopamine and serotonin receptor systems. For instance, the most efficacious atypical antipsychotics, including risperidone, olanzapine and clozapine, exhibit the highest affinity for D2 dopamine and 5-HT2A receptors[28]. Recent findings have indicated the potential of peripheral gene expression as a source of diagnostic information in neuropsychiatric diseases, with the capacity to monitor drug responses, despite the presence of methodological and theoretical limitations. In the present study, no statistically significant differences were observed in COMT expression levels in peripheral blood ($p = 0.804$) or their exosome levels ($p = 0.124$) among patients who used either single or multiple drugs. A non-significant difference was found in the expression of the COMT in the peripheral blood of patients receiving clozapine therapy ($p = 0.065$); however, a significant difference was identified in the expression of the COMT gene in the exosome sample ($p = 0.014$). It is estimated that 51.4% of patients who exhibit upregulation at the COMT gene expression level in their periphery would also display upregulation in their extracellular vesicles. Meanwhile, the probability of downregulation in the exosomes of patients who display downregulation in the COMT gene expression level in their periphery is 73.3%.

Clozapine is the most efficacious of all the antipsychotic drugs, and it is a widely recognised treatment option for patients with drug-resistant conditio. This is due to it having the highest efficacy of all the antipsychotic drugs. While there has been a demonstrated correlation between chronic treatment with antipsychotics, such as haloperidol and olanzapine, and activation of the microglia morphology in the brain, the impact of clozapine appears to be distinguishable [29]. Exosomes, which are derived from multivesicular bodies (MVBs) originating from early endosomes carry neurotransmitter receptors on their membranes[30]. Exosomes have been demonstrated to function as vehicles for the transfer of functional proteins and RNA between cell. These vehicles have been shown to significantly influence cellular functions, including those related to cognition. This has led to the consideration that clozapine-induced changes in exosome profiles could be a key factor in the drug's impact on cognitive processes, via the exertion of a part of their therapeutic effect through the modulation of the content of these vesicles[29].

The evaluation of the patients revealed that 10 of them were using Clozapine in their treatment regimen, while 35 patients were not. The evaluation of the COMT gene expression levels obtained from the exosome revealed that the levels of the 10 patients using Clozapine were downregulated. In contrast, among the 35 patients who did not use clozapine, 20 patients exhibited increased COMT gene expression levels, while 15 patients showed decreased expression. In addition, a decline in COMT expression was observed in both blood and exosome samples in patients with schizophrenia who were treated with a combination of aripiprazole, risperidone, and quetiapine.

The treatment of neuropsychiatric disorders is constrained by the presence of the blood–brain barrier, which engenders suboptimal therapeutic outcomes. Furthermore, a significant number of pharmaceuticals, most notably antipsychotic medications, have been observed to induce adverse reactions, thereby complicating their tolerability for patients. The capacity of exosomes to traverse the brain-blood barrier freely enables them to reflect the state of the brain, a property that confers significant advantages in diagnostic and therapeutic applications[31]. Recent studies have explored the distinction of exosome levels across different SCZ a subtypes such as late-onset SCZ, treatment-resistant SCZ and deficit SCZ[32]. This is because the aetiology of these conditions may vary with exosome levels potentially impacting these different pathways. In order to elucidate the underlying mechanism linking the alteration of exosome and SCZ and to verify the long-term therapeutic effects of targeting exosomal contents, further research is required in the form of larger and longitudinal studies.

The importance of conducting studies in this area is paramount. Consequently, this investigation sought to examine the expression levels of the COMT gene, derived from both exosome samples and peripheral blood. Furthermore, this study sought to ascertain whether antipsychotic drugs utilized by individuals diagnosed with SCZ exert a substantial influence on these expression levels. In view of the findings of this study, there is a clear need for further research to elucidate the impact of antipsychotic drugs, particularly Clozapine, on RNA expression levels within the COMT. The present study aims to discern the potential benefits or drawbacks of these drugs on the COMT expression. It is anticipated that this study, which investigates the effect of antipsychotic drugs used by SCZ patients on COMT expression levels, will prove beneficial to researchers working in this field. The study's limitations can be attributed to the heterogeneity of the patient population, which is attributable to the variation in treatment dosage and pharmaceutical agents administered. With the support of larger sample sizes and functional studies, it will be beneficial in the prevention and treatment of the disease in future studies.

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Ethical Approval: Ethics Approval for this study and approval for use of patient data were obtained by the Scientific Committee of Manisa Celal Bayar University Faculty of Medicine (Date and Number of Documents: 29/09/2017-E.75947 and 31/05/2018-E.49230). Written informed consent from the patients' legal guardians was obtained to participate in this study following the national legislation and the institutional requirements.

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