ORIGINAL ARTICLE

CONTENTS

Study of the influence of disease duration on glutatione-dependent ensymes dynamics in patients with paranoid schizophrenia

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ABSTRACT

Aim: The objective of the research was to conduct a comprehensive longitudinal analysis of the temporal dynamics of glutathione system functionality in individuals diagnosed with paranoid schizophrenia. Specifically, the research was focused on investigating variations in the profiles of glutathione-dependent enzymes, with meticulous consideration given to the duration of the illness.

Materials and Methods: The study group comprised 300 individuals officially diagnosed with 'Paranoid Schizophrenia,' subdivided into five subgroups, each consisting of 60 patients. The subgroups were defined as follows: Subgroup I included 60 patients with a disease duration ranging from 3 to 5 years; Subgroup II comprised 60 patients with a duration of 6 to 10 years; Subgroup III consisted of 60 patients with a duration of 11 to 15 years; Subgroup IV included 60 patients with a duration of 16 to 20 years; and Subgroup V encompassed 60 patients with a duration of 21 years and older. The comparison group comprised 20 patients diagnosed with "Primary psychotic episode".

Results: The research demonstrates a consistent and noteworthy reduction in the enzymatic activities of glutathione peroxidase, glutathione reductase, and glutathione-S-transferase in various Subgroups of paranoid schizophrenia patients. The observed declines are particularly prominent within the first 3-5 years of the illness, show casing statistically significant reductions. Patients with prolonged illness durations, especially surpassing 21 years, display substantial reductions in all three enzymes, suggesting a cumulative enzymatic impact associated with prolonged illness.

Conclusions: The identification of critical periods of inhibition in the glutathione protection chain, provides valuable information about potential therapeutic interventions for individuals with paranoid schizophrenia.

KEY WORDS: schizophrenia, antioxidant defense system, oxidative stress, glutathione-dependent enzymes, neuroinflammation, neuroplasticity

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INTRODUCTION

The most prevalent contemporary mental illnesses include schizophrenia and schizotypal disorders. Schizophrenia is a severe mental illness and affects approximately 1% to 2.5% of the global population, according to various estimates [1-3]. Unfortunately, the lack of dependable biomarkers poses a formidable challenge to the early-stage detection of chizophrenia. Consequently, the development of effective early diagnosis and therapy for schizophrenia holds paramount significance in medicine, particularly within the field of psychiatry.

According to scientific data, oxidative stress (OS) becomes a convergence point for the influence of genetic, environmental and behavioral risk factors for schizophrenia during neuro-development. OS is caused by an excess of free radicals generated due to cellular metabolic stress and impairment of the antioxidant defense system (ADS), known to cause membrane dysfunction involved in the pathophysiology of schizophrenia. Although it cannot be the primary cause of schizophrenia, there is substantial evidence indicating that OS can complicate the course and cause difficulties in the treatment of patients within this category. Numerous scientific works indicate the complex dynamics of redox regulation mechanisms and their modulation in schizophrenia [1, 4].

The antioxidant system as a pivotal defense mechanism within the human body, assumes a fundamental role by actively participating in metabolic processes, thereby maintaining balance across all phases of free radical peroxidation (FRP) [5]. This intricate defense system orchestrates interconnected reverse redox reactions (RR), involving metal ions, glutathione, tocopherols, phospholipids, "trigger hormones", and other bioactive substances. Otherwise speaking, the human body is endowed with a precision-targeted system designed to protect it from oxidative damage induced by reactive oxidants. The glutathione system plays a key role in providing antioxidant protection.

This system comprises reduced glutathione (GSH) and enzymes of its metabolism, including glutathione peroxidase (GPx), glutathione-S-transferase (GST), and glutathione reductase (GR) [6]. This intricate network of molecules and enzymes underscores the sophisticated and finely tuned nature of the body's defense against OS, contributing significantly to the maintenance of cellular homeostasis.

AIM

To conduct a comprehensive longitudinal analysis of the temporal dynamics of glutathione system functionality in individuals diagnosed with paranoid schizophrenia. Specifically, the research was focused on investigating variations in the profiles of glutathione-dependent enzymes, with meticulous consideration given to the duration of the illness. Through this investigation, the objective was to provide nuanced insights into the relation between disease progression and the complicated molecular alterations within the glutathione system, thereby contributing to a deeper understanding of the pathophysiological mechanisms underlying paranoid schizophrenia.

MATERIALS AND METHODS

The study involved the analysis of glutathione-dependent enzymes indicators in the blood serum of 320 patients in order to achieve the research objective. The study group comprised 300 individuals officially diagnosed with 'Paranoid Schizophrenia,' subdivided into five subgroups, each consisting of 60 patients. The criteria for such distribution were based on the duration of the primary disease, according to official medical documentation. The subgroups were defined as follows: Subgroup I included 60 patients with a disease duration ranging from 3 to 5 years; Subgroup II comprised 60 patients with a duration of 6 to 10 years; Subgroup III consisted of 60 patients with a duration of 11 to 15 years; Subgroup IV included 60 patients with a duration of 16 to 20 years; and Subgroup V encompassed 60 patients with a duration of 21 years and older. The comparison group comprised 20 patients diagnosed with "Primary psychotic episode". The clinical assessment of patients was performed by using the Positive and Negative Syndrome Scale (PANSS).

Blood plasma from study participants was collected at a fixed time, between 07:00 and 09:00, following an overnight fast. Subsequent to sample centrifugation, the plasma was transferred to 1.5 ml Eppendorf tubes and frozen at -30 °C. Biological markers were determined by a specialist from the accredited biochemical laboratory of the Center for Microelementology of the Ivano-Frankivsk National Medical University at the Department of Biological and Medical Chemistry named after Academician Babenko G.O. The analysis was conducted by standard methods and strictly adhered to established protocols.

The functional state of the enzymatic system of detoxification and the antioxidant defense system (ADS) was assessed through the activity levels of glutathione-dependent enzymes. Specifically, the activity of glutathione peroxidase was determined by the reaction of reduced glutathione with tert-Butyl hydroperoxide (tBuOOH) and calculated in micromoles of oxidized glutathione per milligram of protein per minute (μmol/(min×mg)). Glutathione reductase activity was measured by the rate of change in optical density at 340 nm due to the oxidation of Nicotinamide adenine dinucleotide phosphate (NADPH) and expressed in nmol/(min × ml). The activity of glutathione-S-transferase was determined by the rate of formation of glutathione-S-conjugates between reduced glutathione and 1-chloro-2,4-dinitrobenzene (CDNB), expressed in nmol/(min×mg).

The research adhered to ethical guidelines, including the Declaration of Helsinki (1964-2013), GCP (1996), ICH GCP (1996), the Council of Europe Convention on Human Rights and of Biomedicine (from 04.04.1997), EU Directive No. 609 (from 11.24.1986), and relevant Ukrainian Ministry of Health orders. Patient participation was possible upon signing the 'Voluntary Informed Consent of the Patient to Participate in the Study' form. The clinical and laboratory research protocol received approval from the Ivano-Frankivsk National Medical University Ethics Committee (protocol N 135/23 dated 05/24/2023).

The «STATISTICA 8.0» software package (StatSoft, Serial STA862D175437Q) and the statistical functions of «Microsoft Excel 2016» were used for the statistical analysis of the obtained results. The reliability of the data was confirmed by calculating errors for relative values. The probability of data differences between the compared groups was established using the t (Student) coefficient, with the accuracy of the error-free prediction determined according to the table. Quantitative characteristics were described using the arithmetic mean (M) and standard error (±m) [7].

RESULTS

According to Fig. 1, a probable decrease in the value of glutathione peroxidase was observed in all experimental subgroups. Subgroups I and II, including patients with a disease duration of 3-5 and 6-10 years, respec-

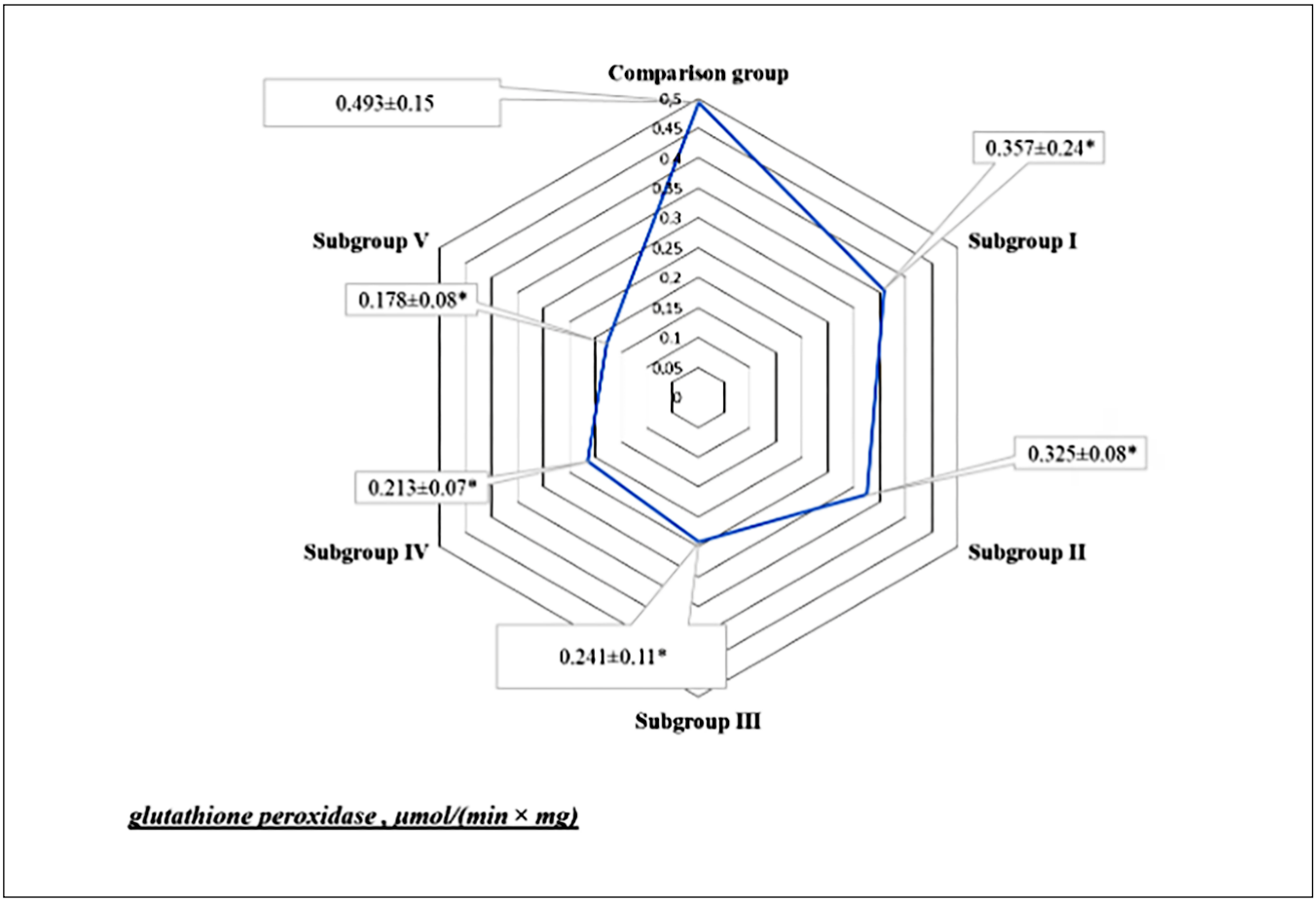

Fig. 1. Dynamics of the glutathione peroxidase indicator in patients with paranoid schizophrenia depending on the disease duration.
Note: * – ($p<0.05$) the data are reliable between the Comparison and Study Subgroups.

tively, demonstrated statistically significant reductions, emphasizing early changes in these enzymatic activities. This indicator was 0.357±0.24 μmol/(min×mg) in Subgroup I which was 27.5% less than in the Comparison group ($p<0.05$). In Subgroup II, this indicator constituted 0.325±0.08 μmol/(min×mg), representing 34.07% decrease compared to the Comparison group ($p<0.05$). In Subgroup III, the value of glutathione peroxidase was 0.241±0.11, more than 50% lower, respectively ($p<0.05$). A sharp decrease in the level of glutathione peroxidase by more than half was observed in patients who suffered from the paranoid form of schizophrenia for more than 10 years: by 51.11% in Subgroup III, by 56.79% in Subgroup IV, by 63.89% in Subgroup V.

Monitoring the dynamics of this indicator, we notice that the first "downfall" occured within the first 3-5 years of the disease, constituting a decrease of 27.58%. Over the next five years, this indicator decreased by another 6.49%. The second sharp "downfall" was observed after 10 years of the disease. The indicator decreased by more than 17% in the examined patients of Subgroup III compared to patients in Subgroup II. In patients of Subgroup IV, the history of the main disease was 16-20 years, and the value of glutathione peroxidase was 0.213±0.07 μmol/(min×mg), being 56.79% lower than in the Comparison group and 5.67% lower than in patients from Subgroup III. In Subgroup V patients suffering from paranoid schizophrenia over 21 years, this indicator was 0.178±0.08 μmol/(min×mg), which was 63.89% and 7.09% lower, respectively ($p<0.05$).

According to the data presented in Fig. 2, the glutathione reductase indicator was 0.089±0.002 nmol/(min×ml) in the patients of experimental Subgroup I, who were sick for 3-5 years. This was only 8.24% less than in the Comparison group ($p>0.05$). In Subgroup II, this indicator was 10.30% less than in the Comparison group and amounted to 0.073±0.004 nmol/(min×mg) (<0.05). In Subgroup III it was by 42.26% lower and constituted 0.056±0.007 nmol/(min×mg), respectively (<0.05). The glutathione reductase indicator was 0.038±0.005 nmol/(min × mg) in the patients of Subgroup IV, where the history of the main disease was 16-20 years. This was 60.82% less than in the Comparison group (<0.05). The indicator amounted 0.029±0.003 nmol/(min×mg), 70.10%, respectively (<0.05) in Subgroup V patients suffering from paranoid schizophrenia over 21 years.

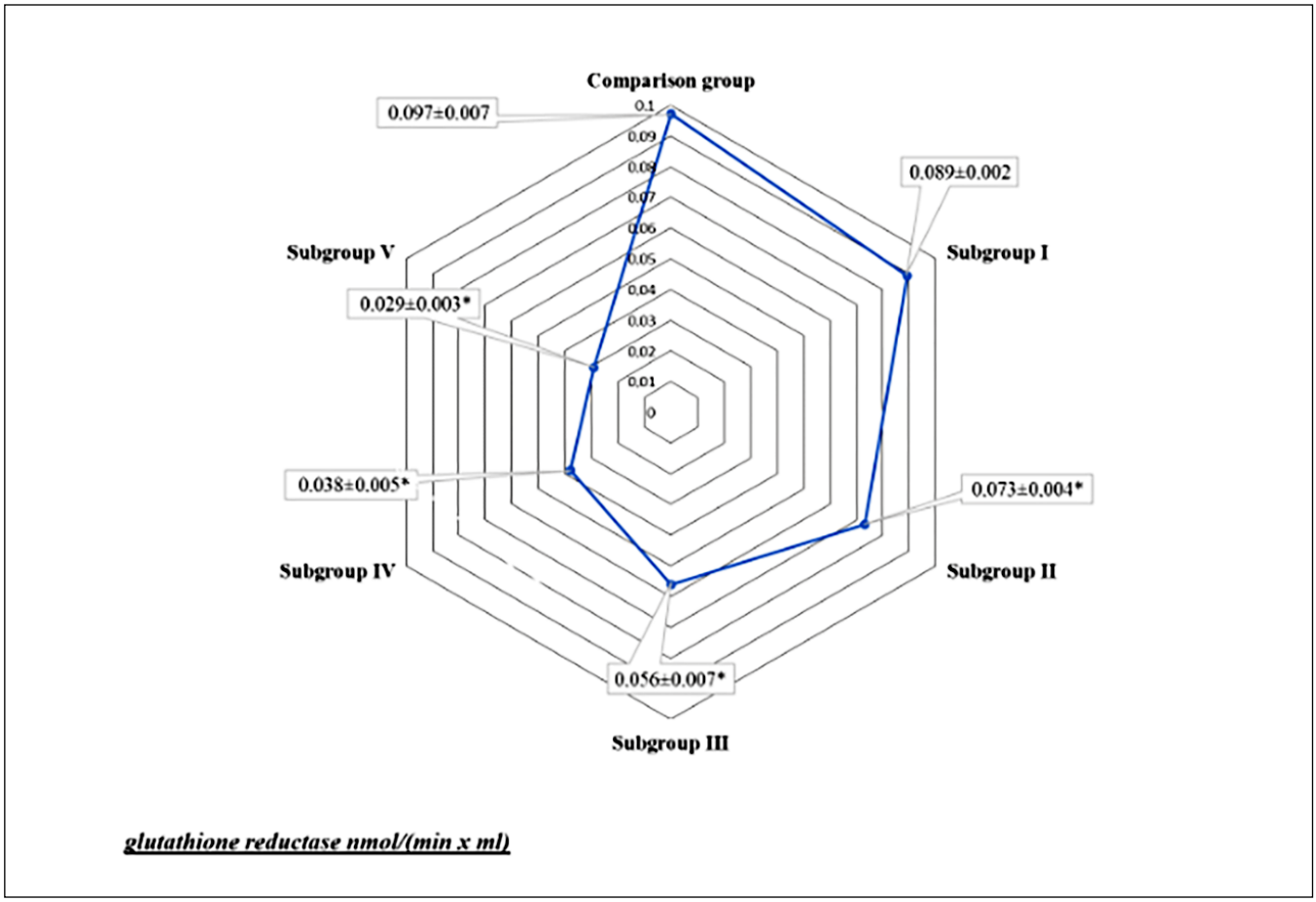

Fig. 2. Dynamics of the glutathione reductase indicator in patients with paranoid schizophrenia depending on the disease duration.
Note: * – ($p<0.05$) the data are reliable between the Comparison and Study Subgroups.

The obtained results, showing a decrease in the activity of the glutathione reductase enzyme, may suggest a physiological deficiency of reduced glutathione as a crucial component of the glutathione pool. Consequently, this deficiency could weaken the antioxidant defense system, leading to an increase in peroxide levels, the onset of oxidative stress, and potential tissue damage.

The study of the enzyme glutathione-S-transferase, responsible for the detoxification segment of the glutathione antioxidant system, also revealed a decrease in its activity. Similar to glutathione peroxidase and glutathione reductase, a consistent decrease in glutathione-S-transferase activity was observed in all subgroups, with significant decreases becoming apparent after 5 years of the disease (Fig.3). This indicator was 1.072±0.122 nmol/(min×mg) in Subgroup I which was 21.80% less than in the Comparison group ($p>0.05$). In Subgroup II, this indicator was 36.32% less than in the Comparison group and amounted to 0.873±0.162 nmol/(min × mg) (<0.05).). In Subgroup III, where the history of the main disease was from 11 to 15 years, it was 65.28% lower and constituted 0.476±0.090 nmol/(min×mg), respectively (<0.05). The glutathione-S-transferase index was 0.432±0.042 nmol/(min×mg) in patients of Subgroup IV who suffered from paranoid schizophrenia from 16 to 20 years, which was 68.49% lower, respectively (<0.05). This indicator was 0.324±0.075 nmol/(min×mg) in the patients of Subgroup V suffering from paranoid schizophrenia over 21 years, which was 76.36% less than in the Comparison group (<0.05). This significant decrease of enzyme, especially in Subgroups IV and V, reinforces the notion of cumulative enzymatic changes with prolonged illness duration.

DISCUSSION

The glutathione system functions cohesively, combining diverse biochemical detoxification mechanisms to preserve a state of normal redox homeostasis. Its components participate in both enzymatic (GPx, GR, GST) and non-enzymatic (GSH) reactions of antioxidant defense. The first ones are genetically programmed, specialized, and the most effective: GPx is responsible for the inactivation of free radicals, and GR, GST are responsible for the recovery of oxidized compounds.

The study of glutathione-dependent enzymes in patients with paranoid schizophrenia reveals interesting

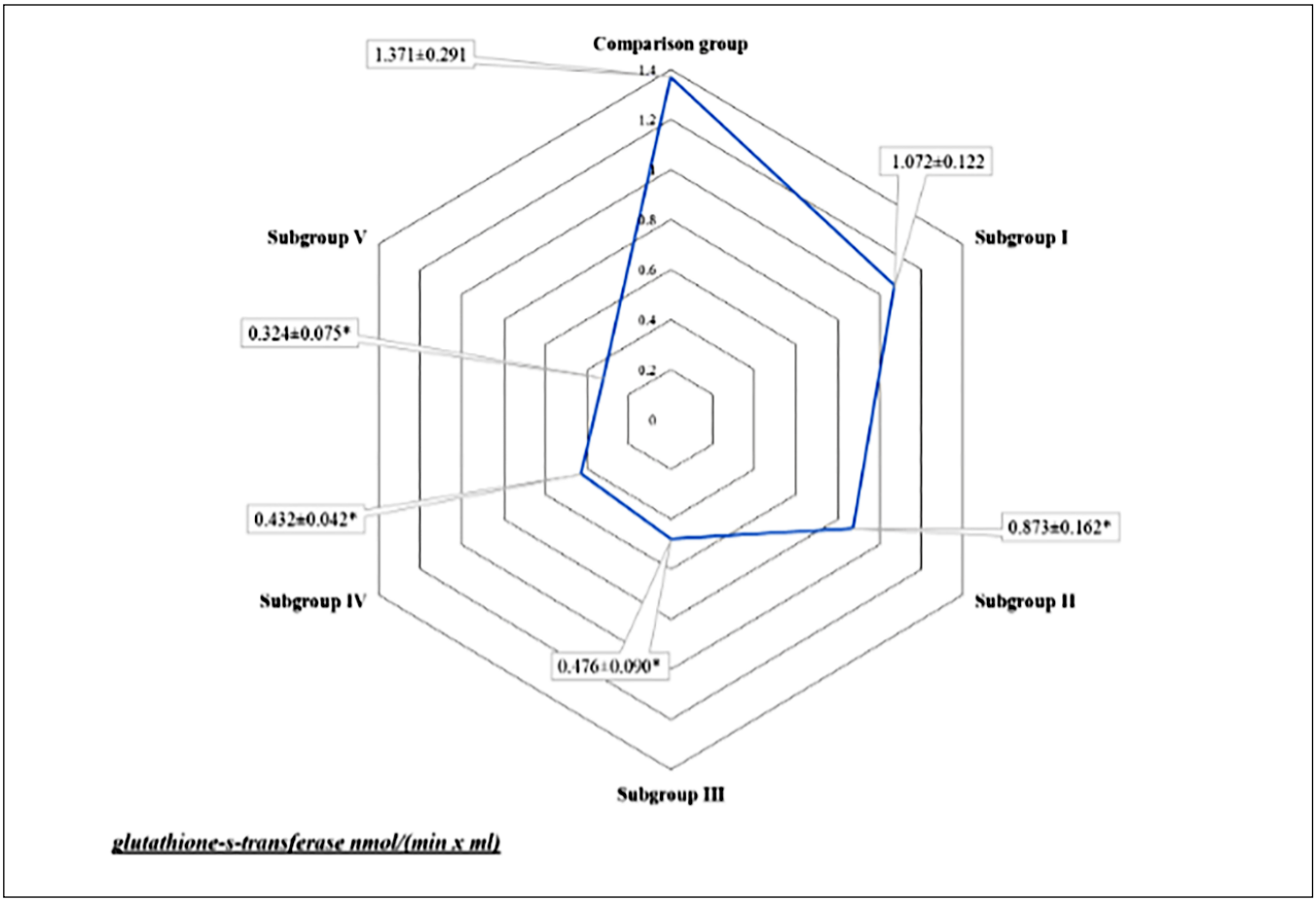

Fig. 3. Dynamics of the glutathione-S-transferase indicator in patients with paranoid schizophrenia depending on the disease duration.
Note: * – ($p<0.05$) the data are reliable between the Comparison and Study Subgroups.

aspects of changes in the enzymatic system during the long course of the disease. According to the analysis of the obtained data, the paranoid form of schizophrenia is accompanied by the development of insufficient functioning of the glutathione system components, and the dynamics of its indicators testifies to the exhaustion of antioxidant protection mechanisms, as a result of which the level of peroxide compounds significantly increases, and antioxidant homeostasis is disturbed

The data we obtained indicated a consistent decrease in the level of glutathione peroxidase in all experimental subgroups. Subgroups I and II, including patients with a disease duration of 3-5 and 6-10 years, respectively, showed a statistically significant decrease, highlighting the early changes in these enzymatic activities. It is noteworthy that the most dramatic decrease in the level of glutathione peroxidase was observed in the patients suffering for more than ten years, and a decrease of more than 50% was observed in subgroup III. This process was even more intensified in subgroups IV and V, demonstrating a significant decrease in the activity of this enzyme. The analysis revealed distinct periods of decline, highlighting potentially critical stages in the progression of paranoid schizophrenia. Our findings align with research conducted by Djordjević V. and colleagues. In their study, scientists observed a significant decrease in GPx activity in the patients who had more than one episode and in those experiencing schizophrenia for over a year. In addition, their results confirmed the existence of OS in the early years of clinically evident schizophrenia, with its correlation to the number of psychotic episodes and the illness duration [8].

In contrast to GPx, reduced levels of GR were evident mainly in patients with a long history of the disease. A significant decrease in its activity was observed in subgroups III, IV, and V. Similar to GPx and GR, a consistent decrease in GST activity was observed in all subgroups with a significant decrease becoming evident after 5 years of the disease. A pronounced decrease in the level of this enzyme, especially in subgroups IV and V, confirms the idea of cumulative enzymatic changes during the long course of the disease. In our opinion, the observed "failures" in enzyme levels indicated complex temporal dynamics emphasizing the need for long-term monitoring and intervention.

Our data partly contradict the findings of Goh, Xue Xin, and their co-authors. In their meta-analysis, includ-

ing studies published from 1964 to 2021, they sought the cause of the disturbance in the antioxidant defense system in case of schizophrenia. In their work, the scientists confirmed that patients with schizophrenia had disorders of the antioxidant defense system, but of the non-enzymatic antioxidant system. Notably, they observed that the antioxidant status was more compromised in drug-naive patients, implying that antipsychotics might enhance the antioxidant defense system [9].

Reactive oxygen species (ROS) are a collective term by their nature including not only radicals but also non-radical molecules of high reactivity, capable of triggering OS in a living organism. Under such conditions, a violation of coordinated metabolic reactions occurs: the formation of peroxides, aldehydes, and ketones. The effectiveness of their detoxification in the body depends on the functional fullness of the enzyme systems responsible for their biotransformation. The primary source of ROS has been scientifically proven to be mitochondria, organelles playing a key role in the energy supply of the cell. In particular, this is caused by a change in the permeability of mitochondrial membranes, namely the appearance of a specific complex of pores. According to the literature, even in a state of rest in the respiratory chain of mitochondria, ROS are formed as intermediate products, some of which are transformed entering the cellular space and start a chain of destructive transformations in biological structures.

OS also affects mitochondrial dysfunction and neuroinflammation [10]. It is well known that all types of neuroplastic processes are inhibited in the conditions of an active inflammatory process. All types of neuroplastic processes are known to be suppressed in conditions of an active inflammatory process. Their role is important in the pathogenesis of schizophrenia [11, 12].

The results of the conducted study emphasize the complexity of the glutathione system response to the progression of paranoid schizophrenia with clear enzyme-specific patterns and temporal dynamics. The observed decrease in enzyme activity indicates a potential relationship between disease duration and changes in the glutathione system, implicating oxidative stress in the pathophysiology of paranoid schizophrenia [9, 12, 13].

LIMITATIONS AND FUTURE DIRECTIONS

While this study provides valuable insights, it is essential to acknowledge certain limitations. The cross-sectional design limits the ability to establish causal relationships, emphasizing the need for longitudinal investigations.

The study only included a relatively small group of participants. Larger studies with longer follow-up periods are needed to confirm these findings.

The study did not explore the potential causes of decreased antioxidant enzyme activity. Investigating the underlying mechanisms could provide valuable insights into the pathogenesis of schizophrenia. In addition, external factors influencing enzyme activities should be considered in future research.

The focus was on paranoid schizophrenia; however, it would be interesting to compare the activity of glutathione-dependent enzymes to other subtypes of schizophrenia to see if there are any differences.

CONCLUSIONS

The identification of critical periods of inhibition in the glutathione protection chain, as found in this study, provides valuable information about potential therapeutic interventions for individuals with paranoid schizophrenia. Understanding these enzymatic changes is crucial for the development of targeted strategies that can eliminate the observed imbalances in the glutathione antioxidant system. The significance of reduced enzyme activity in the pathogenesis of paranoid schizophrenia highlights the need for more detailed investigations. Further research in this direction comprise the development of specific interventions that could enhance the overall treatment and management of paranoid schizophrenia, ultimately improving outcomes for affected individuals.

REFERENCES

1. Mathers CD. History of global burden of disease assessment at the World Health Organization. Archives of Public Health. 2020;78:77. doi: 10.1186/s13690-020-00458-3. DOI
2. Matiashova L, Tsagkaris C, Essar M et al. Achilles in Ukraine: Concerns and priorities over the long-term implications of trauma. The International Journal of Health Planning and Management. 2022;37(6):3369-3371. doi:10.1002/hpm.3562. DOI
3. Romash I. The role of metformin hydrochloride in complex therapy of disorders of carbohydrate metabolism in patients with paranoid schizophrenia treated with atypical antipsychotics. Mental Health: Global Challenges Journal. 2020;3(1):45-51. doi: 10.32437/mhgcj.v3i1.93. DOI
4. Romash IR. Assessment of comorbid profile, quality of life and social functioning in patients with schizophrenia and schizotypal disorders. Medicni perspektivi. 2023;28(1):131–41. doi:10.26641/2307-0404.2023.1.276043. DOI

5. Kovalova O, Pasiieshvili T. Biolohichne ta medychne znachennya systemy antyoksydantnoho zakhystu orhanizmu lyudyny [Biological and medical significance of the antioxidant defense system of the human body]. Medytsyna sohodni i zavtra. 2021;90(1):21-32. doi:10.35339/msz.2021.90.01.03. (Ukrainian). DOI
6. Ali SS, Ahsan H, Zia MK et al. Understanding oxidants and antioxidants: Classical team with new players. Journal of Food Biochemistry. 2020;44(3):e13145. doi:10.1111/jfbc.13145. DOI
7. Mintser O, Voronenko Yu, Vlasov V. Informatsiyni tekhnolohiyi v okhoroni zdorov'ya ta praktychniy medytsyni [Information technologies in health care and practical medicine]. Navch. posib. dlia stud. vyshch. navch. Zakl. Kyiv: Vyshcha shkola. 2003, p.350. (Ukrainian)
8. Djordjević VV, Kostić J, Krivokapić Ž et al. Decreased Activity of Erythrocyte Catalase and Glutathione Peroxidase in Patients with Schizophrenia. Medicina. 2022;58(10):1491. doi:10.3390/medicina58101491. DOI
9. Goh XX, Tang PY, Tee SF. Effects of antipsychotics on antioxidant defence system in patients with schizophrenia: A meta-analysis. Psychiatry Research. 2022;309:114429. doi:10.1016/j.psychres.2022.114429. DOI
10. Picca A, Calvani R, José H et al. Mitochondrial Dysfunction, Oxidative Stress, and Neuroinflammation: Intertwined Roads to Neurodegeneration. Antioxidants. 2020;9(8):647. doi:10.3390/antiox9080647. DOI
11. Song T et al. Mitochondrial dysfunction, oxidative stress, neuroinflammation, and metabolic alterations in the progression of Alzheimer's disease: A meta-analysis of in vivo magnetic resonance spectroscopy studies. Ageing Research Reviews. 2021;72:101503. doi:10.1016/j.arr.2021.101503. DOI
12. Picca A, Guerra F, Calvani R et al. Mitochondrial Dysfunction, Protein Misfolding and Neuroinflammation in Parkinson's Disease: Roads to Biomarker Discovery. *Biomolecules*. 2021;11(10):1508. doi:10.3390/biom11101508. DOI
13. Romash I, Romash I, Tsona A, Pustovoyt M. Peculiarities of indicators of proteins oxidative modification and matrix metalloproteinase-9 activity in patients with paranoid schizophrenia depending on the disease duration. Wiad Lek. 2023;76(12):2625-31. doi:10.36740/WLek202312111. DOI

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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