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Original Article

Biochemistry Section

Evaluating Non Enzymatic Antioxidants as Potential Biomarkers for Oxidative Stress in Schizophrenia: A Cross-sectional Study

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ABSTRACT

Introduction: Schizophrenia significantly impacts patients and their families. Oxidative stress is implicated in various neurological disorders, including schizophrenia. By studying the levels of antioxidants and oxidative stress markers in schizophrenic patients, the pathophysiology of the disease can be examined in depth.

Aim: To investigate the relationship between non enzymatic antioxidants (albumin, bilirubin and uric acid) and oxidative stress markers {Malondialdehyde (MDA)} in first-episode and chronic schizophrenic patients, compared to healthy controls.

Materials and Methods: A cross-sectional study was conducted at Indira Gandhi Government Medical College and Mayo Hospital, Nagpur, Maharashtra, India, from June 2008 to May 2009, with 150 participants, including 50 first-episode schizophrenic patients (Group A), 50 chronic schizophrenic patients (Group B) and 50 healthy controls (Group C). Blood samples were analysed for serum albumin, bilirubin and uric acid using a fully automated clinical biochemistry analyser and plasma MDA levels were measured with a validated biochemical assay. Statistical analyses were performed to explore the associations between antioxidants and oxidative stress markers.

Results: The mean age of participants was 30.88±6.50 years in Group A, 32.98±4.36 years in Group B and 33.74±9.14 years in Group C. Significant positive correlations were observed among albumin, bilirubin and uric acid within each group, suggesting their synergistic antioxidant effects. In controls, all three antioxidants showed strong negative correlations with MDA levels (albumin: r=-0.468, p=0.001; bilirubin: r=-0.805, p=0.001; uric acid: r=-0.694, p=0.001), indicating their role in mitigating oxidative stress. This inverse relationship between non enzymatic antioxidants and MDA weakened with disease progression, as evidenced by a decrease in the coefficient of determination (r^2) from Group A to Group B for all three antioxidants. These findings highlight the potential of albumin, bilirubin and uric acid as biomarkers for monitoring oxidative stress in schizophrenia.

Conclusion: The present study demonstrates significant decreases in serum albumin, bilirubin and uric acid in individuals with schizophrenia, alongside strong inverse correlations between these parameters and MDA, a marker of oxidative stress. Bilirubin's known antioxidant properties suggest its potential as a valuable biomarker for disease progression. These findings underscore the crucial role of the antioxidant system in mitigating oxidative damage in schizophrenia and highlight the potential therapeutic benefits of targeting this system to alleviate oxidative stress-related symptoms.

Keywords: Free radicals, Lipid peroxidation, Malondialdehyde, Reactive oxygen species

INTRODUCTION

Schizophrenia, a chronic psychiatric disorder characterised by delusions, hallucinations, disorganised speech and negative symptoms, significantly impacts patients and their families [1,2]. Oxidative stress, a state of imbalance between Reactive Oxygen Species (ROS) and antioxidant defences, has been implicated in various neurological disorders, including schizophrenia [3]. Approximately 1% of the global population is affected by schizophrenia. A population-based study in India found the lifetime prevalence of schizophrenia spectrum disorders to be 1.41% and the current prevalence to be 0.42% [4]. The annual incidence rate of schizophrenia is estimated to be around 15-20 cases per 100,000 people [4].

Malondialdehyde (MDA), a product of lipid peroxidation, is a widely used biomarker of oxidative stress. Non enzymatic antioxidants, such as albumin, bilirubin and uric acid, provide crucial defence against oxidative damage by neutralising ROS [5]. To elucidate the potential contribution of oxidative stress to the pathogenesis of schizophrenia, the relationship between these antioxidants and MDA levels was examined.

Albumin, a multifunctional plasma protein, plays a pivotal role in mitigating oxidative stress by directly scavenging ROS, chelating redox-active metals and indirectly protecting through bilirubin transport. Bilirubin, a by-product of heme catabolism, exhibits antioxidant properties by neutralising free radicals and preventing lipid peroxidation. Uric acid, a potent endogenous antioxidant, effectively scavenges ROS and protects Superoxide Dismutase (SOD), a key enzyme in ROS detoxification [1-3,5].

Accumulating evidence suggests that schizophrenia is characterised by alterations in the quality and quantity of phospholipids, particularly those containing Essential Polyunsaturated Fatty Acids (EPUFAs) [6]. Oxidative stress has been implicated in the degradation of phospholipids, especially EPUFAs, in schizophrenia [7]. Studies have consistently demonstrated elevated levels of lipid peroxidation products, such as MDA, in individuals with schizophrenia [1-7]. However, past studies [1-7] do not clearly reflect the interrelationships among the non enzymatic antioxidants and their role in disease progression.

Schizophrenia is associated with dysregulation of neurotransmitter systems, including dopamine, glutamate and serotonin [3]. Oxidative stress can further exacerbate these imbalances by damaging neurotransmitter receptors and reducing their sensitivity [5]. For example, oxidative stress can lead to the formation of advanced Glycation End Products (AGEs), which can impair the function of dopamine receptors [6,7].

The existing literature [1-7] primarily focuses on enzymatic antioxidants in schizophrenia, with limited research on non enzymatic

antioxidants. The present study will address this gap by investigating the role of albumin, bilirubin and uric acid in first-episode and chronic schizophrenia. By comparing these groups, we aim to understand disease progression, identify potential biomarkers and inform novel therapeutic strategies.

The present study aimed to investigate the relationship between non enzymatic antioxidants (albumin, bilirubin and uric acid) and oxidative stress markers (MDA) in individuals with first-episode and chronic schizophrenia compared to healthy controls. By understanding the interplay between these factors, the present research seeks to gain insights into the pathophysiology of schizophrenia and potentially identify novel biomarkers for disease progression and therapeutic targets.

MATERIALS AND METHODS

A cross-sectional study was conducted at Indira Gandhi Government Medical College and Mayo Hospital, Nagpur, Maharashtra, India, from June 2008 to May 2009. Ethical approval was obtained from the Institutional Ethics Committee. Participants were recruited from the outpatient psychiatry department of the study Institute.

Inclusion criteria: Individuals aged between 20 and 50 years with first-episode schizophrenia {according to Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) criteria, with no prior antipsychotic medication}, chronic schizophrenia (according to DSM-IV criteria, currently on atypical antipsychotic medication) and healthy controls were included in the study [8].

Exclusion criteria: Participants with acute infectious or inflammatory diseases, liver, pulmonary, renal or ischaemic heart disease, neoplastic diseases, diabetes, smoking, hypertension, alcohol abuse, or those on antioxidant or vitamin supplementation were excluded from the study.

Sample size: The study included 150 participants, comprising 50 individuals with first-episode schizophrenia, 50 with chronic schizophrenia and 50 healthy controls aged 20-50 years.

Study Procedure

Blood samples were collected and analysed for serum albumin {Bromocresol green (BCG) method, end-point}, bilirubin (Diazo method, end-point) and uric acid {uricase/Peroxidase (POD) method, end-point assay} using a fully automated clinical biochemistry analyser, which adhered to acceptable internal and external quality performance standards. Plasma MDA was estimated using a validated biochemical assay.

STATISTICAL ANALYSIS

Statistical analysis was performed using Microsoft Office Excel 2021 and GraphPad 10.3. Descriptive statistics were calculated to summarise the demographic and clinical characteristics of the study participants. Pearson's correlation coefficient was employed to assess the linear relationship between non enzymatic antioxidants (albumin, bilirubin and uric acid) and the oxidative stress marker (MDA) in each study group: first-episode schizophrenia, chronic schizophrenia and healthy controls. The Pearson correlation coefficient 'r' measures the strength and direction of the linear relationship between two variables, ranging from +1 (perfect positive) to -1 (perfect negative), with 0 indicating no correlation. Values between ±0.50 and ±1 suggest a strong correlation, values between ±0.30 and ±0.49 indicate a moderate correlation and values below +0.29 imply a weak correlation. A value of zero implies no relationship. A p-value of less than 0.05 was considered statistically significant. The coefficient of determination (r^2) was calculated to quantify the proportion of variance in one variable explained by the other.

RESULTS

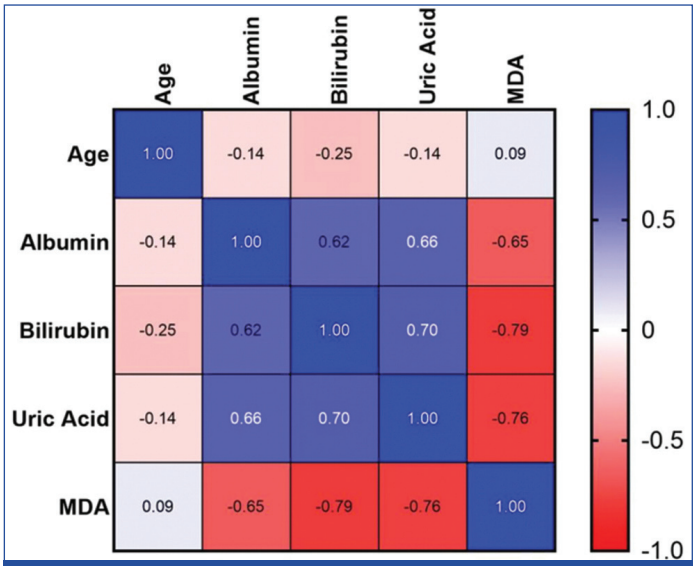
In patients with schizophrenia, the present study explored the interrelationships between oxidative stress and non enzymatic antioxidant levels. The Gaussian distribution of the data was verified and tested for normality.

Group A (First-episode Schizophrenia) included 31 males and 19 females, with a mean age of 30.88±6.50 years. Group B (Chronic Schizophrenia) included 30 males and 20 females, with a mean age of 32.98±4.36 years, while Group C (Controls) included 28 males and 22 females, with a mean age of 33.74±9.14 years. The age and gender distribution in the study groups is depicted in [Table/Fig-1].

Variables	Group A First episode schizophrenics	Group B Chronic schizophrenics	Group C Controls
Age (years)	30.88±6.50	32.98±4.36	33.74±9.14
Gender (Male:Female)	1.63:1	1.5:1	1.27:1
Serum albumin (g/dL)	3.84±0.25	3.90±0.24	4.34±0.31
Serum bilirubin (mg/dL)	0.52±0.17	0.56±0.17	0.69±0.19
Serum uric acid (mg/dL)	4.16±0.73	4.20±0.67	5.29±1.03
Serum MDA (μmoL/litre)	4.44±0.80	4.23±0.65	3.34±0.71

[Table/Fig-1]: Age and gender distribution in study groups.

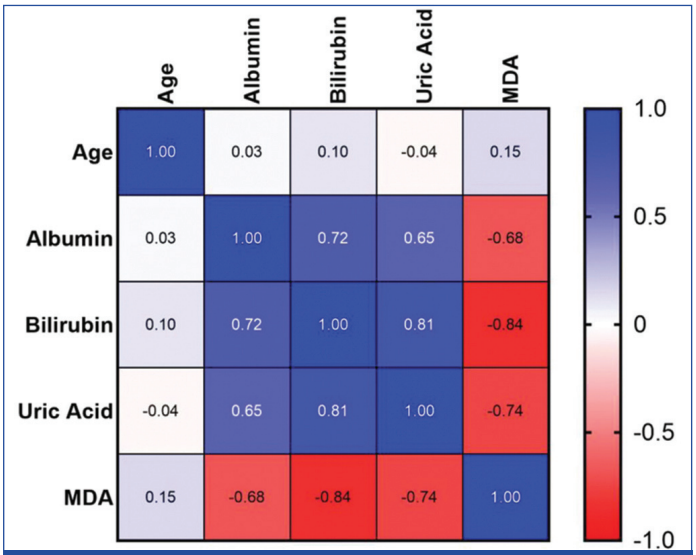
Pearson's correlation coefficient was estimated to assess the relationship between albumin, bilirubin and uric acid and, MDA in each study group. In the cohort of first-episode schizophrenic patients (Group A), age demonstrated very weak correlations with all other parameters, signifying minimal associations. Significant positive correlations were found between albumin and both bilirubin (r=0.62, p=0.001) and uric acid (r=0.66, p=0.001), suggesting a potential synergistic effect of these antioxidants in mitigating oxidative damage. Furthermore, all three antioxidants (albumin: r=-0.65, p=0.001; bilirubin: r=-0.79, p=0.001; uric acid: r=-0.76, p=0.001) exhibited strong negative correlations with MDA, indicating their involvement in reducing oxidative stress. These findings underscore the complex interplay between antioxidant defence mechanisms and oxidative processes in schizophrenia [Table/Fig-2].



[Table/Fig-2]: Correlation amongst serum Albumin, serum Bilirubin, serum Uric acid and plasma MDA levels in first episode of schizophrenia patients (Group A).

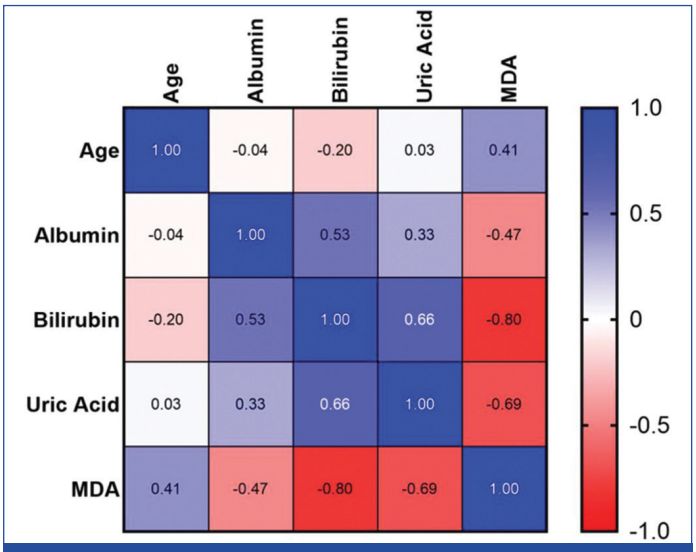
Group B (Chronic Schizophrenic Patients) found that age was a non significant predictor of non enzymatic antioxidant levels and oxidative stress in the study population, suggesting that age-related factors do not substantially influence these biomarkers. Strong positive correlations were observed between albumin and bilirubin (r=0.72, p=0.001), albumin and uric acid (r=0.65, p=0.001) and bilirubin and uric acid (r=0.81, p=0.001). These findings support the hypothesis that these antioxidants may act synergistically to protect against oxidative damage. Inverse relationships were identified between albumin (r=-0.68, p=0.001), bilirubin (r=-0.84, p=0.001)

and uric acid (r=-0.74, p=0.001) with MDA. These results indicate that higher levels of these non enzymatic antioxidants are associated with lower levels of oxidative stress as measured by MDA. These findings further corroborate the protective role of these antioxidants in schizophrenia [Table/Fig-3].



[Table/Fig-3]: Correlation amongst serum Albumin, serum Bilirubin, serum Uric acid and plasma MDA levels in chronic schizophrenia patients (Group B).

Group C, consisting of age- and gender-matched controls, exhibited negligible correlations of age with all other biomarkers, suggesting minimal influence on the studied parameters. A significant positive correlation was observed between albumin and bilirubin (r=0.534, p=0.001) indicating a potential shared physiological function or regulatory mechanism. A moderate positive correlation was identified between albumin and uric acid (r=0.33, p=0.01), suggesting a possible association or indirect relationship. Bilirubin and uric acid showed a robust positive correlation (r=0.66, p=0.001), suggesting a potential shared regulatory mechanism or synergistic effect. Strong negative correlations were demonstrated between albumin and MDA (r=-0.47, p=0.001), bilirubin and MDA (r=-0.80, p=0.001) and uric acid and MDA (r=-0.69, p=0.001), suggesting a potential role of these antioxidants in reducing oxidative stress [Table/Fig-4].

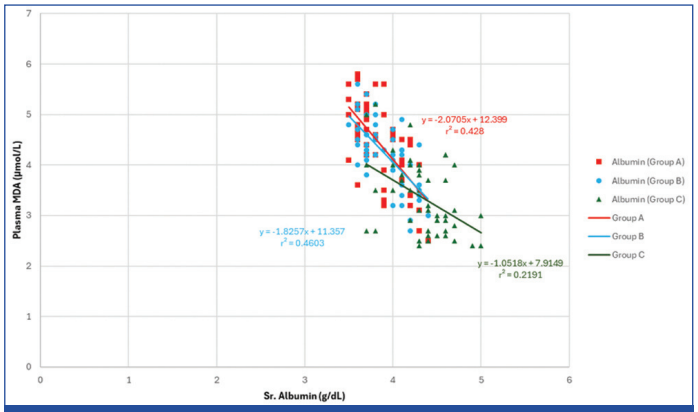


[Table/Fig-4]: Correlation amongst serum Albumin, serum Bilirubin, serum Uric acid and plasma MDA levels in controls (Group C).

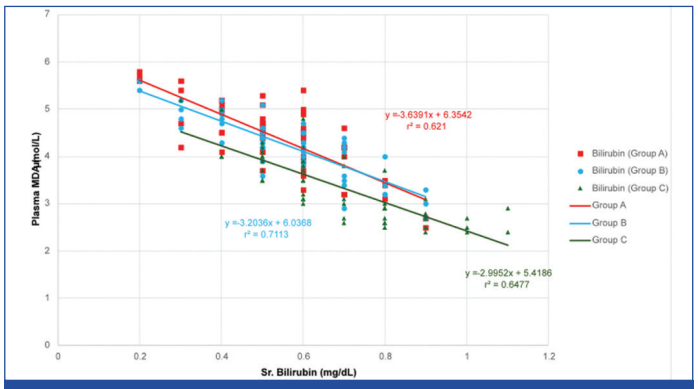
The coefficient of determination (r^2) values for the correlations between non enzymatic antioxidants (albumin, bilirubin and uric acid) and the oxidative stress marker (MDA) across the three groups: Group A (first-episode schizophrenia), Group B (chronic schizophrenia) and Group C (healthy controls) is displayed in [Table/Fig-5]. It exhibits the classical interplay amongst the parameters, typically showcasing the inverse relationship between non enzymatic antioxidants and MDA, which tends to strengthen as the disease progresses [Table/Fig-6-8].

Antioxidants	Oxidative stress (Plasma MDA)		
	Group A	Group B	Group C
Serum albumin	0.428	0.4603	0.2191
Serum bilirubin	0.621	0.7113	0.6477
Serum uric acid	0.58	0.5476	0.4814

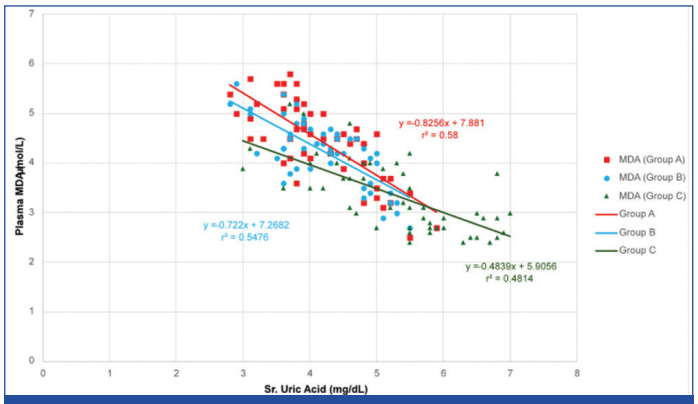
[Table/Fig-5]: Coefficient of determination (r^2) studied for correlation amongst serum albumin, bilirubin, uric acid and plasma MDA in all three groups.



[Table/Fig-6]: Correlation of serum albumin and plasma MDA across the study groups.



[Table/Fig-7]: Correlation of serum bilirubin and plasma MDA across the study groups.



[Table/Fig-8]: Correlation of serum uric acid and plasma MDA across the study groups.

DISCUSSION

Schizophrenia, a complex neuropsychiatric disorder, is increasingly recognised as being influenced by oxidative stress. This condition arises from an imbalance between ROS and antioxidant defences, leading to cellular damage and dysfunction. The brain, with its high metabolic rate and rich lipid content, is particularly vulnerable to oxidative damage. Previous studies have consistently reported alterations in the oxidant-antioxidant balance in patients with schizophrenia [1-7].

Non enzymatic antioxidants, including albumin, bilirubin and uric acid, play a critical role in defending against oxidative stress. These molecules circulate in the plasma, protecting various tissues and organs from oxidative damage. The present study found that age and gender had minimal impact on the levels of non enzymatic antioxidants or oxidative stress in schizophrenia. However, significant differences were observed between the three study groups: first-episode schizophrenia, chronic schizophrenia and healthy controls.

Serum albumin, a major plasma protein with antioxidant properties, was found to be significantly decreased in both first-episode and chronic schizophrenia compared to healthy controls. This reduction suggests a potential link to the increased oxidative stress associated with schizophrenia [3-7]. The decrease in serum albumin levels may be a consequence of immunological responses, acute-phase protein changes, or direct oxidative damage [7].

Bilirubin, a potent antioxidant, was also significantly reduced in both patient groups. This finding aligns with previous research, indicating a potential association between decreased bilirubin levels and the pathophysiology of schizophrenia [6,7]. The reduction in bilirubin may reflect increased consumption of this antioxidant to counteract oxidative stress [1,2].

Serum uric acid levels were also significantly lower in both patient groups compared to controls. This finding is consistent with previous studies suggesting a potential association between decreased uric acid levels and oxidative stress in schizophrenia [3,5].

Positive correlations among the antioxidants suggest potential synergistic interactions, implying that these molecules may work together to enhance their antioxidant activity. Significant negative correlations were observed between all three antioxidants and MDA, suggesting their involvement in reducing oxidative stress [6]. These findings align with previous research indicating the protective role of antioxidants in neurological disorders, including schizophrenia [1-6,9].

Bilirubin consistently shows the highest r^2 values across all groups, suggesting a strong association with MDA. Among the studied antioxidants, bilirubin demonstrated the strongest association with MDA, highlighting its potential as a potent antioxidant in mitigating oxidative stress in schizophrenia. Previous research has consistently demonstrated the protective role of bilirubin in mitigating oxidative stress and chronic inflammation, particularly in conditions like metabolic syndrome and diabetes [9]. A prior study further emphasised this by identifying low unconjugated bilirubin levels as an independent risk factor for severe inflammation and liver fibrosis in individuals with steatohepatitis [10]. This finding strongly suggests that insufficient antioxidant protection, due to low bilirubin levels, significantly contributes to the progression of diseases. It highlights the potential importance of bilirubin as a potent antioxidant in mitigating oxidative stress in schizophrenia.

Uric acid exhibits moderate r^2 values, suggesting a less pronounced but still significant role in reducing oxidative stress compared to bilirubin. While uric acid shows a moderate association with MDA, it also contributes to the antioxidant defence system. Albumin, although a major plasma protein, showed a relatively limited impact on MDA levels, suggesting that its antioxidant role may be less pronounced compared to bilirubin and uric acid.

Albumin exhibits the lowest r^2 values, indicating a relatively limited impact on MDA levels. This may be due to its multiple functions beyond antioxidant activity.

Overall, the interplay underscores the importance of non enzymatic antioxidants in mitigating oxidative stress in schizophrenia, with bilirubin emerging as a particularly potent contributor. Further research is needed to explore the underlying mechanisms and potential therapeutic implications of these findings.

The observed alterations in antioxidant levels and their correlation with oxidative stress provide valuable insights into the pathophysiology of schizophrenia. These findings may have potential clinical implications, as targeting the antioxidant system could represent a promising therapeutic strategy to alleviate oxidative stress-related symptoms and improve cognitive function in individuals with schizophrenia.

The coefficient of determination (r^2) values generally increase from Group A to Group B, indicating a stronger inverse relationship between antioxidants and MDA as the disease progresses. This relationship strengthens as the disease progresses from the first-episode to the chronic phase. This observation suggests that the protective role of these antioxidants may diminish over time, potentially contributing to the exacerbation of oxidative stress in chronic schizophrenia [3]. This may lead to increased and uncompensated oxidative stress, as indicated by elevated MDA levels [5].

The present study provides robust evidence for the protective role of non enzymatic antioxidants against oxidative stress in schizophrenia. The findings highlight the complex interplay between antioxidant defence mechanisms and oxidative processes in this disorder. Further research is necessary to elucidate the specific mechanisms underlying the antioxidant activity of these molecules in schizophrenia and to explore their potential therapeutic implications.

Limitation(s)

The present cross-sectional study has limitations. Firstly, its design prevents the establishment of causal relationships. Longitudinal studies are needed to track changes over time. Secondly, the relatively small sample size may limit generalisability. Larger studies with diverse populations are required. Additionally, the focus on a limited number of antioxidants necessitates further exploration of a wider range. Finally, the influence of confounding factors like medication and diet was not fully controlled. Future studies should consider these factors to improve accuracy.

CONCLUSION(S)

The present study provides robust evidence for the involvement of non enzymatic antioxidants in mitigating oxidative stress in schizophrenia. Significant reductions in serum albumin, bilirubin and uric acid levels were observed in both first-episode and chronic schizophrenia compared to healthy controls. These findings align with previous research indicating the protective role of these antioxidants against oxidative damage. A strong inverse correlation between non enzymatic antioxidants and MDA was observed, further supporting their involvement in reducing oxidative stress. Bilirubin emerged as a particularly potent antioxidant, highlighting its potential as a biomarker for disease progression. The observed alterations in antioxidant levels and their correlation with oxidative stress may have potential clinical implications, as targeting the antioxidant system could represent a promising therapeutic strategy to alleviate oxidative stress-related symptoms and improve cognitive function in individuals with schizophrenia.

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