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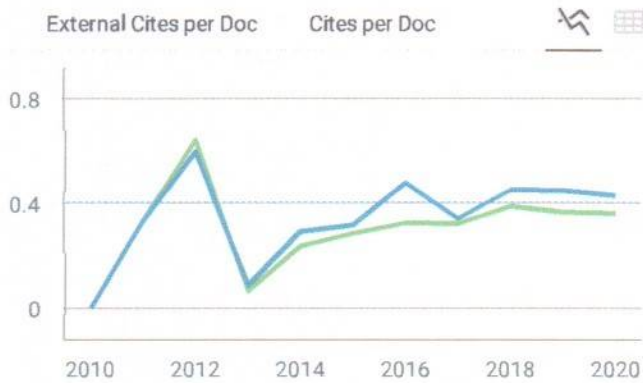
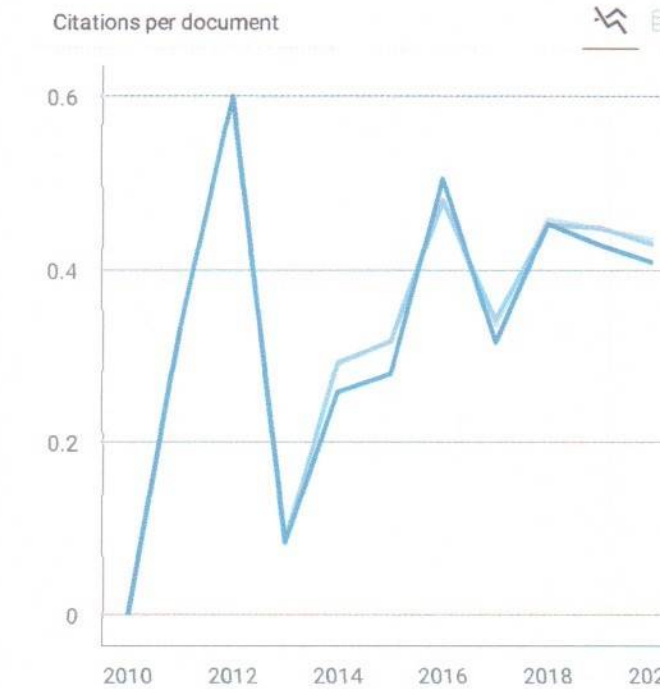
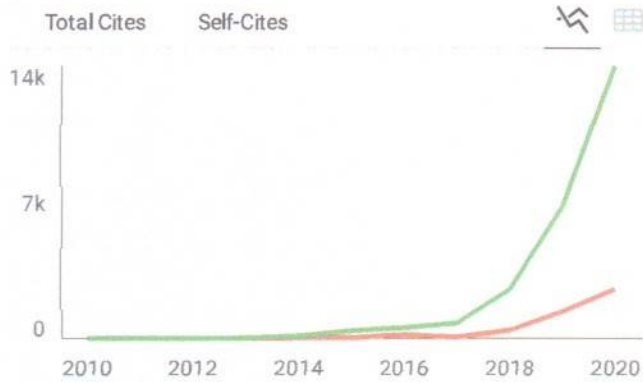
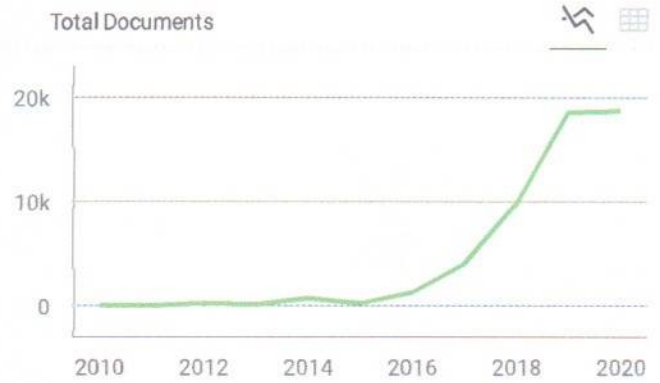
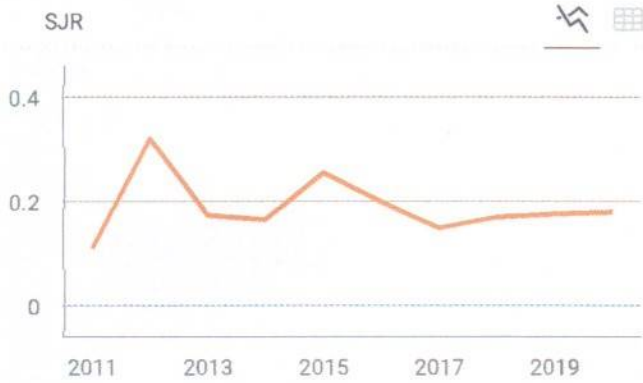
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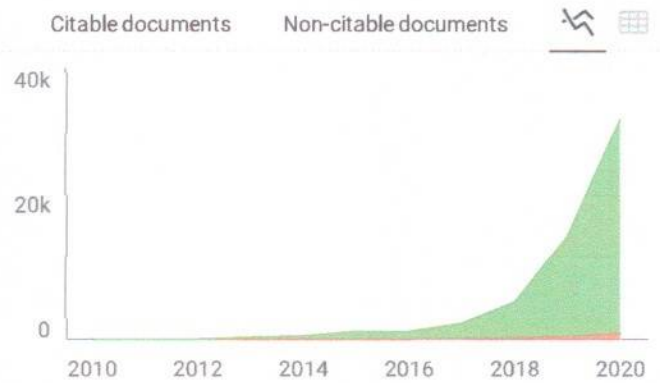
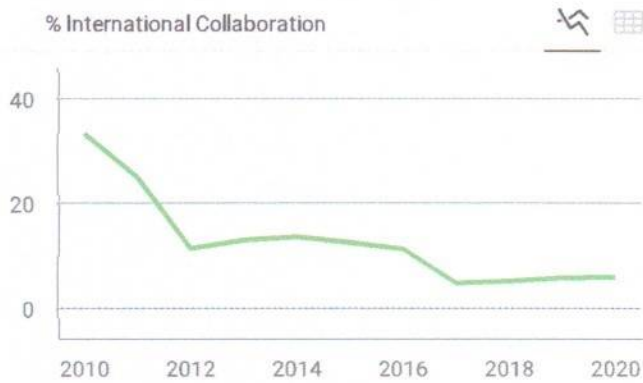
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Second International Conference “Collaboration Seminar of Chemistry and Industry (CoSCI 2018) in conjunction with 23<sup>rd</sup> Indonesian Society for Biochemistry and Molecular Biology (ISBMB) seminar was taken place at Universitas Airlangga, Surabaya-Indonesia on October 11-12, 2018. The event presented a theme” Recent Development of Omics Technology For Human Prosperity”.

"Omics is a general term for a broad discipline of science and engineering for analyzing the interactions of biological information objects in organism. The main focus is on: 1) mapping information objects such as genes, proteins, and ligands; 2) finding interaction relationships among the objects; 3) engineering the networks and objects to understand and manipulate the regulatory mechanisms; and 4) integrating various omes and omics subfields.

The event was held to facilitate for the scientists, scholars, engineers and students from universities, research institutes and industries to present ongoing research activities, especially in Biochemistry, Biology, Chemistry, Medicine and other in related fields. It was also to encourage future collaboration among all participants.

The conference of CoSCI and ISBMB created proceedings from the papers that were submitted by participants, after they were reviewed by committee members and international reviewers. This volume intends to provide readers with the recent advances in the Omics Technology such as Chemistry, Biochemistry and Molecular Biology, and Medicine field.

We would like to thank to all authors who contributed to the proceedings and also to the organizing committee, reviewers, speakers, sponsor, and all the conference participants for their supporting in the conference of CoSCI 2018 and 23<sup>rd</sup> Seminar of ISBMB.

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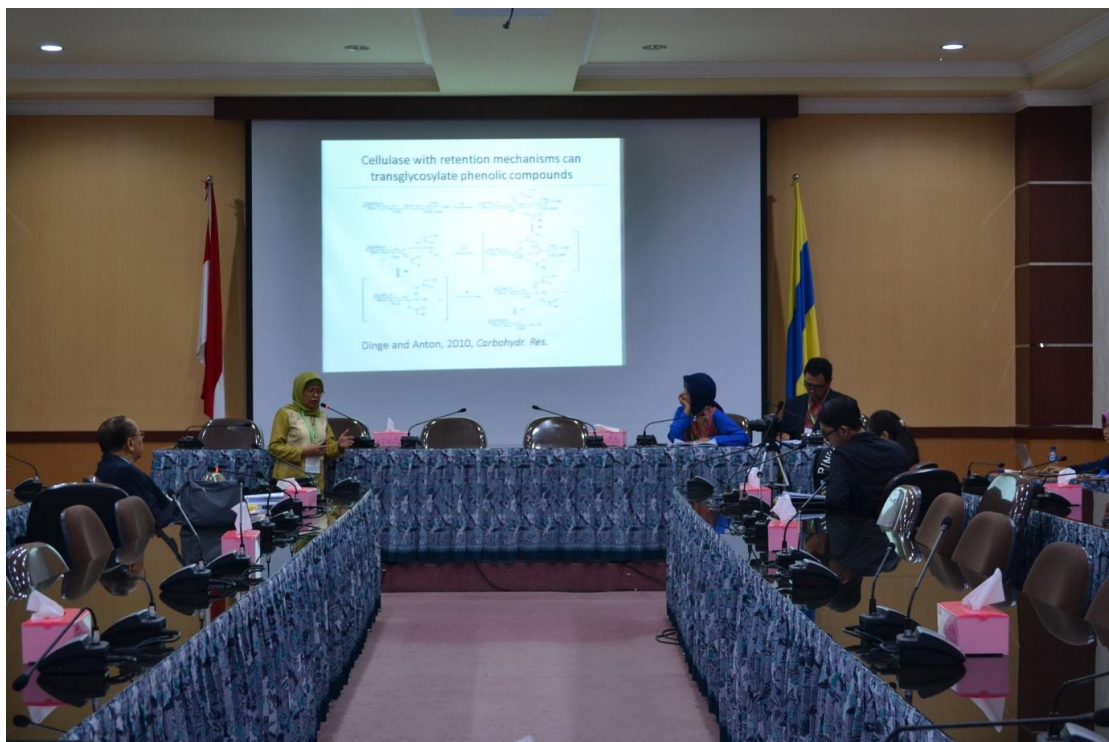
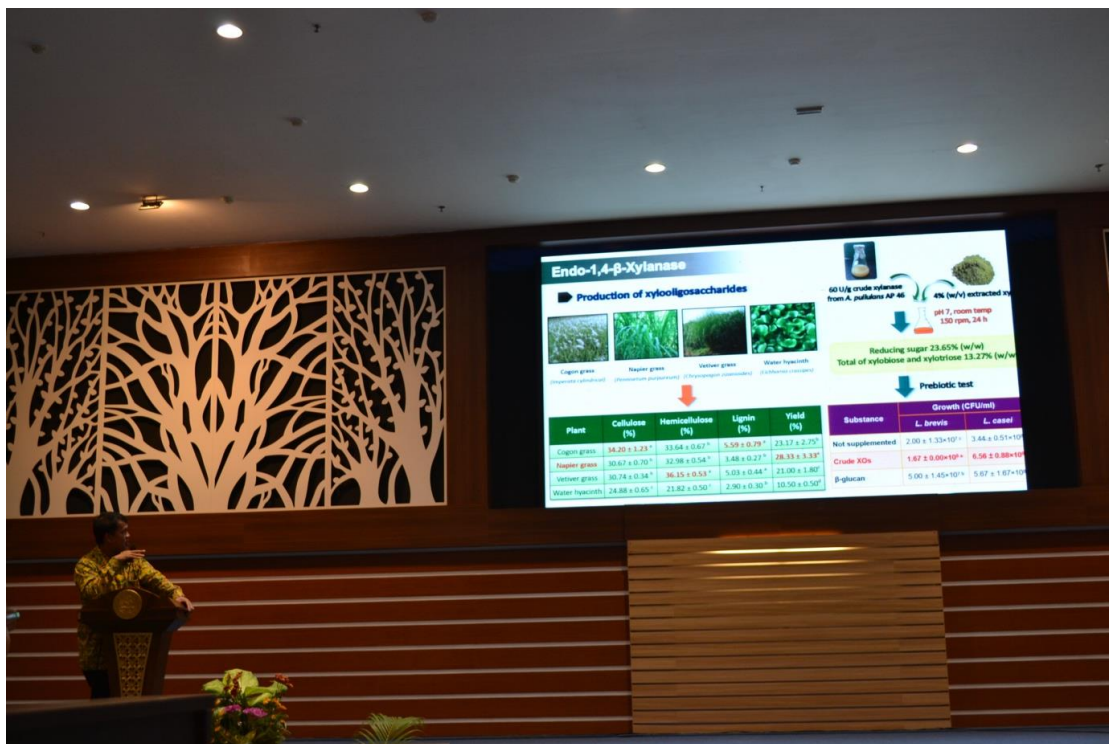
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# Table of contents

Volume 217

2019

◀ Previous issue    Next issue ▶

**The 12th Congress of Indonesian Soc. for Biochemistry and Molecular Biology in Conjunction With The 2nd Int. Conf. "Collaboration Seminar of Chemistry and Industry (CoSCI)" and AnMicro Workshop 11-12 October 2018, Universitas Airlangga, Indonesia**

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Counselling and Screening of Hepatitis B Virus Infection In Dukuh Kupang Community, Dukuh Pakis District, Surabaya

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G I Prabowo, M M Maramis, E Yulianti, A Zulaikha, Z B Syulthoni, C D K Wungu, H M Margono and R Handajani

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Detection Of Hepatitis C Virus (Hcv) Infection And Its Genotype In Patients At Hepatology Outpatient Clinic, Dr Soetomo General Hospital, Surabaya.

R Handajani, C D K Wungu, I Humairah, G I Prabowo, U Cholili, M Amin, P B Setiawan and Soetjipto

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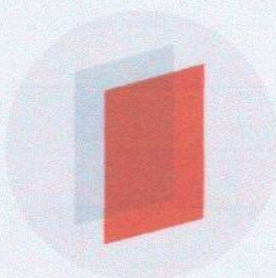


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# Correlation Between Oxidative Stress With Clinical Symptoms In Chronic Schizophrenic Patients In Psychiatric Unit of Dr Soetomo General Hospital Surabaya

G I Prabowo<sup>1\*</sup>, M M Maramis<sup>2</sup>, E Yulianti<sup>2</sup>, A Zulaikha<sup>2</sup>, Z B Syulthoni<sup>2</sup>, C D K Wungu<sup>1</sup>, H M Margono<sup>2</sup>, R Handajani<sup>1,3</sup>

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**Abstract. Background:** Schizophrenia is a psychiatric disorder found worldwide, including Indonesia. In people with schizophrenia, there is an increase in Reactive Oxygen Species (ROS) / Reactive Nitrogen Species (RNS) production and / or a decrease in antioxidants which results in oxidative stress that can be detected by measuring F2-isoprostane levels as gold standard tests. Oxidative stress results in disruption of neuronal function. It is associated with the severity of clinical symptoms of schizophrenia which can be measured by Positive and Negative symptom Scale (PANSS). The purpose of this study was to determine the association between F2-isoprostane levels and clinical symptoms of schizophrenia.

**Methods:** This study was an observational analysis study with a case-control study design in 30 people with chronic schizophrenic Javanese patients and 30 healthy Javanese people as the control group with equal sex and age. In all study subjects, F2-isoprostane level was examined by ELISA technique, while PANNS scoring was only measured for people with chronic schizophrenic.

**Results:** Total PANNS scores in male schizophrenic patients ( $40.71 \pm 16.07$ ) were higher than female schizophrenic patients ( $40.31 \pm 11.42$ ). Plasma F2-isoprostane level in schizophrenic group ( $171.69 \pm 14.62$ ) was significantly higher ( $p < 0.05$ ) when compared to control group ( $92.54 \pm 8.08$ ).

**Conclusion:** In this study we found a significant increase of plasma F2-isoprostane level in schizophrenic patients compared with control group. The F2 isoprostane level in schizophrenic patient were not related to the severity of schizophrenia clinical symptoms.

**Keyword :** PANSS score; schizophrenia; oxidative stress; F2-isoprostane

## 1. Introduction

Schizophrenia is a worldwide health problem, because it can be found in all society and various geographical areas with a life time prevalence around 1% [1]. According to 2013 Basic Health Research data in Indonesia, the schizophrenia prevalence is 1.7 per 1000 population [2].

Schizophrenia is a complex and chronic severe mental disorder, characterized by various clinical symptoms, disease progression and response to therapy [3]. The underlying mechanisms of schizophrenia are still not entirely clear, some evidence suggests that this disease is related to a multifactorial and polygenic etiology. One of the important factors that play an important role in schizophrenia occurrence are oxidative stress [4].

In schizophrenic patients, the increased of Reactive Oxygen Species (ROS) / Reactive Nitrogen Species (RNS) and / or decreased antioxidants production can result to oxidative stress that causes viability disruption and dysfunction of neuronal cells [4,5].

The brain is an organ very sensitive to oxidative stress damage, because in physiological conditions it contains phospholipids and unsaturated fatty acids [6]. In schizophrenic brain tissue, there is some change in phospholipids, including: a decrease in phosphatidylcholine and phosphatidylethanolamine



and a large amount of lipofuscin-like material in oligodendrocytes. The presence of lipofuscin-like material on the cell membrane results in unstable cell membrane structure and permeability and fluidity cell membrane disruption that causes the interference of signal transduction [7].

Oxidative stress in schizophrenic people, mainly occurs in brain tissue [4,7], because the oxygen consumption is ten times higher when compared to the other tissues, high oxidative phosphorylation activity, changes in antioxidant activity in the brain, high dopamine auto oxidation [7], contain many metals (*Ferrous, Zinc, Cobalt dan Mangan*) and the high rate of catecholamine metabolism activity, which contributes to the formation of ROS / RNS [5]. In addition, the brain tissue neuron has prolonged life and the presence of nitric oxide in the brain tissue also plays a dominant role in ROS generating. Neurons are highly susceptible to free radicals toxic effect, because many neuronal membranes contain Polyunsaturated Fatty Acids (*PUFA*) [4,8].

Reactive Oxygen Species (ROS) can cause lipid peroxidation which can damage the cell membranes lipid components. The ROS levels in human body are very difficult to detect, because they have very short half-life. Damage to cell membrane lipid components due to lipid peroxidation reaction can be detected by many methods, for instance by measuring levels of malondialdehyde and F2-isoprostane [9].

The oxidative stress level in schizophrenic patients widely varies and may be related to clinical symptoms severity and therapy effectiveness. Clinical symptoms measurement can be performed by PANSS scoring. Taking into account the above matters, the purpose of this study was to examine the levels of F2 isoprostane and PANSS scoring, to analyze the relationship between the levels of oxidative stress and clinical symptoms in chronic schizophrenic patients at the Psychiatric Unit of Dr. Soetomo General Hospital Surabaya.

## 2. Experimental Methods

This study was an observational research to detect the plasma level of F2-isoprostane in chronic schizophrenic patients at Psychiatric Unit of Dr. Soetomo General Hospital Surabaya. Its research design was case-control study.

The diagnosis of schizophrenia based on psychiatric history and mental checkup by psychiatrist and were conducted under the Guidance of Mental Disorder Categorization and Diagnosis in Indonesia III (PPDGJ 3<sup>rd</sup> edition) [10] and the criteria of Positive and Negative Symptom Scale (PANSS) [11].

Subjects in this study were schizophrenic patients enrolled from the Psychiatric Unit of Dr. Soetomo General Hospital Surabaya. They had to meet some inclusion criterias: age of  $\geq 18$  years old, Javanese, male and female schizophrenic patients, being diagnosed with schizophrenia, and having persistent symptoms at least for 6 months.

Families of schizophrenic patients / control subjects who were willing to take part in this study first filled out the informed consent form. The population of schizophrenia patients diagnosed by a psychiatrist in the Psychiatric unit of the RSUD Dr. Soetomo Surabaya during the period of 4 months was obtained as many as 355 subjects. After questionnaires and laboratory examinations for screening the history of the disease, 30 subjects were got according to the inclusion criteria.

Blood sampling was conducted after obtaining an ethical clearance from the research ethics committee of Dr. Soetomo General Hospital Surabaya. Prior to the research, the families of schizophrenic patients were given an explanation about the research and asked to sign an Informed Consent statement.

The blood samples were collected from schizophrenic patients and put into 5 mL vacutainer with EDTA anticoagulant. The examination of F2 isoprostane in blood plasma used 8-iso-PGF<sub>2</sub> $\alpha$  ELISA kit (abcam, ab 175819), performed by Enzyme Linked Immunosorbent Assay (ELISA)

## 3. Results

In all study subjects, data collection was collected by gender, mean  $\pm$  standard deviation (SD) and age range with year units. A summary of data on gender, mean  $\pm$  SD and age range of study subjects (Table 1)



**Table 1.** Gender distribution, mean  $\pm$  SD and age range of research subject

Gender	N [%]	Chronic Schizophrenia		Control	
		Age (Years)		Age (Years)	
		Mean $\pm$ SD	Age range	Mean $\pm$ SD	Age range
Male	17 (57,0%)	38,53 10,32	$\pm$ 25 - 55	38,94 10,08	$\pm$ 25 - 56
Female	13 (43,0%)	41,08 7,44	$\pm$ 31 - 55	41,15 $\pm$ 7,13	32 - 53
Total [%]	30 (100,0%)	39,63 9,13	$\pm$ 25 - 55	39,90 $\pm$ 9,31	25 - 56

In this study, PANSS scores were only assessed in the chronic schizophrenic group. A summary of gender distribution data and PANSS scores in chronic schizophrenic patients (Table 2)

**Table 2.** Gender distribution and PANSS score in chronic schizophrenic patients

PANSS	Positive (P)	Negative (N)	General (G)	Total (T)
Gender	Mean $\pm$ SD /range	Mean $\pm$ SD /range	Mean $\pm$ SD /range	Mean $\pm$ SD /range
Male	10,71 $\pm$ 4,98 (7-20)	9,88 $\pm$ 5,18 (7-28)	20,12 $\pm$ 8,24 (16-49)	40,71 $\pm$ 16,07 (28-87)
Female	9,62 $\pm$ 2,87 (7-15)	10,62 $\pm$ 6,74 (7-28)	20,08 $\pm$ 3,90 (16-28)	40,31 $\pm$ 11,42 (30-68)
N total	10,23 $\pm$ 4,17 (7-20)	10,20 $\pm$ 5,81 (7-28)	20,10 $\pm$ 6,61 (16-49)	40,53 $\pm$ 14,02 (28-87)

**Note:** P: total positive symptom score, N: total negative symptom score, G: total general symptom score, T: total score ( P+N+G)

In chronic schizophrenic group of men in this study, the total mean of positive symptom score, total general symptom score and total score was higher than female. In chronic schizophrenic group the total mean of negative symptom score was higher than men and in further statistic analysis, the difference was not significant ( $p > 0,05$ ).

In this study, levels of F2-isoprostane in the chronic schizophrenia group were higher than in the control group and F2-isoprostane levels in women were higher than in men. F2-isoprostane levels in the study subjects (Table 3).

**Table 3.** F2-isoprostane level in research subject

F2-Isoprostane (ng/mL)	level	N	Minimum	Maximum	Mean $\pm$ SD	P value
Chronic Schizophrenia	Male	17	152,28	197,65	166,94 $\pm$ 13,75	0,079
	Female	13	154,07	201,22	177,90 $\pm$ 13,81	
	Subtotal	30	152,28	201,22	171,69 $\pm$ 14,62	
Control	Male	17	82,01	99,83	95,51 $\pm$ 5,00	0,072
	Female	13	82,01	99,82	88,66 $\pm$ 9,78	
	Subtotal	30	73,49	99,83	92,54 $\pm$ 8,08	

The results of the analysis showed differences in F2-isoprostane levels between the chronic schizophrenic group and the control group ( $p < 0.05$ ). In men and women with chronic schizophrenia, there was no difference in F2-isoprostane levels ( $p > 0.05$ ).

In patients with schizophrenia, oxidative damage is associated with a worse clinical outcome. To evaluate clinical outcomes in chronic schizophrenic patients in this study we used PANSS scores. The correlation table between PANSS scores and F2-isoprostane levels in chronic schizophrenic patients (Table 4).

**Table 4.** The correlation between PANSS score and F2-isoprostane level

PANSS score	Correlation	
	$r_s$	P
P	0,315	0,090
N	0,163	0,389
G	0,253	0,177
T	0,264	0,158

**Note:** P: total positive symptom score, N: total negative symptom score, G: total general symptom score, T: total score ( P+N+G)

The results of statistical analysis showed no correlation between all PANSS score categories with F2-isoprostane levels ( $p > 0.05$ ).

#### 4. Discussion

Oxidative stress will arise when there is an imbalance between oxidants and antioxidants [4]. Isoprostane is a biomarker gold standard for oxidative stress levels detection [9,12,1]. Isoprostane is a prostaglandin-like compound, which is formed from a nonenzymatic arachidonic acid peroxidation reaction. The increase of F2-isoprostane levels has a positive correlation with the severity of the disease, including schizophrenia [14].

In this study, the F2 isoprostane levels in chronic schizophrenic patients were significantly increased compared with the control group. The results of this study are supported by the results of previous studies, that obtained an increase of ROS production and decreased antioxidants in schizophrenic patients [4]. Direct evidence of oxidative injury in schizophrenic people is an increase of lipid peroxidation and protein modification [15]. The results of this study are in line with previous studies, which F2-isoprostane levels in women with chronic schizophrenic is higher ( $p > 0.05$ ) when compared to men [15].

Brain tissue is strongly influenced by oxidative stress, because it has low antioxidant levels, high PUFA content, high oxygen demand [16] and high energy demand [15]. In eukaryotic cells organisms, mitochondria produces about 95% of energy that we needs and 5 % ROS as by product are produced [15]. High mitochondria are found in brain tissue, due to high brain energy requirements [15]. Hypothesis from the previous studies states that the occurrence of various processes which take place in mitochondria are changed in schizophrenic patients, for example: changes in calcium homeostasis, transport of neurotransmitter, synaptic plasticity and exacerbations of ROS production [15].

The increase of ROS does not occur in the early stages of schizophrenia development, because of some key processes of the oxidative stress response, for example: impaired purine catabolism that causes DNA damage, impaired energy production, genetic disorders and new protein expression occurs in the later stages. In the advanced stages, increasing ROS can cause apoptosis which causes cell death and neuronal plasticity disorders and disrupt neurotransmission, so the manifestation of schizophrenic symptoms generally occurs during early adulthood [15].

Evidence that Oxidative stress can induce apoptosis is a disorder of glutamate metabolism that induces a decrease in glutathione in immature embryonic cortical neurons. Decreased glutathione can cause chromatin hypercondensation and fragmentation into spherical and irregular forms, accompanied by the formation of [17] multiple oligonucleosome DNA fragments that mark apoptosis [18].

Increased oxidative stress in schizophrenia is a trigger for the onset of an inflammatory process. Oxidative stress and inflammation both underlie the emergence of a pathology process in people with schizophrenia [16]. The reports of oxidative stress biomarker measurement in schizophrenic patients when compared with the control group from previous researchers have inconsistent results [19].

The results of this study reinforce that in chronic schizophrenic patients there is damage due to oxidative stress that occurs by an increase in oxidative stress and a decrease in the body's ability to protect oxidative stress [15]. Nonetheless, F2 isoprostane is not a specific biomarker for schizophrenia, because the clinical symptoms in schizophrenic people are influenced by some factors, as genetic factors, environmental factors and antipsychotic drugs used by the patients [15].

The increased of oxidative stress in schizophrenic people is related with the dopamine metabolic disorders, the abnormal electron transport chains in mitochondria and the genetic changes [7]. It affects the cell-signaling cascades which regulate several neurotransmitter systems [20]. Changes in neurotransmitters metabolism can affect the oxidative stress and modify the function of cell membranes [15].

Oxidative stress that occurs in schizophrenic people can be related to side effects induced by typical antipsychotic drugs. Other researchers found that the administration of long term typical or atypical antipsychotic therapy in schizophrenic people did not reveal any differences in oxidative stress biomarkers [7].

The severity of clinical symptoms in schizophrenic people does not have an association with oxidative stress [21]. Oxidative damage in schizophrenic patients related to worse clinical outcomes [15] and because of the complication of antipsychotic drugs [22]. The inconsistent result of this study with the previous research because of the clinical symptoms in schizophrenic patients are influenced by multifactorial, such as: genetic factors, environmental factors and the type of antipsychotic and the used of antipsychotic period [19].

The results of this study can be used as a basis for the development of additional antioxidant therapies to anticipate oxidative damage and clinical sequelae in schizophrenic patients [15].

## 5. Conclusions

Oxidative stress is supposed to have important role in the pathophysiology of schizophrenia, because this research found that *F2-isoprostane* level in schizophrenic group was higher than control group, but it had no association with severity of clinical symptoms. In this research we found no significant difference of PANSS score and *F2 isoprostane* level between women and men in schizophrenic patients.

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### Conflict of Interest

There is no conflict of interest.