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RESEARCH ARTICLE
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THE EFFECT OF ADJUVANT OMEGA-3 THERAPY ON THE IMPROVEMENT OF CLINICAL SYMPTOMS AND TUMOR NECROSIS FACTOR ALPHA (TNF-ALPHA) SERUM LEVELS IN SCHIZOPHRENIC PATIENTS TREATED WITH RISPERIDONE

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Abstract

Objectives: The pathogenesis of schizophrenia remains unclear; However, neuro inflammation is considered a potential factor. Some researchers have also proposed that immune disorders may be involved in the etiology and pathophysiology of schizophrenia. Abnormal levels of cytokines and pro inflammatory receptors have been found in peripheral blood and cerebrospinal fluid in schizophrenic patients. In recent years, growing attention has concentrated on the role of nutritional interventions in neurological and mental diseases to maximize the effects of nutritional components on brain function, neural plasticity, and mental health including administration of adjuvant therapy to help schizophrenic patients in achieve better life functions. This research conducted to determine the effectiveness of omega-3 to improve clinical symptoms and levels of TNF-αserum in schizophrenic patients.

Method: This research is an experimental analysis, with a pre-posttest with control group approach (pretest-posttest randomized design), which was conducted at the Dadi Regional Special Hospital in South Sulawesi Province, Indonesia in January-April 2023 and sample testing was carried out at the UNHAS RSPTN Research Laboratory .The subjects of the study were 44 inpatient schizophrenia patients who received a therapeutic dose of risperidone and were randomly allocated to the treatment group and control group with a total of 22 people each. The treatment group was given Omega-3 capsules at a dose of 2400 mg (1 capsule)/24 hours/orally for 8 weeks. Improvement in clinical symptoms was measured using the PANSS (Positive and Negative Syndrome Scale) at week 0 (baseline), week 4, and week 8. Serum TNF-alpha markers of inflammation were measured at week 0 (baseline) and week 8 using the ELISA method.

Results: There was an improvement in clinical symptoms in the form of a decrease in the PANSS score, positive symptoms, negative symptoms and general psychopathology in both the treatment and control groups. However, better results occurred in the treatment group compared to the control group,

especially in the first 4 weeks of the intervention, where in the treatment group the total PANSS score decreased by 29.6%, while in the control group the total PANSS score decreased by only 14.9%. As for the inflammatory marker TNF-alpha, there was a significant decrease of 45.9% after 8 weeks of giving omega-3, whereas in the control group who were not given omega-3 there was no significant change in TNF-alpha levels.

Conclusion: Administration of adjuvant omega-3 therapy contributed to better clinical symptom improvement in the first 4 weeks of therapy and reduced markers of TNF-alpha inflammation compared to the group that only received conventional antipsychotic therapy.

Keywords: Schizophrenia, Risperidone, TNF-α, Omega-3.

1. Introduction

In addition to genetic factors as an important etiology (Lichtenstein et al., 2009), environmental factors such as an unhealthy lifestyle with poor diet may be the cause (Samele et al., 2007). Previous studies have shown that low consumption of fish and seafood during pregnancy may increase the risk of low IQ and impaired neurodevelopmental outcomes in childhood which in turn may be associated with an increased risk of mental disorders such as schizophrenia in adulthood. Omega-3s are abundant in oily fish such as mackerel and sardines. A recent meta-analysis study found that the prevalence of schizophrenia was greater for the group with low fish consumption (Kinney et al., 2009). in recent years, growing attention has concentrated on the role of nutritional interventions in neurological and mental diseases to maximize the effects of nutritional components on brain function, neural plasticity, and mental health (Fang et al., 2017). A study was conducted in randomized, double blind, during the 8th week in 60 patients with schizophrenia divided into two groups: omega-3 (1000 mg/day) (n=30) and placebo (n=30). The efficacy of omega-3s in reducing general psychopathological and total scores was significant compared to the placebo group from 4 and 6 weeks after treatment onset, respectively ($p<0.05$) (Jamilian et al., 2014). omega-3 (1000 mg/day) (n=30) and placebo (n=30). The efficacy of omega-3s in reducing general psychopathological and total scores was significant compared to the placebo group from 4 and 6 weeks after treatment onset, respectively ($p<0.05$) (Jamilian et al., 2014). omega-3 (1000 mg/day) (n=30) and placebo (n=30). The efficacy of omega-3s in reducing general psychopathological and total scores was significant compared to the placebo group from 4 and 6 weeks after treatment onset, respectively ($p<0.05$) (Jamilian et al., 2014).

Thus, there have been no consistent results regarding TNF-α levels being associated with clinical symptoms of schizophrenia and given the potential correlation basis between schizophrenia and low fatty acid levels in the brains of schizophrenic patients, it seems worthwhile to test the idea that dietary omega -3 supplementation may be beneficial in the treatment of these patients. In addition, omega-3 supplementation may increase the efficacy of commonly used antipsychotic drugs due to changes in neurotransmission. Based on this, this study seeks to analyze the effect of adjuvant omega-3 therapy on improving clinical symptoms and tumor necrosis factor alpha (TNF-α) serum levels in schizophrenic patients. The general objective of this study was to determine the effectiveness of omega-3 in improving clinical symptoms and serum TNF-α levels in schizophrenic patients. The specific objectives of this study were to measure the clinical symptoms of schizophrenic patients who only received risperidone antipsychotics at the beginning of the study, 4th week and 8th week, to measure the clinical symptoms of schizophrenic patients who received antipsychotics risperidone and adjuvant omega-3 therapy at the beginning of the study, 4th week and 8th week, to measure serum TNF-α levels in schizophrenic patients who only received antipsychotic risperidone at the beginning of the study and 8th week, to measure serum TNF-α levels in schizophrenic patients. he received the antipsychotic risperidone and adjuvant omega-3 therapy at the start of the study and at 8th week. Furthermore, this study attempted to compare changes in clinical symptoms in the intervention and control groups at the beginning of the study, 4th week and 8th week, compare changes in TNF-α

levels in the intervention and control groups at the start of the study, 4th week and 8th week and determine the correlation between clinical symptoms and serum TNF-α levels in schizophrenic patients who received the antipsychotic risperidone and omega-3 adjuvant therapy. The hypothesis of this study was "Adjuvant omega-3 therapy can improve clinical symptoms and reduce serum TNF-α levels in schizophrenic patients receiving the antipsychotic risperidone. this study attempted to compare changes in clinical symptoms in the intervention and control groups at the beginning of the study, 4th week and 8th week, compare changes in TNF-α levels in the intervention and control groups at the start of the study, 4th week and 8th week and determine the correlation between clinical symptoms and serum TNF-α levels in schizophrenic patients who received the antipsychotic risperidone and omega-3 adjuvant therapy. The hypothesis of this study was "Adjuvant omega-3 therapy can improve clinical symptoms and reduce serum TNF-α levels in schizophrenic patients receiving the antipsychotic risperidone. this study attempted to compare changes in clinical symptoms in the intervention and control groups at the beginning of the study, 4th week and 8th week, compare changes in TNF-α levels in the intervention and control groups at the start of the study, 4th week and 8th week and determine the correlation between clinical symptoms and serum TNF-α levels in schizophrenic patients who received the antipsychotic risperidone and omega-3 adjuvant therapy. The hypothesis of this study was "Adjuvant omega-3 therapy can improve clinical symptoms and reduce serum TNF-α levels in schizophrenic patients receiving the antipsychotic risperidone. 4th week and 8th week and determine the correlation between clinical symptoms and serum TNF-α levels in schizophrenic patients who received the antipsychotic risperidone and omega-3 adjuvant therapy. The hypothesis of this study was "Adjuvant omega-3 therapy can improve clinical symptoms and reduce serum TNF-α levels in schizophrenic patients receiving the antipsychotic risperidone. 4th week and 8th week and determine the correlation between clinical symptoms and serum TNF-α levels in schizophrenic patients who received the antipsychotic risperidone and omega-3 adjuvant therapy. The hypothesis of this study was "Adjuvant omega-3 therapy can improve clinical symptoms and reduce serum TNF-α levels in schizophrenic patients receiving the antipsychotic risperidone.

2. Research Methods

This research is an experimental analysis research, using a pre- and post-test research design with non-random group selection, in which variable measurements are carried out before and after the intervention. This research was conducted in January-April 2023. This research was conducted at Dadi Regional Special Hospital, South Sulawesi Province. The population in this study were all patients who were hospitalized at Dadi Regional Special Hospital, South Sulawesi Province. The sample in this study were all patients with stable phase schizophrenia who were treated at the Dadi Regional Special Hospital in South Sulawesi Province who met the inclusion and exclusion criteria. The required sample size for a two-tailed test is obtained by the following formula:

$$n = 2(\frac{(Z\alpha + Z\beta)S}{x1 - x2})^2$$

$$n = 2(\frac{(1{,}96 + 0.84)4{,}73}{60.20 - 56{,}23})^2$$

$$n = 22$$

Based on the calculation results, the minimum number of samples for each group is 22 people. This research was conducted on two sample groups so that the total sample was 44 people.

Information:

n = Minimum number of subjects per group

α = Type one error, set at 5%, one-way hypothesis

Zα = The standard value of α is 5%, which is 1.96

β = Type two error, set at 20%

Zβ = Standard value of 20% β, which is 0.84
x1-x2 = difference in the mean value of the PANSS value in the control and intervention groups of 3.97
S = Combined standard deviation = 4.73

The sampling technique for each group was carried out by means of Consecutive Sampling, namely all patients who met the Selection Criteria. Inclusion criteria were diagnosed with schizophrenia according to ICD X, patients aged 20-45 years, patients who had passed the acute phase (PANSS-EC <15) and a total PANSS score of 90-100, received the antipsychotic risperidone 4-6 mg/day, disease onset ≤ 3 years, and willing to participate in the study. The exclusion criteria were having organic co-morbidities, having a history of consuming drugs for at least 6 months before being admitted to the hospital, using anti-inflammatory drugs and antibiotics. The criteria for dropping out included an increase in the PANSS or PANSS-EC (Moving to the acute room), the study subjects refused to continue the study and the research subjects died.

3. Results

In this study, a sample of 44 subjects was taken which was divided into 2 groups, namely the intervention group of 22 subjects and the control group of 22 subjects where all of them met the inclusion criteria. To provide descriptive data related to the frequency distribution of research subjects, statistical descriptive analysis was carried out. All subjects in this study were male. The age of the intervention subjects was found to be an average age of 32.68 ± 5.95 years, while in the control group it was found to be an average age of 34.09 ± 6.62 years.
Data on the level of education of the study subjects showed that the intervention group had the highest level of junior high school education (36.4%) while in the control group the highest number was junior high school education (45.5%). In the intervention group, the most subjects (72.7%) were not working, while in the control group most were not working (68.2%). Marital status in the intervention group was mostly unmarried (63.6%), while in the control group the most were unmarried (59.1%). After the homogeneity test was carried out, the p values for all variables were greater than 0.05 (p> 0.05) so that it could be concluded that the subjects in this study were homogeneous or not significantly different from the T-Independent test in the age group and the chie square test in the marital status group , education and employment.

Table 1. Demographic Characteristics of Research Subjects

Variables	intervention	control	p-values
	n = 22	**n = 22**	
Age (Mean ± Elementary School)	32.68 ± 5.95	34.09 ± 6.62	0.321*
Marital status:			
Married	8 (36.4%)	9 (40.9%)	0.531**
Single	14 (63.6 %)	13 (59.1%)	
Education:			
No. School	3 (13.6%)	0 (0.0%)	
Elementary school	7 (31.8%)	8 (36.4%)	0.349**
Junior High School	8 (36.4%)	10 (45.5%)	
senior high school	4 (18.2%)	4 (18.2%)	
Employment:			
employed	6 (27.3%)	7 (31.8%)	1,000**
Unemployed	16 (72.7%)	15 (68.2%)	

* Independent t test; **Chi Square test

In both groups clinical symptoms were measured based on the total PANSS value, with positive symptom domains, negative symptoms and general psychopathology in the early week or baseline

which can be seen in table 4. In the intervention group the mean baseline PANSS total was 94.82 and in the control group 96.09 after it was done independent t-test showed no different results. Meanwhile, for baseline TNF-α levels in the two groups, values varied with a large standard deviation, however, the TNF-α level in the intervention group was 97.87, which was higher than the TNF-α level in the control group, which was 81.33, and the difference was after the Mann Whitney test found no significant difference between the treatment and control groups ($p>0.05$). Thus, the research subjects are homogeneous. In both groups clinical symptoms were measured based on the total PANSS value, with the domains of positive symptoms, negative symptoms and general psychopathology in the early weeks or baseline which can be seen in Table 2. In the intervention group the mean baseline total PANSS value was 94.82 and in the control group 96.09 after an independent t-test, no different results were obtained. Meanwhile, for the baseline TNF-α levels in the two groups, values varied with a large standard deviation, but the TNF-α level in the intervention group was 97.87, which was higher than the TNF-α level in the control group, which was 81.33, and after the Mann Whitney test, no significant difference was found between the treatment and control groups ($p>0.05$). Thus, the research subjects are homogeneous. with the domains of positive symptoms, negative symptoms and general psychopathology in the early weeks or baseline which can be seen in Table 2. In the intervention group the mean baseline total PANSS value was 94.82 and in the control group 96.09 after an independent t-test , no different results were obtained. Meanwhile, for the baseline TNF-α levels in the two groups, values varied with a large standard deviation, but the TNF-α level in the intervention group was 97.87, which was higher than the TNF-α level in the control group, which was 81.33, and after the Mann Whitney test, no significant difference was found between the treatment and control groups ($p>0.05$). Thus, the research subjects are homogeneous. with the domains of positive symptoms, negative symptoms and general psychopathology in the early weeks or baseline which can be seen in Table 2. In the intervention group the mean baseline total PANSS value was 94.82 and in the control group 96.09 after an independent t-test , no different results were obtained. Meanwhile, for the baseline TNF-α levels in the two groups, values varied with a large standard deviation, but the TNF-α level in the intervention group was 97.87, which was higher than the TNF-α level in the control group, which was 81.33, and after the Mann Whitney test, no significant difference was found between the treatment and control groups ($p>0.05$). Thus, the research subjects are homogeneous. In the intervention group the mean baseline total PANSS value was 94.82 and in the control group 96.09 after an independent t-test, no different results were obtained. Meanwhile, for the baseline TNF-α levels in the two groups, values varied with a large standard deviation, but the TNF-α level in the intervention group was 97.87, which was higher than the TNF-α level in the control group, which was 81.33, and after the Mann Whitney test, no significant difference was found between the treatment and control groups ($p>0.05$). Thus, the research subjects are homogeneous. In the intervention group the mean baseline total PANSS value was 94.82 and in the control group 96.09 after an independent t-test, no different results were obtained. Meanwhile, for the baseline TNF-α levels in the two groups, values varied with a large standard deviation, but the TNF-α level in the intervention group was 97.87, which was higher than the TNF-α level in the control group, which was 81.33, and after the Mann Whitney test, no significant difference was found between the treatment and control groups ($p>0.05$). Thus, the research subjects are homogeneous. but the TNF-α level in the intervention group was 97.87, which was higher than the TNF-α level in the control group, which was 81.33, and after the Mann Whitney test, no significant difference was found between the treatment and control groups ($p>0.05$). Thus, the research subjects are homogeneous. but the TNF-α level in the intervention group was 97.87, which was higher than the TNF-α level in the control group, which was 81.33, and after the Mann Whitney test, no significant difference was found between the treatment and control groups ($p>0.05$). Thus, the research subjects are homogeneous.

Table 2. Results of Measurement of Clinical Symptoms Based on Total PANSS Values, Positive Symptoms, Negative Symptoms, General Psychopathology and TNF-α Baseline Levels in the Intervention and Control Groups

Group	control Mean ± SD	intervention Mean ± SD	P-values
Baseline- PANSS Total	94.82±3.25	96.09±3.24	0.200*
Baseline- Positive Symptoms	36.95±1.70	37.5±2.41	0.390*
Baseline- Negative Symptoms	33.5±2.28	34.23±1.82	0.402**
Baseline- General Psychopathology	24.36±3.55	24.14±3.00	0.660**
Baseline-TNF-α	97.87±91.12	81.33±58.34	0.760**

* Independent t test; ** Mann Whitney test

After measuring clinical symptoms at baseline, both groups were given the antipsychotic risperidone, in the intervention group besides the antipsychotic risperidone an additional omega-3 capsule was given at a dose of 2400 mg/day. To see changes in clinical symptoms in the intervention and control groups, PANSS values were examined at the 4th week and 8th week. From Table 3 the results of measurements in the intervention group. In table 5 the total PANSS value decreased by an average of 9.18 points from baseline to 4th week ($p < 0.001$), from 4th week to 8th week the total PANSS value decreased by an average of 14.68 points ($p < 0.001$) and from baseline to 8th week the total PANSS value decreased by an average of 33.86 points ($p < 0.001$).

Table 3. Comparison of Clinical Symptoms Based on PANSS Total Intervention Group Values at Baseline, 4th week and 8th week

PANSS Total	Mean ± SD	std. Dev.	P-values
baseline	94.82 ± 3.25	19.18 (6.78)	0.001*
4th week	75.64 ± 7.77		
4th week	75.64 ± 7.77	14.68 (8.32)	0.001*
8th week	60.95 ± 7.59		
baseline	94.82 ± 3.25	33.86 (7.50)	0.001*
8th week	60.95 ± 7.59		
*Paired t test			

Table 4 shows the results of measuring clinical symptoms in the control group. In table 9 the total PANSS value decreased by an average of 9.86 points from baseline to 4th week ($p < 0.001$), from 4th week to 8th week the total PANSS value decreased by an average of 10.73 points ($p < 0.001$) and from baseline to 8th week the total PANSS value decreased by an average of 20.59 points ($p < 0.001$).
Table 4. Comparison of Clinical Symptoms Based on PANSS Total Control Group Values at Baseline, 4th week and 8th week

Table 5. Comparative Analysis of Clinical Symptoms Based on Total PANSS Values Between Intervention and Control Groups at Baseline, 4th week and 8th week

PANSS Total	Mean ± SD	std. Dev	P-values
baseline	96.09 ± 3.24	9.86 (4.68)	0.001*
4th week	86.23 ± 6.17		
4th week	86.23 ± 6.17	10.73 (3.67)	0.001*
8th week	75.50 ± 5.78		
baseline	96.09 ± 3.24	20.59 (4.79)	0.001*
8th week	75.50 ± 5.78		
*Paired t test			

Group	PANSS Total Baseline (Mean ± SD)	PANSS Total 4th week (Mean ± SD)	Value Change in PANSS Total	Difference Mean Decrease (IK95%)	P-values
intervention	94.82 ±3.25	75.64 ± 7.77	- 19.18	-9.38	0.001**
control	96.09 ± 3.24	86.23 ± 6.17	- 9.86	(5.77;12.86)	
P-values	0.200*	0.001*			
	PANSS Total 4th week (Mean ± SD)	**PANSS Total 8th week (Mean ± SD)**	**Value Change in PANSS Total**	**Difference Mean Decrease (IK95%)**	**P-values**
intervention	75.64 +7.77	60.95 ± 7.59	- 14.68	-3.95	0.226**
control	86.23 ± 6.17	75.50 + 5.78	- 10.73	(0.04;7.86)	
P-values	0.001*	0.001*			

	PANSS Total Baseline (Mean ± SD)	PANSS Total 8th week (Mean ± SD)	Value Change in PANSS Total	Difference Mean Decrease (IK95%)	P-values
intervention	94.82 +3.25	60.95 ± 7.59	- 33.87	-13.28	0.001**
control	96.09 ± 3.24	75.50 + 5.78	- 20.59	(9.44;17.10)	
P-values	0.200*	0.001*			
*Independent test, **Mann Whitney test					

From Table 5, the p value for the mean difference in the decrease in the total PANSS value from baseline to 4th week and from baseline to 8th week in the treatment group and the control group was p <0.001, so it can be concluded that there was a significant change in the total PANSS value in the control and treatment groups. However, the change in total PANSS values in the intervention group was greater than that of the control group, where the total PANSS values decreased by an average of 9.38 points better than the control group with 95% confidence intervals ranging from 5.77 to 12.86 from baseline to 4th week. And the total PANSS value decreased by an average of 13.28 points better than the control group with a 95% confidence interval ranging from 9.44 to 17.10 from baseline to 8th week. From the table it is also obtained that the p value for the mean difference in the decrease in the total PANSS score from the 4th week to the 8th week in the treatment group and the control group is p> 0.05 so it can be concluded that the change in the total PANSS value was not significant, both in the control group as well as treatment. However, the change in total PANSS values in the intervention group was greater than that of the control group, where the total PANSS values decreased by an average of 3.95 points better than the control group with 95% confidence intervals ranging from 0.04 to 7.86. both in the control group as well as treatment. However, the change in total PANSS values in the intervention group was greater than that of the control group, where the total PANSS values decreased by an average of 3.95 points better than the control group with 95% confidence intervals ranging from 0.04 to 7.86. both in the control group as well as treatment. However, the change in total PANSS values in the intervention group was greater than that of the control group, where the total PANSS values decreased by an average of 3.95 points better than the control group with 95% confidence intervals ranging from 0.04 to 7.86.

Table 6. Improvement in Clinical Symptoms Based on the Percentage of Decrease in PANSS Scores in the Intervention and Control Groups from Baseline, 4th week and 8th week

Group	intervention						control					
	Mean ± SD	% Value Changes Baseline-4th week	% Value Decrease Baseline-4th- 8th week	% Value Changes Baseline-8th week	Interpretation	P-values	Mean ± SD	% Value Changes Baseline-4th week	% Value Decrease Baseline-4th- 8th week	% Value Changes Baseline-8th week	Interpretation	P-values
Baseline-PANSS Total	97.82±3.25	29.6%	22.7%	52.3%	Much Improvement	00.001**	96.09±3.24	14.9%	16.3%	31.2%	Moderate improvements	0.001**
4th week-PANSS Total	75.64±7.77						86.23±6.17					
8th week-PANSS Total	60.95±7.59						75.50±5.78					
Baseline- Positive Symptoms	36.95±1.70	30.6%	21.6%	52.2%		0.001**	37.50±2.41	13.7%	18.9%	32.6%		0.001**
4th week-Positive Symptoms	27.77±4.79						33.32±3.82					
8th week-Positive Symptoms	21.32±4.03						27.55±5.38					
Baseline- Negative Symptoms	33.50±2.28	30.9%	20.84%	51.8%		0.001**	34.23±1.82	11.0%	13.1%	24.1%		0.001**
4th week-Negative Symptoms	25.32±4.79						31.23±4.05					
8th week-Negative Symptoms	19.77±4.82						27.68±4.43					
Baseline- General Psychopathology	24.36±3.55	20.1%	33.7%	53.8%		0.001**	24.14±3.00	30.2%	17.3%	47.5%		0.001**
4th week- General Psychopathology	22.68±2.80						21.68±2.46					
8th week- General Psychopathology	19.86±1.55						20.27±1.88					

*Wilcoxon test, ** Repeated ANOVA test, ***Friedman test

After measuring baseline TNF-α levels as can be seen in table 4 and giving additional omega-3 capsules to the intervention group so that at 8th week TNF-α levels were measured again in both groups. The results showed that the TNF-α level in the intervention group was 52.92 pg/ml with an SD of 52.21 pg/ml while in the control group it was 64.61 pg/ml with an SD of 55.70 pg/ml. Then a calculation of the difference in levels between the baseline and the 8th week was carried out with the result that in the intervention group there was a significant decrease in TNF-α levels of 44.95 pg/ml or 45.9% from the baseline to the 8th week, compared to the control group who only received the antipsychotic risperidone even though there appeared to be a decrease in the average baseline TNF-α level to the 8th week of 16.72 pg/ml or 20. 6% but after the Wilcoxon test it could be said that the decrease was not significant or in other words there was no decrease. The results of the data analysis can be seen in Table 7.

Table 7. Changes in Serum TNF-α Levels in the Intervention and Control Groups at Baseline and 8th week

Group	intervention				control			
	Mean ± SD	**The difference in the mean difference in levels of TNF -α**	**% Level Decrease TNF-α Baseline - 8th week**	**P-values**	**Mean ± SD**	**The difference in the mean difference in levels of TNF -α**	**% Level Decrease TNF-α Baseline - 8th week**	**P-values**
TNF-α Baseline	97.87±91.12	44.95	45.9%	0.024*	81.33± 58.34	16.72	20.6%	0.306*
TNF-α 8th week	52.92±52.21				64.61± 55.70			
* Wilcoxon test								

Comparison of the decrease in TNF-α levels between the intervention and control groups from baseline to 8th week can be seen in Figure 1.

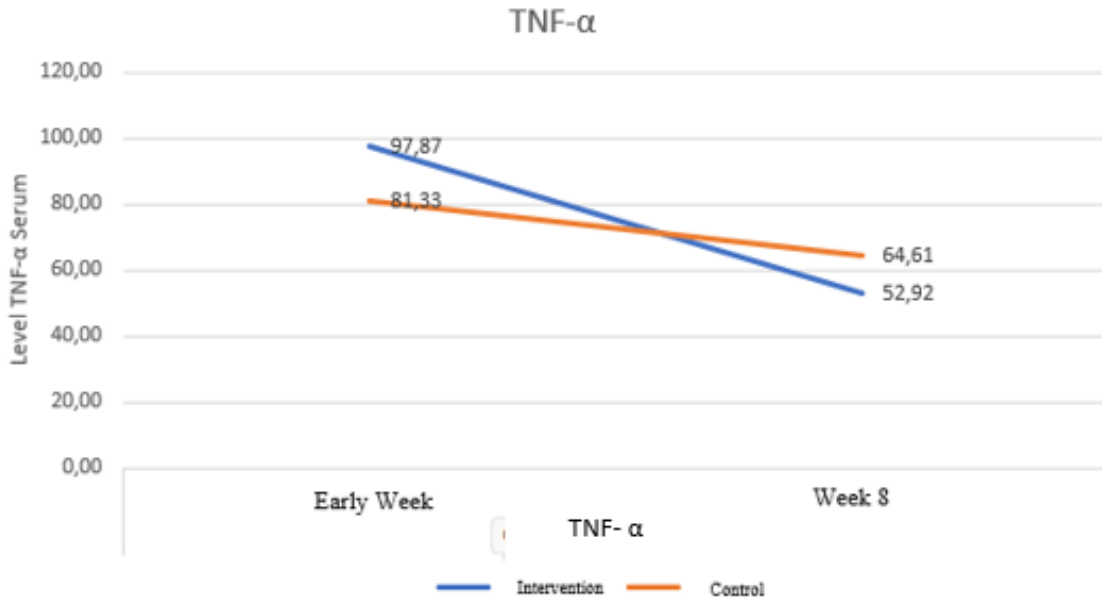

Figure 1. Comparison of the reduction in TNF-α in the intervention group and the control group

The relationship between the improvement of clinical symptoms as measured by the PANSS score, as well as the positive, negative and general psychopathology subscales of TNF-α levels in the two groups can be seen in Table 8.

Table 8. Relationship Between Serum TNF-α Levels and Total PANSS Value, Positive, Negative and General Psychopathology Subscales in the Intervention Group and Control Group

Group	Symptoms	TNF	
		r value	P VALUE
intervention	PANSS Total	0.546	0.000
	Positive Symptoms	-0.143	0.355
	Negative symptoms	0.066	0.669
	General psychopathology	0.377	0.120
control	PANSS Total	0.157	0.310
	Positive Symptoms	-0.153	0.321
	Negative symptoms	-0.009	0.955
	General psychopathology	-0.105	0.492

*Spearman Correlation Test, r = strength of correlation; 0.1 - 0.3 weak; 0.4 - 0.6 medium; 0.7 - 0.9 strong

The results of correlation analysis using the Spearman correlation test showed that the correlation between total PANSS values and TNF-α levels in the intervention group showed a significant relationship ($p < 0.05$) with moderate correlation strength and positive value. The correlation between general psychopathology domain scores and TNF-α levels in the intervention group showed significant results ($p<0.05$) with weak correlation strength and positive value. The correlation between positive and negative symptoms and general psychopathology domain values with TNF-α levels in the intervention group showed no significant results ($p>0.05$). The correlation between total PANSS value, domain of positive symptoms, negative symptoms and general psychopathology with TNF-α levels in the control group showed no significant results ($p>0.05$).

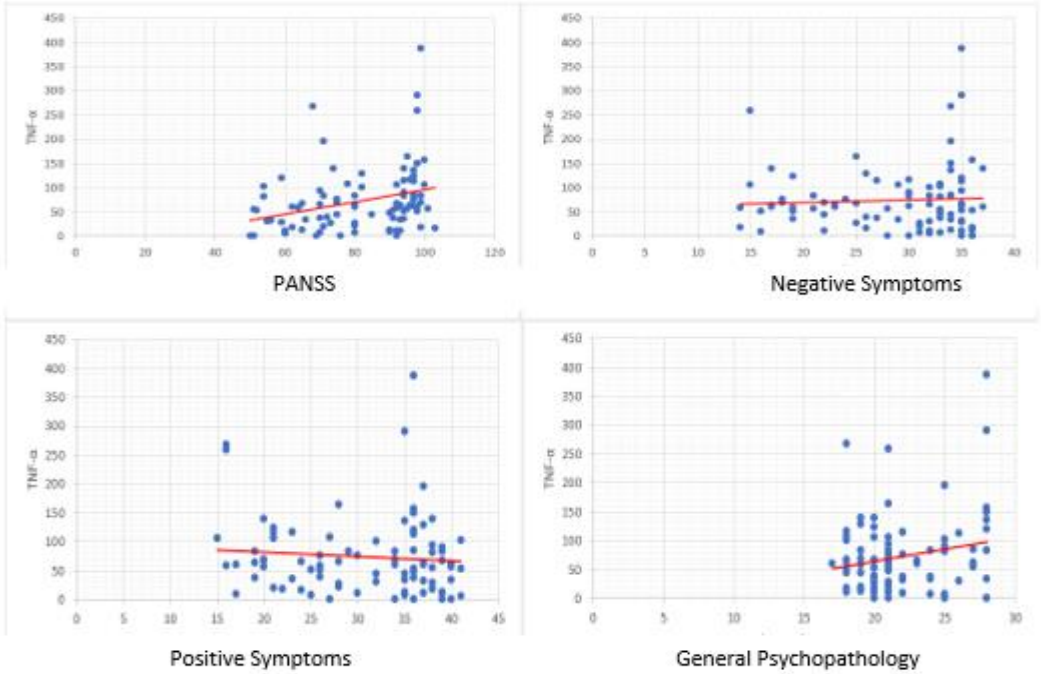

Figure 2. Correlation Between Total PANSS Values, Positive Symptom Domains, Negative Symptoms and General Psychopathology with TNF-α in the Intervention Group.

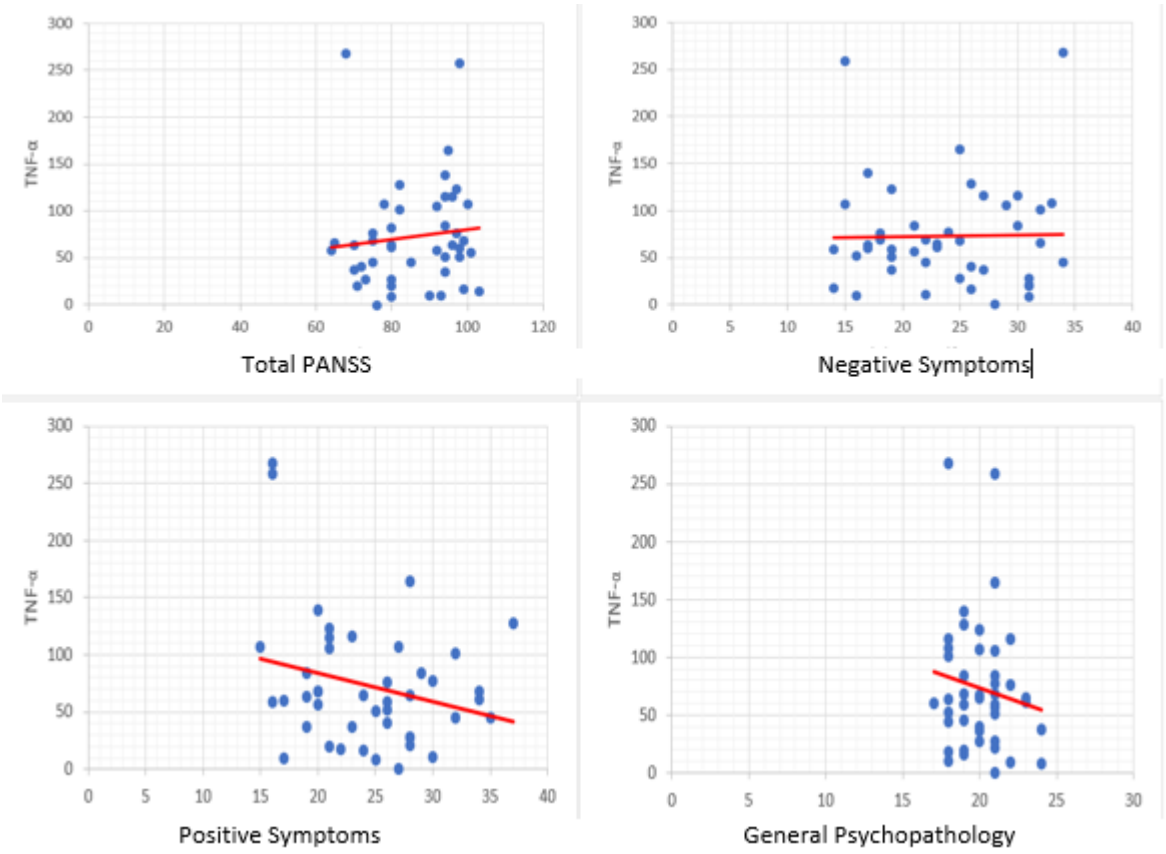

Figure 3. Correlation Between Total PANSS Values, Positive Symptom Domains, Negative Symptoms and General Psychopathology with TNF-α in the Control Group

4. Discussion

To see the effect of adjuvant omega-3 therapy on clinical symptoms as measured by total PANSS along with positive, negative and general psychopathological symptom domains and its effect on TNF-α levels in the blood serum of schizophrenic patients carried out for 8 weeks of administration. The basic characteristics of the research subjects including age, education, occupation and marital status found no significant differences between the intervention and control groups ($p>0.05$). Thus the research subjects are homogeneous.

The mean age of the study subjects was 32.68 ± 5.95 in the intervention group and 34.09 ± 6.62 in the control group. In this study, male subjects were taken as research subjects because of the distribution of men who were temporarily hospitalized during the study, more men were hospitalized at the hospital where the study took place. In theory, earlier onset is usually found in males than females. Where more than half of schizophrenics are men but only a third are women (Sadock, 2015). There are several hypotheses that can explain sex differences in schizophrenia. A theory of schizophrenia that implicates gonadal hormones, such as estrogen, playing a neuroprotective role preventing schizophrenic pathology in women. Estrogen deficiency at menopause is strongly associated with the severity of psychiatric symptoms in women. A negative correlation between patients' plasma estrogen levels and schizophrenic symptoms was also reported in men. Gender-specific association of certain dopaminergic genes (catechol-O-methyltransferase, monoamine oxidase) is also associated with schizophrenia. Whereas dopamine deficits and excesses have been associated with both positive and negative symptoms of schizophrenia in general(Li et al., 2016)

Based on the last education level of schizophrenics in the last educational intervention group, the most were junior high school students, 36.4% and the control group, the most were junior high school

students, 45.5%. If seen from these data individuals with schizophrenia are also less likely to have a higher education than individuals without schizophrenia. Poor academic achievement before the age of 16 years associated with the appearance of prodromal symptoms may represent a premorbid cognitive marker that is susceptible to schizophrenia later in life to undergo an educational process.(Dickson et al., 2020).

In this study, 72.7% of research subjects did not work in the intervention group and 68.2% in the control group. People with schizophrenia who are employed in normal jobs generally get terminated due to the dissatisfaction of the private sector with the results of the work they do.(Dickson et al., 2020). Factors of stigmatization and discrimination from society cause patients to lack self-motivation and limit their right to get a job. Some schizophrenic patients cannot work because of difficulties in getting job opportunities and the rest decide not to continue working from their jobs because of their illness, because people who work have confidence in the future and have a greater zest for life compared to those who don't work.(Saperstein et al., 2011).

Based on marital status, most of the subjects were unmarried by 14 participants or 63.6% for the intervention group and for the control group 13 participants or 59.1%. This is in line with other research which states that someone who is not married may be at greater risk of experiencing schizophrenia than someone who is married, although statistically there was no significant difference between marital status and the incidence of schizophrenia. Sadock stated that the prognosis of schizophrenics who are unmarried, do not have a partner, divorced, widowed, or widowed tends to be worse than schizophrenics who have partners who support the patient's recovery (Sadock, 2015).

Clinical symptoms in both groups were measured by the total PANSS value along with the domains of positive symptoms, negative symptoms and general psychopathology at baseline and the mean initial total PANSS values between the intervention and control groups were almost the same, namely 94.82 and 96.09, while TNF- The baseline α in the intervention group and the control group obtained varied values even though the TNF-α level in the intervention group was higher, namely 97.87 ±91.12pg/mlwhile the level of TNF-α in the control group was81.33 ± 58.34pg/ml. The levels of TNF-α in the two groups which varied were in line with several previous studies. The results of the analysis test by Hasbi, 2020 showed that the median TNF-α level in the group with schizophrenia was 3.40 pg/ml with a minimum value of 0.65 pg/ml and a maximum value of 43.8 pg/ml while in the healthy control group the median value TNF-α level was 14.75 pg/ml with a minimum value of 5.18 pg/ml and a maximum value of 31.1. pg/ml(Y. Luo et al., 2019).A study by Kim in Korea found an average TNF-α of 870.82 pg/ml in schizophrenic patients and 577.51 pg/ml in normal controls, as well as schizophrenic patients after being hospitalized and then checked again at discharge with the results of TNF-α levels having decreased to an average of 850.97 pg/ml(YK Kim et al., 2009).

Elevated TNF-α serum levels indicate that the body is in an active state and oxidative stress may be present. Brain microglia activation may play a key role in this immune response. When the body encounters various factors that cause peripheral immune dysfunction, inflammatory substances such as cytokines activate the migration of brain microglia through pathways such as the peripheral nervous system via signaling, secondary messengers, or directly through the blood-brain barrier. Activation of microglia alters the balance between excitatory and inhibitory signaling neurons, triggering immune dysfunction in the cerebral cortex, affecting neurotransmitters, especially glutamic and aminobutyric acids, and thereby causing psychotic symptoms. This may explain why the neuroinflammatory response plays an important role in the etiopathogenesis of schizophrenia(Lin et al., 2021). Possible causes of changes in cytokine levels in blood and CSF include polymorphisms in their genes. This polymorphism may contribute to the subsequent sensitivity to the influence of other factors, such as early childhood trauma and HPA axis dysregulation; or disturbances in the composition of the gut microbiota. The role of immunological dysfunction in the etiopathogenesis of schizophrenia has also

been suggested by its association with underlying autoimmune disorders, polymorphisms in the TNFR2 gene encoding the TNF-α receptor, depending on the variant, may be associated with an increased risk of schizophrenia, or may have a protective effect. Experiencing any type of trauma in childhood appears to lead to increased peripheral levels of TNF-α and IL-6 in adults, while being a victim of sexual abuse only increases peripheral levels of TNF-α in adulthood. The nature of the association between early childhood trauma and cytokine levels also appears to depend on a number of factors, such as the nature of the trauma, the diagnostic category applied, age, menopause, and sex. Meanwhile, gut microbiome dysbiosis influences behavior as well as microglia function and maturation. Disturbances in the composition of the gut microbiome are associated with increased permeability of the gut epithelium to bacteria, their antigens, and pathogen-associated molecular patterns (PAMPs), eg LPS. Greater permeability, in turn, correlates with increased peripheral cortisol levels and may lead to activation of the immune system associated with secretion of pro-inflammatory cytokines, activation of the HPA axis, and establishment of a positive feedback loop. such as the nature of the trauma, the diagnostic category applied, age, menopause, and gender. Meanwhile, gut microbiome dysbiosis influences behavior as well as microglia function and maturation. Disturbances in the composition of the gut microbiome are associated with increased permeability of the gut epithelium to bacteria, their antigens, and pathogen-associated molecular patterns (PAMPs), eg LPS. Greater permeability, in turn, correlates with increased peripheral cortisol levels and may lead to activation of the immune system associated with secretion of pro-inflammatory cytokines, activation of the HPA axis, and establishment of a positive feedback loop. such as the nature of the trauma, the diagnostic category applied, age, menopause, and gender. Meanwhile, gut microbiome dysbiosis influences behavior as well as microglia function and maturation. Disturbances in the composition of the gut microbiome are associated with increased permeability of the gut epithelium to bacteria, their antigens, and pathogen-associated molecular patterns (PAMPs), eg LPS. Greater permeability, in turn, correlates with increased peripheral cortisol levels and may lead to activation of the immune system associated with secretion of pro-inflammatory cytokines, activation of the HPA axis, and establishment of a positive feedback loop. Disturbances in the composition of the gut microbiome are associated with increased permeability of the gut epithelium to bacteria, their antigens, and pathogen-associated molecular patterns (PAMPs), eg LPS. Greater permeability, in turn, correlates with increased peripheral cortisol levels and may lead to activation of the immune system associated with secretion of pro-inflammatory cytokines, activation of the HPA axis, and establishment of a positive feedback loop. Disturbances in the composition of the gut microbiome are associated with increased permeability of the gut epithelium to bacteria, their antigens, and pathogen-associated molecular patterns (PAMPs), eg LPS. Greater permeability, in turn, correlates with increased peripheral cortisol levels and may lead to activation of the immune system associated with secretion of pro-inflammatory cytokines, activation of the HPA axis, and establishment of a positive feedback loop. (Dawidowski et al., 2021). The role of external influences on the cytokine network in patients schizophrenia it can be concluded that there are at least three important factors: stress, smoking and psychotropic substances (eg, clozapine). (Kronfol, 2003).Some of these factors may contribute to variations in TNF-α levels in schizophrenic patients.

In the 4th and 8th weeks clinical symptoms were measured with PANSS in both groups. From the results of measuring the PANSS value, it is obtained that the value changes from*baseline*,4th and 8th week. Changes in the PANSS value from *baseline* to the 4th week, there was an average decrease of 19.18 in the intervention group and a decrease of 9.86 in the control group. Changes in PANSS values from week 4 to week 8 with an average decrease of 14.68 in the intervention group and a decrease of 10.73 in the control group. While the change in the PANSS value from *baseline* to the 8th week with an average decrease in value of 33.86 in the intervention group, while in the control group the decrease in PANSS score was 18.47, then the percentage of decrease in PANSS score was calculated to assess the interpretation of the improvement in clinical symptoms that occurred in both groups. In the intervention group that received adjuvant omega-3 capsules, there was a decrease in the total PANSS

value of 52.3%, which meant a lot of improvement in clinical symptoms, whereas in the control group, which only receiving the antipsychotic risperidone therapeutic dose obtained a decrease of 31.2% with an interpretation of moderate improvement in clinical symptoms. Furthermore, the difference in the decrease in the PANSS value in the two groups, namely 13.27, after the significance test was carried out, obtained significant results with a p value <0.001.

Studies addressing structural brain abnormalities on magnetic resonance imaging examinations frequently reveal reduced brain volume and cortical thickness in patients with schizophrenia, particularly abnormalities of the frontotemporal and hippocampal areas of the brain and similar enlargement of the lateral ventricles, even in individuals at high clinical risk of schizophrenia and in those with schizophrenia. early stage of the disease. In 2018, Pawe Lczyk et al., published a paper aiming to confirm whether the use of supplemental polyunsaturated fatty acid therapy, with its neuroprotective effects, can sustain this loss of cortical volume in patients with schizophrenia. The authors note that individuals who received polyunsaturated fatty acids demonstrated reduced cortical thickness loss in cortical areas that control functions known to be impaired in schizophrenia. Specifically, the study conducted by PaweLczyk observed a reduction in cortical thickness loss associated with polyunsaturated fatty acids, compared with placebo in schizophrenic patients treated with antipsychotics. Differences were observed in the left parieto-occipital cortex, particularly Brodmann areas 7 and 19, as cortical areas located near the temporo-parieto-occipital junction, which integrate information from both the external environment and from within the body. The temporo-parieto-occipital junction is also involved in another difference: a process that is damaged in schizophrenia and one of the main features of this disease. Brodmann's area 7 is described as the somatosensory association cortex and is thought to play a role in visuomotor coordination, semantic categorization tasks, and recognition of temporal context. Brodmann's area 19 is described as the associative visual cortex and is involved in light intensity detection, feature attention and pattern detection, and is represented in spatial working memory. Many of these activities, such as visuomotor coordination, attention, pattern detection, and working memory, are generally impaired in schizophrenia. There is substantial evidence from animal studies addressing the topic of the effect of polyunsaturated fatty acids on cortical thickness; only one placebo-controlled study has been reported. The authors evaluated the effect of 8 weeks of polyunsaturated fatty acid supplementation on structural changes in the brains of aged mice in a high-resolution magnetic resonance imaging examination. Significant enlargement of gray matter volume has been described in the hippocampus, medial prefrontal cortex, and retrosplenial cortex of the rat brain. However, the reported increase in gray matter volume correlated significantly with increased cognitive task performance. In a previous study, the same research group found that polyunsaturated fatty acid supplementation in aged mice was associated with improved hippocampal cognitive function that occurred in the context of increased cellular plasticity and reduced neurodegeneration. Polyunsaturated fatty acid supplementation enhances hippocampal neurogenesis and dendritic arborization of nascent neurons, increasing neuronal volume and density, together with microglial cell count. In addition, it also reduces apoptosis, astrocytosis, and lipofuscin accumulation in the hippocampus. The increased blood acetyl-l-carnitine levels and brain polyunsaturated fatty acid concentrations found in mice supplemented with polyunsaturated fatty acids also illustrate effective neuroprotection.(Mazza et al., 2019)

The 44 study subjects were examined for TNF-α at baseline and the 8th week, and a comparison of baseline serum TNF-α levels was obtained in the intervention group with levels of 97.87 pg/ml ±91,12whereas in the control group, the baseline serum TNF-α level was 81.33 ± 58.34pg/ml. In the 8th week there was a decrease in TNF-α serum levels in both groups, a significant reduction was found in serum TNF-α levels in the intervention group which was 44.95 pg/ml or 45.9% compared to the control group which, although the mean value the change from baseline to week 8 showed a decrease in TNF-α levels of 16.72pg/ml or 20.6%, but after being tested for significance, the results were not significant which could be interpreted as no significant decrease.

The greater reduction in TNF-α levels in the group receiving adjuvant omega-3 therapy compared to the control group could be attributed to omega-3 fatty acids being thought to have some effect on inflammation by modulating the amount and type of eicosanoids made, and other effects elicited by independent mechanisms. eicosanoids, including actions on intracellular signaling pathways, transcription factor activity, and gene expression. Simopoulos, 2002). EPA and docosahexaenoic acid (DHA), the two main omega-3 fatty acids in fish oil, have important roles in the CNS. DHA is a key structural component of nerve membranes, which is important for cognitive and behavioral function. EPA on the other hand has important physiological functions such as proper growth, development and functioning of the brain (Peet and Stokes, 2005). EPA and DHA are thought to exert their anti-inflammatory effects via mediators called resolvins and protectins. Resolvin is biosynthesized from EPA and DHA and reduces inflammation by binding to G-protein coupled receptors on leukocytes and blocking the production of prion flammatory mediators. Resolvin also stops leukocyte migration and reduces cytokine expression by microglial cells. Protectin is synthesized exclusively from DHA via a pathway separate from resolvin. Protectins reduce inflammation by stopping leukocyte infiltration and reducing cytokine production by glial cells Resolvin also stops leukocyte migration and reduces cytokine expression by microglial cells. Protectin is synthesized exclusively from DHA via a pathway separate from resolvin. Protectins reduce inflammation by stopping leukocyte infiltration and reducing cytokine production by glial cells Resolvin also stops leukocyte migration and reduces cytokine expression by microglial cells. Protectin is synthesized exclusively from DHA via a pathway separate from resolvin. Protectins reduce inflammation by stopping leukocyte infiltration and reducing cytokine production by glial cells(Keller et al., 2013)

In addition, it has been argued that dysfunctional fatty acid metabolism may be involved in the etiology of schizophrenia, based on the finding of reduced omega-3 polyunsaturated fatty acids (PUFAs) in individuals considered to be at very high risk for psychotic disorders.(Amminger et al., n.d.). This is due to the effect of their changes on membrane fluidity and receptor response after their incorporation into cells. Omega-3 PUFAs are also thought to interact with the dopaminergic and serotonergic systems, which have been linked to the pathophysiology of schizophrenia. In addition, the omega-3 PUFA, eicosapentaenoic acid (EPA), can increase glutathione in the temporal lobes of first-episode psychotic patients. Glutathione protects neurons from excitotoxicity and oxidative stress. Several studies have shown that glutathione levels may be low in schizophrenic patients. This suggests that omega-3 PUFAs may have neuroprotective or anti-inflammatory properties. In addition to the evidence presented, many recent trials have supported the hypothesis of a role for inflammation and nutritional deficiencies in the pathogenesis of schizophrenia(Raffa et al., 2011).

While the decrease in TNF-α levels in the control group who only received risperidone antipsychotic therapy, can be associated with several studies that have shown that typical or atypical antipsychotic drugs can affect cytokine concentrations. Flupentixol, queitiine, risperidone, aripiprazole, chlorpromazine, clozapine, and olanzapine can reduce serum concentrations of IL-10, IL-6, IL-1β, IL-2, and TNF-α, thereby demonstrating anti-inflammatory effects associated with antipsychotics in patients with schizophrenia. It is unclear whether changes in cytokine concentrations are directly due to the effects of antipsychotic drugs or whether they are the result of improvement in clinical symptoms. It has been reported that antipsychotic drugs such as chlorpromazine, haloperidol, risperidone, or clozapine can directly affect the action of macrophage cells and microglial cells that produce cytokines. In addition, various neurotransmitters are produced by immune cells, and neurotransmitters such as dopamine, serotonin, and opioid receptors can alter the actions of these immune cell cytokines.(Y. Luo et al., 2019)

After measuring the total PANSS value including the domains of positive symptoms, negative symptoms and general psychopathology and TNF-α levels in the intervention and control groups, a

correlation test was then performed to see the relationship between PANSS values and TNF-α levels, a significant correlation was found between decreased levels the total PANSS value for the decrease in TNF-α levels, while the decrease in values for the domains of positive, negative symptoms and general psychopathology found no significant relationship to TNF-α levels in the group of schizophrenic patients who received the antipsychotic risperidone and omega-3 adjuvants, whereas in the control group who received received the antipsychotic risperidone on the total PANSS value, positive, negative symptom domains and general psychopathology found no significant relationship to the decrease in TNF-α levels.

The effects of polyunsaturated fatty acids are very important for overall health. The proportion of polyunsaturated fatty acids in serum and erythrocyte phospholipids, which are dependent on endogenous metabolism controlled by genetic polymorphisms and dietary intake, are important determinants of health and disease. Kiecolt-Glaser and co-workers assessed the effect of 4 months of supplementation with omega-3 fatty acids in healthy individuals on indicators of inflammation and oxidative stress. Together with the mitigation of pro inflammatory cytokine levels (TNFα and IL-6), the omega-3 fatty acid regimen substantially reduced lipid peroxide levels. Possible mechanisms in polyunsaturated fatty acid modulation of the inflammatory response were investigated in a number of studies. Based on a randomized, double-blind, placebo-controlled parallel group study, PaweLczyk et al., postulated that the potential mechanisms underlying the therapeutic action of omega-3 fatty acids may be neuroprotective, acting through changes in the physical and biochemical properties of cell membranes modulating inflammatory responses and intracellular antioxidant defense systems and reducing dopamine toxicity; it may operate by reducing inflammation through modulating cytokine production. It is possible that the effect of polyunsaturated fatty acid supplementation may depend on the direct interaction between eicosapentaenoic acid or decosahexaenoic acid and glutamatergic neurotransmission.(Mazza et al., 2019)

5.Conclusion

The findings showed that there were much improvements in clinical symptoms in schizophrenic patients who received risperidone therapy and omega-3 adjuvants and moderate improvement in clinical symptoms in schizophrenic patients who received risperidone therapy without omega-3 adjuvants. There was a decrease in serum levels of the pro-inflammatory cytokine TNF-α in schizophrenia patients two times more in the group given omega-3 adjuvants than in the group without omega-3 adjuvants. Improvement in clinical symptoms had a moderate positive correlation with decreased levels of the proinflammatory cytokine TNF-α in schizophrenic patients who received omega-3 adjuvants. The lower the TNF-α, the lower the PANSS score, the more clinical symptom improvement.

The practical benefit of this research is as a reference material for Psychiatrists/Psychiatrists in the management of schizophrenic patients. Theoretical benefit is to increase knowledge and understanding regarding the effect of adjuvant omega-3 therapy on clinical symptoms especially at the 4th week of therapy and decrease serum TNF-α levels in schizophrenia patients, and to make a scientific contribution, especially in the psychosocial approach regarding the effect of adjuvant omega-3 therapy on clinical symptoms and serum TNF-α levels. schizophrenic patients. As a suggestion, giving omega-3 adjuvant to schizophrenic patients is considered because it is proven to improve clinical symptoms and reduce levels of the pro-inflammatory cytokine TNF-α. in addition,

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