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EFFECT OF ADJUVANT ACETYLSALICYLIC ACID THERAPY ON IMPROVEMENT OF CLINICAL SYMPTOMS & TNF-A LEVELS IN PATIENTS WITH SCHIZOPHRENIA

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Abstract
The newest adjuvant treatment concept is acetylsalicylic acid which contains anti-inflammatory properties and is proven with proper administration to provide physiological and psychological benefits. Most studies suggest administration acetylsalicylic acid reduces inflammation and clinical improvement of schizophrenia and research involving clinical trials is still very limited regarding administration acetylsalicylic acid on schizophrenic patients. This study is to determine the effect of adjuvant therapy of acetylsalicylic acid on the improvement of clinical symptoms and TNF-α serum levels in schizophrenic patients. This study uses Experimental Double Blind conducted at the South Sulawesi Provincial RSKD Indonesia in February-March 2023 and sample testing was carried out at the UNHAS RSPTN Research Laboratory.The research sample was schizophrenia patients undergoing hospitalization who received therapeutic doses of risperidone as many as 23 treatment people and 23 control people. Research subjects were measured by PANSS weeks 0, 4, & 8 then the treatment group received acetylsalicylic acid ½ tablet/12 hours/oral for 8 weeks, and the control group received a placebo ½ tablet/12 hours/oral for 8 weeks. And 2 measurements were carried out TNF-α with ELISA in both groups, namely at the beginning of week 0 and at the end of week 8. The results showed that decreased PANSS levels in the schizophrenia group after receiving antipsychotic doses of therapy with tablets acetylsalicylic acid greater decline compared to the group that did not receive acetylsalicylic acid ($p<0.001$). The percentage of clinical symptom improvement was calculated with the results of clinical symptom improvement from the percentage reduction in the PANSS score in the treatment group of 73.46% with the interpretation of clinical symptoms very much improvement, while in the control group, the percentage decrease in the PANSS score was 35.5% with the interpretation of clinical symptoms only moderate improvement. As well as decreased levels of TNF-α baseline in the treatment group of 95.09 ng/l to 60.52 ng/l after 8 weeks with a margin of 34.57 ng/l or 36,3% ($p < 0.001$) compared to the control group on TNF-α baseline from 97.00 ng/l to 87.82 ng/l with a margin of 9.18 ng/l or 9.46% ($p < 0.001$). The reduction in TNF-α levels that occurred was greater in the treatment group than in the control group ($p<0.001$). This indicates a significant

improvement in the levels TNF-α treatment group compared to the control group. There was an effect of adjuvant therapy tablet acetylsalicylic acid who received risperidone in the schizophrenia group showed a greater decrease in serum TNF-α levels than those who were only given risperidone and there was an effect of Adjuvant therapy tablet Acetylsalicylic acid who received risperidone in the group of schizophrenic patients who showed a reduction in clinical symptoms were very much in schizophrenic sufferers than those who were only given risperidone.

Keywords: TNF-α, PANSS, Acetylsalicylic acid, Risperidone, Schizophrenia

1. Introduction

The etiology of schizophrenia has not been identified, but several possible causes have been identified.[1] This includes several dysfunctions of the neurotransmitters involved in signaling dopaminergic, glutamatergic, and asam gamma-aminobutyrate (GABA), impaired neuronal myelination, impaired function of the prefrontal cortex of the brain, and increasing evidence linking inflammation in the brain to the central nervous system.[2] The study of inflammatory mechanisms in psychosis has progressed over the last decades, but further investigations are needed directing at clinical models and cell cultures from human subjects.[3] Individuals with schizophrenia identified as having higher levels of inflammatory markers may benefit from the development of adjuvant therapeutic targets. Seeing the lack of predictor biomarkers of treatment response in schizophrenia, one of the suggested applications is the use of cytokines to predict benefits in treatment.[4]

Acetylsalicylic acid also has many benefits. Acetylsalicylic acid works by inhibiting the production of COX-1 enzymes and modifying COX-2 activity, thus disrupting the neurotoxic inflammatory cascade and suppressing the production of prostaglandins, thromboxane, and other inflammatory molecules including cytokines. Acetylsalicylic acid stimulates endogenous production 'braking signals' arrangement anti-inflammatory, including lipoxins, which dampen the inflammatory response and reduce levels of inflammatory biomarkers. A recent study shows that acetylsalicylic acid reduces the core symptoms of schizophrenia. Even though this study had a relatively small number of participants (n = 20), it does show the benefits of Acetylsalicylic acid compared with a placebo over 2 months of treatment using the Positive and Negative Symptom Scale (PANSS).[5,6] Role Acetylsalicylic acid has properties that inhibit the proinflammatory status of the brain and has beneficial effects in preclinical studies in schizophrenic patients.[7,8] However, research involving clinical trials is still very limited about the role of Acetylsalicylic acid on schizophrenic patients and currently, there is no research conducted in Indonesia.

2. Material and Methods

2.1. Subjects

The subjects in this study were all schizophrenic male subjects who were treated at Dadi Hospital in South Sulawesi Province and met the inclusion criteria. The inclusion criteria were male subjects diagnosed with schizophrenia according to ICD-10 criteria, aged 20-45 years, and willing to participate in the study. Exclusion criteria include having serious physical illness and other illnesses based on history, and a history of abuse of psychotropic drugs. Sample drop out The research subjects could not continue the research because they were hospitalized and research subjects have hypersensitivity to drugs. Blood samples were obtained from the subjects in the morning (around 08.00). Serum was separated, collected, and stored at -80C before use. Concentration TNF-α was measured in the subjects' serum using the EnzymeLinked Immunosorbent Assay (ELISA) method, according to the manufacturer's instructions. Values are expressed as ng/L.

2.2. Procedure

Each subject who met the criteria for schizophrenia according to the ICD-10 criteria and met the inclusion criteria in the study group was recorded and their medical history was taken. The researcher

then explained to the family and the subject about the aims and objectives of the research. If agreed, the subject will be involved in the research (informed consent). The research subjects were divided into two groups (the treatment group was given risperidone therapy and adjuvant therapy Acetylsalicylic Acid, and the control group was given risperidone therapy without therapy Acetylsalicylic Acid). PANSS scores were measured in both groups at study entry, in the 4th week, and in the 8th week. Much TNF-α in the blood in both groups was recorded at the start of the study and at week 8.

2.3. Statistical Analysis

Data were analyzed using the SPSS 24.0 computer program and Microsoft Excel to obtain the expected statistical results with homogeneity test, independent t-test, Mann-Whitney test, Friedman test, and Spearman test.

2.4. Research Ethics

This research, was approved by the Ethics Committee for Biomedical Research in Humans, Faculty of Medicine, Hasanuddin University, Number: **1702/UN4.24.1.1/PT.01.04/2023**. Informed consent was provided from the subject the confidentiality was kept.

3. Results

3.1. Characteristics of Patients

This research was conducted at the Dadi Hospital in South Sulawesi Province in the month of February 2023 to March 2023 and blood serum was analyzed at Hasanuddin University Medical Research Center (HUMRC). The research subjects were 54 people. Eight patients did not meet the criteria so only 46 subjects participated in the study. There were 46 subjects in this study divided into 2 groups (23 subjects in the treatment group and 23 subjects in the control group). The demographic characteristics of the research subjects were all male (100%). Most of the education levels were elementary school (47.8% for the treatment group, 39.1% for the control group). For work, both the control group (69.6%) and the treatment group (60.9%) were more unemployed and marital status, and both the control group (91.3%) and the treatment group (78.3%) were more unmarried. (Paired T-Test).

Table 1. Sociodemographic Characteristics by Frequency (N=46)

	Treatment	Control	*p*
	N=23	N=23	
Age (mean ± SD)	33.09 ± 7.17	33.26 ± 6.88	0.934
Education Level			*0.873*
Elementary School	11(47.8%)	9(39.1%)	
Junior High School	4(17.4%)	9(39.1%)	
Senior High School	8(34.8%)	5(21.7%)	
Occupation			*0.277*
Unemployed	14(60.9%)	16(69.6%)	
Farmer	2(8.7%)	3(13%)	
Employee	3(13%)	3(13%)	
Businessman	4(17.4%)	1(4.3%)	
Marital Status			*0.311*
Married	5(21.7%)	2(8.7%)	
Single	18(78.3%)	21(91.3%)	

**Significant $p < 0.05$ (Paired T Test)*

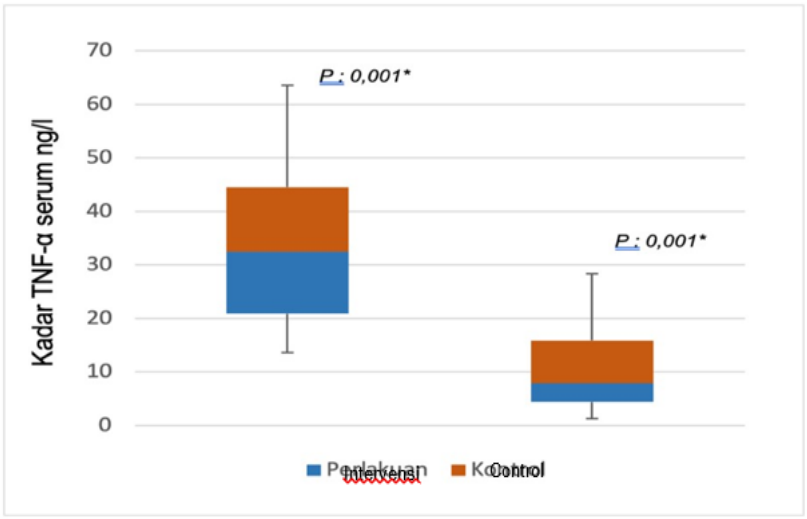
3.2. *Comparison The Mean Values of TNF-α serum*

Comparison the mean values of TNF-α serum in both the treatment group and the control group at baseline, at the 4th week and at the 8th week

Table 2. Comparison The Mean Values of TNF-α serum

	Intervensi			**Control**		
	n = 23			*n* = 23		
	(ng/l)	% decline		*(ng/l)*	% decline	
TNF-α serum	*mean, SD*	TNF-α	*p*	*mean, SD*	TNF-α	*p*
Baseline	95.09 ± 12.84	36,3%	*< 0.001**	97.00 ± 13.89	9,46%	*< 0.001**
8th week	60.52 ± 12.67			87.82 ± 14.81		

***signifikan *p* < 0.05 (*Paired T Test*)**



*Signifikan p < 0,05

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

3.3. *Comparison The Mean Levels of TNF-α serum*

Comparison the mean levels of TNF-α serum in both group at the 4th week and at the 8th week.

Tabel 3. Comparison The Mean Levels of TNF-α serum

	Mean, SD	*Mean, SD*	*P*
	Treatment n=23 (ng/l)	***Control=23 (ng/l)***	
Baseline	95,09 ± 12,84	97,00 ± 13,89	*0,630*
8th week	60,52 ± 12,67	87,82 ± 14,82	*0,001*
Decline TNF-α	34.569± 15.29	9.178± 9.52	*0.001*

(Independent sample test)

Table 4. Comparison of Clinical Symptom Improvement based on the difference in PANSS baseline and 8th week

Improvement of Clinical Symptoms	**Treatment** *n = 23*			***p***	**Control** *n = 23*			***p***
	mean, SD	*% Decrease in Baselineweek 8*	*Interpretasi*		*mean, SD*	*% Decrease in Baselineweek 8*	*Interpretasi*	
PANSS	46,39±11,88	73,46%	**Very much improvement**	*<0.001**	22,21 ± 6,12	35,5%	**Moderate improvement**	*< 0.001**

3.4. *Correlation of PANSS and TNF-α Serum Levels*

Correlation of PANSS and TNF-α serum Levels in both group at baseline, at the 4th week and at the 8th week.

Table 5. ***Correlation of PANSS and TNF-α Serum Levels***

Variable	***Treatment*** (n = 23)		***Control*** (n = 23)	
	deviation TNF-α		deviation TNF-α	
	r	*p*	*r*	*p*
PANSS	*0,736*	*<0,001*	*-0,040*	*0,850*

Significant * p<0.05, ** p<0.01, *** p<0.001 (Spearman Test)

4. Discussion

Forty-six study subjects who received risperidone in both the treatment group and the control group had serum TNF-α levels measured in the first week (baseline) and at the end of the 8th week. It was found that there was a decrease in TNF-α levels baseline in the treatment group after 8 weeks with a difference of 34.57 ng/l ($p < 0.001$). While the control group was on TNF-α baseline with a margin of 9.18ng/l ($p < 0.001$). There was a significant decrease in TNF-α levels in the treatment and control groups. This indicates an improvement in TNF-α levels for both groups. The decrease in both groups could have occurred due to the administration of antipsychotic therapy in both groups which caused a decrease in proinflammatory cytokines, namely TNF-α levels after administration for 8 weeks but the decrease that occurred in the treatment group was greater than the control group.

The biomarker of TNF-α levels in schizophrenia describes the process of neuronmicroglia interaction in the pathophysiology of schizophrenia. This supports previous studies where TNF-α has several functions in the inflammatory process, which can increase the prothrombotic role and stimulate adhesion molecules from leukocyte cells and induce endothelial cells, play a role in regulating macrophage activity and immune response in tissues by stimulating growth factors and other cytokines and functions as a regulator of hematopoietic as well as a co-mitogen for T cells and B cells as well as neutrophil and macrophage cell activity.[9,10] Nearly all inflammatory processes result in activation of tissue macrophages and infiltration of blood monocytes. An increased inflammatory response is implicated in the pathophysiology of schizophrenia, and antipsychotic drugs may be involved in the treatment of schizophrenia. It is also possible that some antipsychotics may have anti-inflammatory activity. [11]

For clinical symptoms as measured by the total PANSS value based on the analysis of the treatment group the total PANSS value between the initial week, the 4th week, and the 8th week showed that there was a significant difference ($p<0,05$). This study also obtained a significant decrease in the rate of improvement in clinical symptoms in the treatment group with very much improvement of 73,46% and moderate improvement of 35,5 % in the control group. Decrease in the value of PANSS from baseline to the 8th week which describes the improvement in clinical symptoms of schizophrenic patients after receiving therapeutic doses of antipsychotics and tablets of Acetylsalicylic acid greater than the group that did not get tablets of Acetylsalicylic acid. In the early weeks of PANSS for the treatment group, the conditions were more varied than the control group but after administration of antipsychotics and adjuvant therapy in the form of tablets of Acetylsalicylic acid in the 4th and 8th weeks, the decrease in PANSS values was more consistent and better in the treatment group than the control group. It can also be seen giving adjuvant tablet therapy Acetylsalicylic acid improves after at least 4 weeks of administration in subjects receiving the adjunctive therapy.

The mechanism of action of the effectiveness of the use of Acetylsalicylic acid based on its ability to inhibit the cyclooxygenase enzyme cyclooxygenase/COX), which catalyzes the conversion of arachidonic acid to prostaglandin H2, prostaglandin E2, and thromboxane A2.[12] Acetylsalicylic acid only works on the cyclooxygenase enzyme, not on the lipoxygenase enzyme so it does not inhibit the formation of leukotrienes.[13] Unlike other NSAIDs which inhibit enzymes competitively so that they are reversible, Acetylsalicylic acid irreversibly inhibits COX enzymes. This is caused by acetylsalicylic acid causes acetylation of serine residues on the terminal carbon group of the COX enzyme, so to produce new prostanoids requires the synthesis of a new COX enzyme. Work mechanism Acetylsalicylic acid Mainly is the inhibition of the synthesis of prostaglandin E2, thromboxane A2, and other inflammatory molecules including cytokines.[14] As a result of this inhibition, there are three main actions of acetylsalicylic acid, namely: (1) anti-inflammatory, due to a decrease in the synthesis of proinflammatory prostaglandins, (2) analgesic, because a decrease in prostaglandin E2 will cause a decrease in the sensitization of nociceptive nerve endings to pro-inflammatory mediators, and (3) antipyretic, due to a decrease in prostaglandin E2 which is responsible for an increase inset point temperature regulation in the hypothalamus.[15]

The PANSS value improved in the study subject group, indicating clinical improvement before and after antipsychotic therapy according to the therapeutic dose. Risperidone was used as the main antipsychotic in this study. Risperidone is the first-choice therapy for treating schizophrenic patients with different stages of symptoms. Risperidone has a mechanism of action through interactions between serotonin and dopamine in the 4 dopamine pathways in the brain. This is what causes side effects of Extrapyramidal syndrome (EPS) lower is very effective to overcome negative symptoms. The difference between typical antipsychotics and atypical antipsychotics is that typical antipsychotics only block D2 receptors while atypical antipsychotics simultaneously block serotonin receptors (5HT2A) and dopamine receptors (D2).

Acetylsalicylic acid may improve symptoms of schizophrenia by affecting the phospholipids of neuronal membranes. As suggested by Horrobin the incorporation of arachidonic acid and acid docosahexaenoic into the phospholipid membrane results in a decrease in essential fatty acids, this inhibits nerve development and adult nerve function. This situation may be related to changes in phospholipase A2 activity.[16] Because acetylsalicylic acid Inhibits phospholipase A2 can result in clinical improvement in schizophrenia. Other studies on the use of acetylsalicylic acid in schizophrenia patients previously found a correlation between proinflammatory cytokines and clinical symptoms. The results of this study indicate that acetylsalicylic acid, as an antipsychotic adjunct, has a significant beneficial effect on the main psychopathological symptoms of schizophrenia in men. The use of non-steroidal antiinflammatory drugs (NSAIDs), as (COX) inhibitors, combined with specific first-choice drugs that have been studied previously, seems to improve the clinical symptoms of schizophrenic patients. [17]

Based on the correlation table between PANSS and TNF-α levels in this study, the dynamics of change occurred in a better direction where a decrease in PANSS was accompanied by a decrease in TNF-α levels and changes occurred after the 8th week of administration. And in this study, there is a weak correlation strength in each measurement with a positive and negative correlation direction. This is in accordance with several studies that examined the relationship between TNF-α serum levels. Previous study stated that no relationship was found between the severity of psychopathology assessed by the total PANSS score and the concentration of immune parameters in the schizophrenia group when assessed using the Pearson correlation coefficient. No relationship was seen between immune parameters and the positive, negative, or general psychopathology subscales.[7] In schizophrenic patients, found no significant correlation between TNF-α cytokine levels and Total PANSS values. Studies from other studies have demonstrated increased serum levels of proinflammatory cytokines in schizophrenic patients compared to controls and a correlation between levels of inflammatory

markers and severity of clinical symptoms.[18] This suggests an increase in the inflammatory response and a shift in the ratio to normal has been associated with a positive response to treatment.[1]

Usage of Acetylsalicylic acid can cause dyspepsia and gastrointestinal bleeding. However, researchers estimate that the risk of ulcers or serious bleeding is much lower than the 1% per year observed using similar doses.[19] At each study subject visit, the researcher paid attention to whether there was epistaxis, hematemesis, melena, rectal bleeding, and hematuria. Furthermore, any complaints will be recorded systematically at each visit. Side effects during the study were not found in this study. Researchers provide adjuvant therapy Acetylsalicylic acid in the form of aspirin 325 mg, ½ tablet/12 hours/oral, and the time of administration in the morning and afternoon to monitor the condition of the research subjects if side effects are found. Conclusively that therapy adjuvant Acetylsalicylic acid in the form of aspirin as nonsteroidal anti-inflammatory drugs (NSAIDs) can provide patients with anti-inflammatory benefits in the brain without any side effects. The results of this study must be interpreted taking into account its limitations. The limitations of this study were only carried out in male schizophrenic patients, so it is not known whether the decrease in serum TNF-α levels also occurs in women. Furthermore, the limitations of this study are, Schizophrenia sufferers still have difficulty in conveying their complaints so further examination is needed regarding the bleeding time to see the side effects of the administration of Acetylsalicylic acid. Finally, conclusively that adjuvant therapy Acetylsalicylic acid 325 mg as a non-steroidal antiinflammatory drug (NSAID) can provide anti-inflammatory benefits in the brain without any side effects found and provide benefits in reducing the length of treatment and accelerating remission.

5. Conclusion

There was an effect of adjuvant therapy tablet acetylsalicylic acid who received risperidone in the schizophrenia group showed a greater decrease in serum TNF-α levels than those who were only given risperidone and there was an effect of Adjuvant therapy tablet Acetylsalicylic acid who received risperidone in the group of schizophrenic patients who showed a reduction in clinical symptoms were very much in schizophrenic sufferers than those who were only given risperidone.

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Conflict of Interest None

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