THE EFFECT OF Anti-miRNA 144 AND Anti-miRNA 150 ON THE EXPRESSION OF α GLOBIN CHAINS IN PBMC (PERIPHERAL BLOOD MONONUCLEAR CELL) OF MAJOR β THALASSEMIA PATIENTS (experiment laboratories)

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ABSTRACT

The excess of unbound α globin chains are the basic pathophysiology of the cause of clinical symptoms in major β thalassemia. Recently there are many alternative therapies by increasing γ globin chains to reduced the effects of unbound α globin chains. Alternative therapies by decreasing α globin chains have not been much noticed. α globin expression involves complex regulatory involving transcription factors and miRNAs. It involves GATA-1, KLFD and MYB transcription factors . It also involves miRNA-144 and miRNA-150. The role of miRNA-144 and miRNA-150 to reduce the α globin chains expression in major β thalassemia patients is an alternative therapy. miRNA-144 and miRNA150 activity need to be known by using anti-miRNA 144 and anti- miRNA 150. Analyzed the effect of anti-miRNA 144 and anti-miRNA 150 on the expression of α globin chains. This study was an experimental study using PBMC of a major β thalassemia patients. PBMC divided into four groups that were not transfected, transfected by anti-miRNA 144, transfected by anti-miRNA 150 and transfected by anti-miRNA 144 and anti-miRNA 150. qPCR examinations to find out expression of miRNA-144 and miRNA-150. Western blot examination to find out the expression of α globin chains. There was significantly lower miRNA-144 expression in PBMC of major β thalassemia patients who had been transfected by anti-miRNA 144 than those not transfected. In line with the result of miRNA-144 expression which was 0.17 times lower than the control group. There was significantly lower miRNA-150 expression in PBMC of major β thalassemia patients who had been transfected by anti-miRNA 150 than those not transfected. In line with the result of miRNA-150 expression which was 0.30 times lower than the control group. There was significantly lower α globin chains expression in PBMC thalassemia patients who had been transfected by anti-miRNA 150 than those not transfected. There was significantly lower α globin chains expression in PBMC thalassemia patients who had been transfected by anti-miRNA 150 and anti-miRNA 144 than those not transfected. This is evidenced

by the decreased in the area 10-25 KDa band. Based on this study, the administration of anti-miRNA 150 or anti-miRNA 144 and anti-miRNA 150 is capable of decreasing the expression α globin chains in PBMC of major β thalassemia patients.

Keywords : anti-miRNA 144, anti-miRNA 150, miRNA-144, miRNA-150, α globin chains

INTRODUCTION

β thalassemia in Indonesia is a health problem, with a fairly high carrier rate. Carriers of β thalassemia traits in Indonesia range from 3% -10% (Colah et al., 2010; Freisleben et al., 2011; Tamam et al., 2010) higher than the distribution of traits in the world which ranges from 1.67% (Run & Rachmilewitz, 2005). As a hereditary disease, in Indonesia, premarital genetic counseling and prenatal diagnosis are still not routine activities carried out to overcome these problems (Weatherall, 2011; Williams & Weatherall, 2012)

Mutations of the same gene in β thalassemia can show different phenotypes (Higgs, 2004; Ranjbaran et al., 2014). Several factors involved in these differences in the hematological appearance and clinical symptoms are modifiers. Primary modifiers are various mutations in the β globin gene, secondary modifiers are various factors that affect the balance between the β globin chain and α globin chain as well as other potential modifiers that play a role in reducing the detrimental effects of excess α globin chains (Nienhuis & Nathan, 2012; Thein, 2005).

Three management approaches have been considered recently, namely activation of the fetal globin gene, allogeneic hemopoetic stem cell transplantation (HSCT) or allogeneic bone marrow transplantation and gene therapy. The effectiveness of HU administration was reported only in certain groups of patients with β thalassemia major, because it requires selecting groups of responders and nonresponders. Several studies have even reported failure of HU therapy in patients with β-thalassemia major (Dreuzy et al., 2016; Panja & Basu, 2015; Thein, 2005).

The existence of miRNAs in α globin gene expression and their role can be considered to change α globin gene expression which can reduce the clinical symptoms of β thalassemia. Several miRNAs have been associated with regulation of gene expression at the stage of erythropoiesis through their activity on transcription factors.

The therapeutic approach for hemolytic anemia in β thalassemia major is only symptomatic, while the etiological therapy approach that has recently been developed still encounters various obstacles. Secondary modifiers that affect the balance of α globin chains and β globin chains can be used as an alternative etiological therapy approach in patients with β thalassemia major. The discovery of anti-miRNA is an opportunity to regulate miRNA activity that can be used as an alternative.

METHODS

This research is classified as laboratory experimental research. The research design was the post test only control group design, because no initial measurements were taken. The experimental unit (UE) was PBMC, a patient with β thalassemia major, as a research subject (S) divided into four groups based on the type of treatment, namely control that was not transfected, treatment 1 was transfected with 144 anti-miRNAA, treatment 2 was transfected with 150 anti-miRNA and treatment 3 transfected with anti-miRNA 144 and anti-miRNA 150.

The sample used in this study was PBMC, a patient with β thalassemia major, aged 10 years with a body mass index (BMI) between 18.5-22.9 which was obtained according to research procedures. Blood sampling is carried out when the patient comes for laboratory examination before the transfusion is carried out with the condition of not having a fever, not menstruating (for girls) and the plasma is not lysed or lipemic.

RESULTS

Proving the effect of anti-miRNA-144 and anti-miRNA-150 on the expression of α globin chains in PBMC with β major. The amplification curve is considered optimal if an increase in the curve is obtained which shows an exponential phase and the curve does not reach the plateau phase too quickly at the beginning of the cycle. On the amplification curve, the Ct value is obtained for each examination.

MiRNA-144 data were obtained from PBMC qPCR examination in all groups, namely the control group, the group transfected with anti-miRNA 144, the group transfected with anti-miRNA 150 and the group transfected with antimiRNA 144 and anti-miRNA 150. Distribution of Ct miRNA-144 values for all control and treatment groups can be seen in table 1.

Group	Ct miRNA-144		
	Minimum	Maksimum	Average ±SD
K	39,02	42,63	41,01±1,13
P1	35,22	39,78	36,77±1,13
P2	34,50	42,27	37,82±2,49
P3	35,35	40,52	38,06±1,69

Table 1. Distribution of Ct miRNA-144 values

The results of the normal distribution test for all groups using the Shapiro-Wilk normality test showed that the results of miRNA-144 in all groups were normally distributed ($p>0.05$). The results of the Shapiro-Wilk normality test can be seen in the appendix.

	K	P1	P2	P3
K	-	0,000*	0,002*	0,000*
P1	0,000*	-	0,499	0,112
P2	0,002*	0,499	-	1,000
P3	0,000*	0,112	1,000	-

Table 2. Table of miRNA-144 different tests between groups

Description: significant at α=0.05

Differences between groups (multiple comparisons Games-Howel)

*different meaning

The results of the miRNA-144 test (Multiple Comparisons Games-Howel) between groups, in the group transfected with anti-miRNA 144, in the group transfected with anti-miRNA 150 and in the group transfected with anti-miRNA 144 and anti-miRNA 150, showed expression of miRNA-144 which was significantly lower than the non-transfected group ($p<0.05$).

The distribution of Ct miRNA-150 values for all control and treatment groups can be seen in table 3.

Group	Ct miRNA-144		
	Minimum	Maksimum	Average ±SD
K	26,25	28,24	27,71±0,69
P1	23,47	25,12	24,06±0,43
P2	25,59	26,74	26,23±0,27
P3	2,09	29,47	27,75±0,89

Table 3. Distribution of Ct miRNA-150 values

The relative expression level of miRNA-150 was lower than the control in the anti-miRNA 150 transfected group and the anti-miRNA 144 and anti-miRNA 150 transfected groups.

The results of α globin protein expression in Figure 5.11. From these results, a band between 10-25 KDa was found which was thinner and between 50-75 KDa which was thicker, which based on its molecular weight described an α globin monomer (16 KDa) and an α globin tetramer (64 KDa).

The normal distribution test results for all groups using the Shapiro-Wilk normality test showed that the protein expression results between 10-25 KDa and 50-75 KDa in some groups were not normally distributed ($p<0.05$). The results of the Shapiro-Wilk normality test can be seen in the appendix. So the different test was continued using Kruskal Wallis.

The results of the different test (Kruskal Wallis) band between 10-25 KDa between groups, showed a significant difference ($p <0.05$). The results of the difference test (Kruskal Wallis)

between 50-75 KDa between groups did not show a significant difference (p>0.05).

The results of the difference test (Mann-Whitney) band between 10-25 KDa between groups, in the group that was transfected with anti-miRNA 144 and anti-miRNA 150 showed band expression between 10-25 KDa which was significantly lower than the group that was not transfected and the group that was not transfected. transfected with anti-miRNA 144, the group that was transfected with anti-miRNA 150 showed band expression between 10-25 KDa which was significantly lower than the group that was not transfected and the group that was transfected with anti-miRNA 144 (p<0.05).

Difference test results (Mann-Whitney) band between 10-25 KDa between groups, did not show a significant difference (p.0.05) between the group that was not transfected with the group that was transfected with anti-miRNA 144 and the group that was transfected with anti-miRNA 150 with the anti-miRNA 144 and anti-miRNA 150 transfected groups.

REFERENCES

Colah, R., Gorakshakar, A., & Nadkarni, A. (2010). Global burden, distribution and pevention of thalassemias and hemoglobin E disorders. *Expert Rev. Hematol*, *3*(1), 103–117.

Dreuzy, E., Bhukhai, K., Leboulch, P., & Payen, E. (2016). Current and future alternative therapies for beta-thalassemia major. *Biomedical Journal*, *36*, 24–38.

Freisleben, S., Hidayat, J., Freisleben, H., Poertadji, S., Kurniawan, B., Bo, N., Handayani, S., Wahidiyat, P., & Soegianto, R. (2011). Plasma lipid pattern and red cell membrane structure in β-thalassemia patients in Jakarta. *Med J Indones*, *20*(3), 178–184.

Higgs, D. (2004). Gene regulation in hematopoiesis: new lessons from thalassemia. *American Society of Hematology*, *65*, 801–814.

Nienhuis, A., & Nathan, D. (2012). Pathophysiology and clinical manifestations of the β-Thalassemias. *Cold Spring Harbor Perspectives in Medicine*, *2*, 1–13.

Panja, A., & Basu, A. (2015). Pharmacogenomics of the drugs used for the treatment of thalassemia. *Cytology an Histology*, *06*, 360–362.

Ranjbaran, R., Okhovat, M., Mobarhanfard, A., Aboualizadeh, F., Abbasi, M., Moezzi, L., Golafshan, H., Behzad-Behbahani, A., Bagheri, M., & Sharifzadeh, S. (2014). Relationship between AHSP gene expression, β/α globin mRNA ratio, and clinical severity of β-thalasemia patients. *Annals of Clinical and Laboratory Science*, *44*(2), 189–193.

Run, D., & Rachmilewitz, E. (2005). β-Thalassemia. *The New England Journal of Medicine*, *5*(1), 12–15.

Tamam, M., Hadisaputro, S., Sutaryo,

Setianingsih, I., Astuti, R., & Soemantri, A. (2010). Correlation between mutation type of globin gene and clinical manifestations of thalassemia. *Jurnal Kedokteran Brawijaya*, *26*, 48–52.

Thein, S. (2005). Pathophysiologi of β-Thalassemia a guide to molecular therapies. *American Society of Hematology Education Program*, *5*, 31–37.

Weatherall, D. (2011). The challenge of haemoglobinopathies in resource-poor countries. *British Journal of Haematology*, *154*, 736–744.

Williams, T., & Weatherall, D. (2012). World distribution, population genetics, and health burden of the hemoglobinopathies. *Cold Spring Harbor Perspectives in Medicine*, *2*, 67–68.