

COMPARISON OF PLATELET ANTI AGGREGATION EFFECT ON ACUTE THROMBOTIC STROKE WITH DIABETES MELITUS AND NON-DIBETES MELITUS

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ABSTRAK

Stroke merupakan penyebab kematian tertinggi pada semua umur (15.4%) dan penyebab utama kecacatan di Indonesia. Stroke trombotik memiliki prevalensi 58,3% dari semua jenis stroke. Salah satu faktor resiko yang banyak dijumpai ialah diabetes mellitus (DM). Pada kondisi DM terjadi hiperreaktivitas platelet salah satunya melalui peningkatan produksi tromboksan A2 (TXA2). Hiperreaktivitas platelet dapat mempengaruhi efek antiagregasi platelet asam asetil salisilat (AAS). Dengan dasar tersebut, penelitian ini ditujukan untuk menganalisis efek antiagregasi platelet AAS pada pasien DM dan non-DM. Penelitian ini membandingkan efek antiagregasi platelet AAS 1x100 mg/hari pada pasien stroke trombotik akut dengan DM dan Non DM selama 7-10 hari. Penelitian dilakukan selama bulan Januari-Maret 2013. Efek anti agregasi platelet diukur dengan Tes Agregasi Trombosit (TAT) dua kali yaitu sebelum dan sesudah penggunaan AAS, dilakukan analisis dengan Light Transmission Aggregometry (LTA). Hasilnya memberikan rentang persentase agregasi sebesar 11-48% (pre) dan 7-43% (post) pada kelompok DM (n=9). Sementara pada kelompok Non-DM (n=15) memberikan rentang persentase agregasi sebesar 13-64% (pre) dan 13-39% (post). AAS 1 x 100 mg pada pasien stroke trombotik akut Non DM dan DM memberikan penurunan persentase agregasi yang bermakna (t-berpasangan; $p < 0,05$) namun hasil ini tidak memiliki makna klinis. Pada perbandingan antara kedua kelompok, tidak terdapat perbedaan pada kedua kelompok tersebut (t-independent; $p > 0,05$). Penelitian lebih lanjut perlu dilakukan dengan menggunakan instrumen agregasi platelet lain sebagai pembanding. Instrumen yang dipilih diharapkan dapat memberikan hasil yang memiliki makna klinis. Penelitian ini dapat digunakan sebagai informasi mengenai penggunaan salah satu instrumen TAT. (FMI 2013;49:163-167)

Kata Kunci: asam asetil salisilat (AAS), diabetes, agregasi platelet, trombotik, stroke

ABSTRACT

Stroke was the highest cause of death and disability of all ages (15.4%) in Indonesia based on RISKESDAS 2007. Thrombotic stroke is one of ischemic stroke that had prevalence of 58.3%. Acetosal/ASA is one of anti-platelet which has been used widely as secondary prevention for stroke meanwhile, diabetic melitus (DM) was one of the risk factor. Anti-aggregation platelet effect was measured by Light Transmission Aggregometry (LTA). The test had been done twice, before and after ASA usage for 7-10 days. 24 patients that were involved, hospitalized for acute thrombotic stroke at least 7-10 days, 15 from non-diabetic group and 9 from diabetic group. The results showed that there is no significant difference in demographic and risk factors for those patients (X2 test, $p > 0.05$). The aggregation percentage were analyzed statistically by pair t-test for pre-and post-within group, and independent t-test to compare those two groups. The aggregation percentage of diabetic patients (n=9) was 11-48% (pre) and 7-43% (post). Meanwhile, the aggregation percentage of non-diabetic patients (n=15) was 13-64% (pre) and 13-39% (post). The usage of ASA 1 x 100 mg gave a lowering aggregation percentage ($t < 0,05$). For the comparison between diabetic and non-diabetic patients with acute thrombotic stroke, there were no difference ($p > 0,05$). It can be concluded that there were no difference on anti-platelet aggregation of ASA between diabetic and non diabetic patients with acute thrombotic stroke. For practical purpose, it can be suggested to confirm percentage aggregation ASA with instrument that applied reliable clinical meaning. (FMI 2013;49:163-167)

Keywords: acetyl salicylic acid (ASA), diabetic, platelet aggregation, thrombotic, stroke

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INTRODUCTION

Today, Stroke is the leading cause of death in all age groups (15.4%) and the leading cause of disability in Indonesia. Thrombotic stroke is a type of stroke caused by a blockage are one of the most common type of stroke than the others, up to 58.3% (Lip & Blann 2003, Fagan & Hess 2008). There aren't rules that can predict

the time of occurrence of stroke yet. Thus, early treatment is an important aspect in the management of patients with ischemic stroke. One of common and already known risk factors is DM. Acetyl salicylic acid (ASA) acetylate the Cyclooxygenase (COX) enzyme that inhibits the synthesis of thromboxan A2 (TXA2). That will reduce the potential for thrombus formation (Undas et al 2007). Vascular damage and platelet

hyperreactivity factor may contribute to the lack of effectiveness of ASA in patients with DM (Ferroni et al 2004, Yassine et al 2010). The decrease in platelet response to ASA that is currently being used as an oral antiplatelet phenomenon causes of poor clinical output obtained (Angiolillo 2009). The change of platelet function in stroke patients with diabetes mellitus can affect the dose of ASA as an antiplatelet.

The use of ASA in Dr. Soetomo general hospital as secondary prevention is 1x100 mg for thrombotic stroke patients. Based on the analysis of platelet reactivity and the lack of research on the effects of a dose of 100 mg ASA in thrombotic stroke patients with DM, then is needed a research to see the anti-platelet aggregation effect of ASA. To fulfill that need this study are purposed to compare the platelet anti-aggregation effect of asetosal in acute thrombotic stroke those with and without DM.

MATERIALS AND METHODS

This was a clinical trial with sample of the infarction thrombotic stroke patients who meet the inclusion and exclusion criteria. Inclusion criteria was aged 18 years, with a diagnosis of thrombotic stroke, having ASA antiplatelet therapy for 7-10 days and willing to follow the study by signing the informed consent, specifically for DM group: there was a diagnosis of DM. Exclusion criteria was using drugs that interact with the other antiaggregation platelet effects (eg, clopidogrel), presence of liver and kidney disorders, patients using Nonsteroidal anti-inflammatory drugs (NSAIDs) other than ASA, steroids or drugs that affect the aggregation during the 2 weeks prior to the study. The sample size in this study was based on the limited time sampling method (within a certain time), i.e. for 3 months, from January to March 2013.

Subjects have their blood drawn for Platelet Aggregation Test (TAT) on the first day of the MRS before getting ASA (pre). The subjects received 100 mg of ASA therapy for 7-10 days. Subjects have their blood drawn to do TAT again 2 hours after received 100 mg of ASA therapy for 7-10 days(post). 5 mL blood sample was drawn through the vein, put into blue capped Vacutainer containing Na 3.2% citrate (blood : citrate = 9:1). Homogenized, give the identity of the patient and the sampling time on the label. Citrate blood centrifuged at 1000 rpm for 15 min, plasma was taken as Platelet-Rich Plasma (PRP), and the rest of citrate blood were centrifuged at 3000 rpm for 15 min, the plasma was taken as a Platelet Poor Plasma (PPP). Prepared 1 cuvette filled with 500 mL of PPP, one cuvette filled with 500 mL of PRP + stirr bar, then placed on the

apparatus and incubated for 3 min. The data obtained from the results of TAT was the percentage of aggregation.

The device used was Light Transmission Aggregometry (LTA) with Chronolog aggregometer 490 specifications. The prinsip of this tool was absorbent plasma PRP will be decreased according to platelet aggregation that occurs. The number and rate of the decline will depends on the reaction of platelets to ADP 10 μ M reagent addition. The materials needed are PRP, PPP, 10 μ M ADP reagent, 5 ml of blood citrate (1:9), Na citrate anticoagulant 3.2%. Citrate stabilized blood 2-4 hours after blood sampling (Mousa 2010, Guinto et al 2011). The data will be analyzed by paired t-test to determine the difference between the pre-and post-ASA. To compare the two group's percentage of aggregation independent t-test are used. All data will be processed with the program Statistical Package for the Social Sciences (SPSS) 16.0.

RESULTS

This research show that acute thrombotic stroke patients with DM (n = 9) and non-DM (n = 15) in terms of age and sex have a representation on the population that did not differ significantly ($p > 0.05$). Similarly, the risk factors in both groups, namely HT, dyslipidemia, smoking, and history of stroke, also have a representation on the population that did not differ significantly ($p > 0.05$). The data of TAT with agonist ADP resulted from the use of 1 x 100 mg ASA gave the percentage range of 11-48% aggregation (pre) and 7-43% (post) in acute thrombotic stroke patients with DM. While the non-DM group gives the range of the percentage of aggregation by 13-64% (pre) and 13-39% (post).

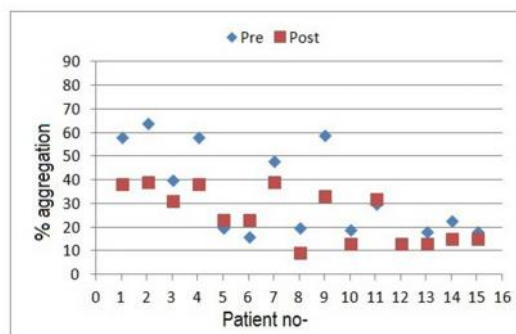


Figure 1. Aggregation Percentation of acute thrombotic stroke patient on Non-DM Group before and after addition of ASA 100 mg during 7-10 days

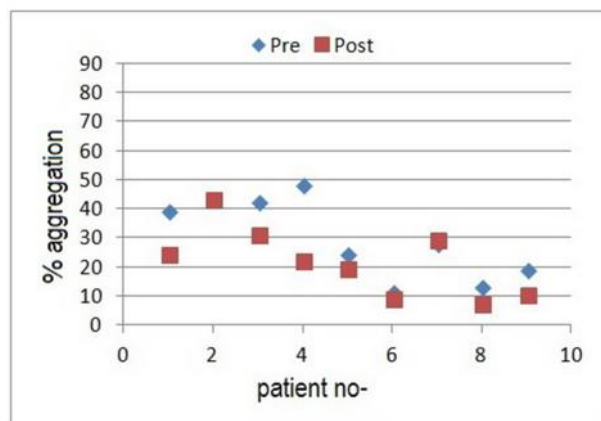


Figure 2. Aggregation Percentation of acute thrombotik stroke patient on DM Group before and after addition of ASA 100 mg during 7-10 days

When referring to the range of recommendations of ADP reagent is used, then the value of 50-82% is called normoaggregated. The majority of patients in graph 1 includes hipoaggregated both before and after ASA, and only 4 patients who are in the category normoaggregated in pre-ASA on Non-DM group. In figure 2 the results obtained are all in state of hipoaggregated both before and after the use of ASA. Aggregation percentage obtained can be calculated on mean in both groups as well as the value before and after the use of ASA.

Table 1 shows that on the paired t-test, there are significant differences in the time before until after the use of ASA on the DM group and Non DM. This was evident from the value of $p < 0.05$. In the independent t-test, it appears that no significant differences found between the two groups ($p > 0.05$). Thus, the use of ASA in patients with acute thrombotic stroke, both group and DM Non DM will decrease antiaggregation effect that statistically significant.

DISCUSSION

From the baseline demographic data of the two groups of patients it is seen that the two groups did not differ significantly. There was no influence of gender, age, even risk factors in the two groups that may affect the analysis of the effects of platelet antiaggregation. To analyze the results of this study, then we do a review of some of the factors that influence the outcome of aggregation percentage. These factors include drug interactions with platelet aggregation, stability samples, reagents and instrument types. For the paired t-test, showed a significant difference ($p < 0.05$) the use of

ASA in patients with acute thrombotic stroke non-DM and DM groups. From this analysis, it appears that the ASA still gives a significant reduction in aggregation. Meanwhile, the independent t-test, the two groups showed no difference ($p > 0.05$). Thus, in the non-DM and DM groups, the use of ASA still gives a significant reduction in aggregation.

These results differ from studies showing that the maximal percentage of aggregation in patients with diabetes will be higher than non-DM patients. Research conducted by DiChiara et al (2007) with the agonists ADP 5 $\mu\text{mol/l}$ and 81 mg ASA resulted in a mean percentage of aggregation by 64% for the DM group and 57% for non-DM group. As for the 162 mg of ASA, the DM group and NonDM had respectively 60% and 57%. DiChiara's study use the same types and brands of LTA, that is Chronolog 490. From drugs used by the patient, generally there are no significant interaction effects both on the ASA as an antiplatelet or on the measurement of TAT. But the use of citicolin as standard supportive therapy in stroke patients and insulin in patients with diabetes has effect to platelets. Insulin therapy is recommended by the American Diabetes Association (ADA) to lower glucose levels to the range 140-180 mg/dL in conditions of acute ischemic stroke (Jauch et al 2013).

Platelets are one of the targets of insulin action. Directly, insulin regulates platelet function through the insulin receptor (insulin receptor/IR). Insulin lowers platelet response to agonists ADP, collagen, thrombin, arachidonic acid and PAF (Vinik et al 2001). Although insulin has been reported to stimulate AMP-activated protein kinase, lower Ca^{2+} of the platelet and decreased activation of the agonists induced platelet, such activities have not been fully associated with the insulin receptor. Insulin also increases the production of NO (normally characterized by an increase in intracellular cGMP) in platelets of healthy people (Randriamboavonjy & Fleming 2009). Insulin binding to its receptor on platelets will be able to give the effect as described above, so it will suppress platelet hyperreactivity. In the preparation before TAT analysis, centrifugation is performed on the sample. The centrifuge process has the risk of injury on platelets thus lowering in platelet count (Dyszkiewicz-Korpanty et al 2005). PRP preparation process (platelet separation from whole blood) in this LTA is also at risk of platelet activation and great platelet aggregation (Mousa 2010). The centrifuge influence seen in studies comparing LTA with WBA (Whole Blood Aggregometry). WBA with the working principle of impedance better than the LTA (Dyszkiewicz-Korpanty et al 2005).

Table 1. TAT on the acute thrombotic stroke in Non DM and DM group before and after the addition of ASA.

Group	Mean % aggregation pre ASA \pm SD	Mean % Aggregation post ASA \pm SD	*p pre-post ASA
Non DM (n=15)	33.60 \pm 18.78	24.93 \pm 11.26	0.005
DM (n=9)	29.67 \pm 13.82	21.56 \pm 11.83	0.021
*p NonDM- DM	0.843		

Reagents ADP used was from Chrono-PAR. Reagents that have been reconstituted stored at -70 °C for one year in a volume which is used in a day. Daily volume estimation of packaging is important, because once opened, the reagent can only be stable at 2-8 °C for 8 hours. But in this study, reagents that have been divided into micro tubes stored at 2-8 °C for several months.

In this study there are some limitations. Many confounding variables that cannot be avoided such as how long smoking, and hyperhomocysteinemia may influence the outcome of the experiment. In risk factors of smoking, there is a relationship between cigarettes dose and response (Goldstein et al 2011). Patients that are accustomed to daily smoking (habitual smokers) have a tendency to platelet aggregation than those who do not regularly smoke (non-habitual smokers) (Glynn et al 1966). Decrease in HDL and increased of TG and LDL induce the formation of a thrombus (Ferreiro et al 2010). Similarly, the data did not reveal homocysteine. A wide variety of risk factors and patient intake can affect each other on the platelet aggregation.

Although there are many theories and research on platelet hyper-reactivity in patients with diabetes, some management guide of stroke therapy still recommend the use of ASA in patients with DM. Revised standard therapy management of patients with diabetes by the ADA (American Diabetes Association) in 2013, recommended dose ASA 75-162 mg/day as secondary prevention of heart disease. Heart disease is a risk factor for thrombotic stroke. The ADA recommendations provide Level of Evidence A for ASA as secondary prevention.

CONCLUSION

From this study it can be concluded that the use of ASA 1 x 100 mg in patients with acute thrombotic stroke non-DM and DM provide a significant reduction in the percentage of aggregation ($p < 0.05$) but this result has no clinical significance. In addition, there is no difference on platelet anti-aggregation effects of ASA 1 x 100 mg in patients with acute thrombotic stroke Non-DM and DM ($p > 0.05$). Further research needs to be done using instruments other platelet aggregation as

selected comparison. That instrument is expected to provide results that can interpret clinical significance. This research can also be used as information regarding the use of TAT.

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