The Effect of Diabetes Mellitus's Duration on Platelet Count and Mean Platelet Volume's Rat Stroke Model

Nabila Nabila[1], Rahma Yuantari[2], and Ety Sari Handayani[3(✉)]

[1] Faculty of Medicine, Islamic University of Indonesia, Yogyakarta, Indonesia
[2] Department of Clinical Pathology, Faculty of Medicine, Islamic University of Indonesia, Yogyakarta, Indonesia
[3] Department of Anatomy, Faculty of Medicine, Islamic University of Indonesia, Yogyakarta, Indonesia
097110415@uii.ac.id

Abstract. Hiperglikemia in Type 2 Diabetes Mellitus (DM) have an impact on increasing the coagulation activity of the human homeostatic system. DM is a comorbidity of stroke. An increase of Platelet Count (PC) and Mean Platelet Volume (MPV) is useful for assessing the prognosis and severity of ischemic stroke. Research about the effect of diabetic duration on PC-MPVs rat stroke model is limited. This study was purposed to identify the effect of Diabetes Mellitus's duration on PC-MPVs rat stroke model. This research was an experimental study with posttest control group design. A total 24 male wistar rats aged 3–4 months were randomly divided into four groups. Group 1,2,3 and 4 are a sham, rat stroke model without DM, stroke + 2 weeks DM and stroke + 3 weeks DM. Diabetic rat was induced by Streptozotocin-Nicotinamide injection, with fasting blood glucose level ≥ 126 mg/dL. Rat stroke model was performed with 20 min Bilateral Common Carotid Artery Occlusion (BCCAO) and 24 h reperfusion. PC and MPV tests were performed in clinical laboratories at the end of study. The data was tested by One Way ANOVA. There was an effect of duration of Type-2 DM on PC ($p = 0.001$) and MPV ($p = 0.047$) in BCCAO. There was a significant difference ($p = 0.009$) PC in group 1 (1095800 ± 169893,202 mm3) vs group 2 (648000 ± 175945,067 mm3) and group 2 vs group 3 (1113857,14 ± 205721,842 mm3, $p = 0.003$). There was a significant difference ($p = 0.048$) MPV in group 1(7,44 ± 0,51fL) vs group 2 (8,03 ± 0,46 fL) and group 1 vs group 4 (8,08 ± 0,6 fL, $p = 0.047$). There is an effect of DM duration to platelet count and MPV on rat stroke models.

Keywords: Platelet · MPV · DM · Stroke

1 Introduction

Diabetes mellitus (DM) is a metabolic disease characterized by hyperglycemia [1]. DM contributes to the main cause of death in the world, namely 1 in 11 people. Patients with DM, 90% of whom suffer from type 2 DM with the main population held by Asia [2].

H. Nurdiyanto et al. (Eds.): ICCvD 2021, AHSR 52, pp. 3–9, 2023.
https://doi.org/10.2991/978-94-6463-048-0_2

The survey by Rikesdas 2013 stated that the number of Indonesian population diagnosed with DM was 5.7% or around 12 million people [3].

Ischemic stroke is a clinical condition in the form of local and or global nerve function disorders caused by a lack of blood flow to the brain so that the oxygen demand in the brain is less [4]. Ischemic stroke ranks as the second leading cause of death and serious disability after ischemic heart in the world. Every year around 5.8 million people die each year due to stroke and 2/3 of them occur in developing countries [5]. The prevalence of stroke in Indonesia diagnosed by health workers or symptoms is 12.1 per 1000 population [4].

One of the factors that influence the prothrombotic state is the Platelet Count (PC) and the Mean Platelet Volume (MPV). Hyperglycemia for a long time in type 2 DM (insulin resistance) has an impact on increasing the coagulation activity of the homeostatic system, causing platelet abnormalities (blood components for coagulation) as assessed from the platelet profile and platelet function abnormalities assessed from MPV. Blood will increase blood viscosity so that it has the potential for endothelial dysfunction and inhibition of the distribution of insulin, glucose and oxygen. This can facilitate the occurrence of plaque or blockage that can cause ischemic stroke [7].

MPV and PC are very useful in assessing the prognosis and severity of ischemic stroke. In the study of Patel et al., (2018), it was stated that there was an increase in MPV in acute phase ischemic stroke. The increase in size (MPV) is used as a marker of platelet function where the larger the platelet size, the greater the solid granule component that functions for the blood aggregation process [7].

This study aims to determine the effect of the duration of type 2 diabetes on the profile of platelets and MPV in rat stroke models.

2 Methods

This research was a quasi-experimental with post-test control group design. The subject was Rattus norvegicus wistar strain. The inclusion criteria were Male sex, 3–4 months old, 150–300 g body weight, healthy (clean, not wet or sticky, active movement, eats, drinks, and sleeps according to its life cycle), does not have physical disabilities. The exclusion criteria were the rats died during the treatment or showed inactive movements or did not want to eat and drink during 7 days of acclimatization or the rats showed abnormal behavior, among others, appeared weak, not agile, and appeared aggressive during the study. The number of rats used in this study were 24 rats. Rats were divided into four groups consisting of group 1 (sham), group 2 (rat stroke model), group 3 (rat stroke model + 2 weeks DM), and group 4 (rat stroke model + 3 weeks DM). Each group consisted of 6 rats.

The research flow is the acclimatization stage. During adaptation, rats were given water ad libitum and pellets every morning at 06.00 a.m. At the induction of Type II Diabetes Mellitus stage, the rats were injected by Nicotinamide (NAD) at a dose of 130 mg/kgbb. Then after 15–30 min Streptozotocin (STZ) was injected with a dose of 65 mg/kgbb. Experimental animals were given 5% glucose solution and fed ad libitum for 24 h and then measured fasting blood sugar levels and body weight of rats within 72 h post-induction. Rats with fasting blood glucose levels 126 mg/dL are classified as rats with diabetes type II [8].

Bilateral common carotid artery occlusion (BCCAO) is performed by previous protocol [15]. Prior to the ligation, the rats were anesthetized using ketamine at a dose of 80–100 mg/kgbb administered intramuscularly to the lateral thighs of the rats. The disinfection stage was aimed at preventing infection in rats. Vertical incision in the anterior medial neck of the rat and explore common carotid arteries were performed. The right and left common carotid arteries were 20 min-ligated with vascular clamps. After the ligation is complete, the vascular clamp is released. The incision was then sutured using silk thread. Then the rats were left to live for 24 h (reperfusion period). The control group (sham) received anesthesia, disinfection, incision, exploration, and suturing without bilateral common carotid artery occlusion. Rats were anesthetized using ketamine at a dose of 80–100 mg/kgbb intramuscular on the lateral thighs of the rats after 24 h reperfusion. The blood that has been stored in the EDTA tube is then taken to the Clinical Laboratory for examination of the PC and MPV [9].

Data analysis was performed with statistical software. Shapiro-Wilk Test was carried out to determine the normality of the data followed by One Way Analysis of Variance (ANOVA) Test followed by Tamhane's Post Hoc. The analysis was also carried out descriptively to determine the mean value and standard deviation of each research variable.

This research has been approved by the Ethic Committee Faculty of Medicine Islamic University of Indonesia no 6/Ka/Kom.Et/70/KE/IV/2019.

3 Result

The results of OneWay ANOVA analysis showed that there was an effect of duration of Type-2 DM on PC ($p = 0.001$) and MPV ($p = 0.047$) in rat stroke model. The results of the Post Hoc PC value showed that there was a significant average difference (Fig. 1) in group 1 (sham (1095800 ± 169893,202 mm3) vs group 2 (rat stroke model (648000 ± 175945,067 mm3)) with a p value = 0.009 and group 2 vs group 3 (rat stroke model + 2 weeks DM (1113857,14 ± 205721,842 mm3)) with a value of $p = 0.003$. While the results of Post Hoc MPV values showed that there was a significant average difference (Fig. 2) in group 1 vs group 2 with p value = 0.048 and group 1 vs group 4 (rat stroke model + 3 weeks DM (8,08 ± 0,6 fL)) with p value = 0.047.

4 Result and Discussion

The results of this study showed that there was a significant effect of the duration of Type-2 DM on PC and MPV in rat stroke models. Platelets are a major component in the homeostatic process. Vascular injury causes platelet aggregation to form a hemostatic plug [9]. In this study, there were significant differences between groups 1 and 2 ($p = 0.009$), groups 2 and 3 ($p = 0.003$). Meanwhile, the average PC scores between groups from the highest to the lowest are group 3, group 1, group 4, group 2. Research that is in line with this research includes the research of Akinsegun et al. (2014) using a case control study method involving 200 Type-2 DM patients and 100 non-DM patients [10]. The results showed a significant increase in PC values among DM patients compared to normal controls. This study is not in line with the research of Buch et al. (2017)

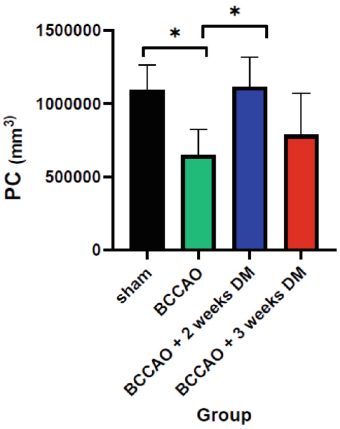

Fig. 1. Platellet Count (PC). Rat stroke model (BCCAO group) is the lowest PC (648.000 ± 175.945,067). * $p < 0,05$.

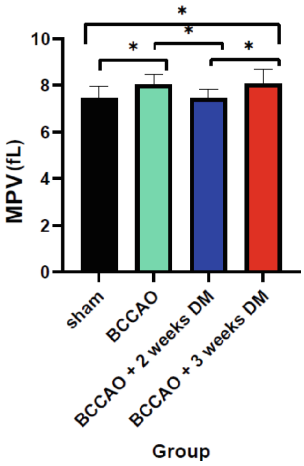

Fig. 2. MPV. Diabetes for 3 weeks increases MPV in stroke rate (8,08 ± 0,60 fL). * $p < 0,05$.

using a case control study method with 300 patients with Type-2 DM and 200 non-DM patients as controls. The results showed a significant decrease in the mean value of PC among DM patients compared to normal [11]. In addition, the study of Hekimsoy et al. (2004) with 145 patients with Type-2 DM and 100 non-DM patients as controls with the result that there was a significant decrease in the mean value of PC among DM patients compared to normal [12].

Platelets play an important role in homeostasis, atherosclerosis and cardiovascular disease events. Type 2 diabetes can cause macrovascular complications. High thromboxane in type 2 DM with poor glycemic control. The number of platelets circulating in the blood is influenced by both activation and inhibitory signals. Inhibitory signals are released from intact vasculature in the form of NO and prostacyclin, while activation

signals are triggered by vascular damage so that platelets experience increased activity for the form of hemostatic plugs. Hyperglycemia and insulin resistance in Type 2 DM can cause increased platelet reactivity so that there is a signal for platelet production and causes an increase in circulating platelets in the bloodstream [13].

MPV is a parameter of morphology and activity or function of platelets (Bath et al., 2004). In this study, there were significant differences between groups 1 and 2 ($p = 0.048$), groups 1 and 4 ($p = 0.047$), groups 2 and 3 ($p = 0.037$), groups 3 and 4 ($p = 0.038$). While the order of MPV average values from highest to lowest is group 4, group 2, group 3, group 1. [14].

The results of this study are in line with the research of Kodiatte et al. (2012), a cross-sectional study method involving 300 Type-2 DM patients and controls with the result that there is a significant increase in MPV values in Type-2 DM patients compared to control patients [15]. Research by Vaddatti, et al. (2016), the case control study method involved 171 type-2 DM patients and 37 healthy patients. The results of the study stated that there was a significant increase in MPV values in Type-2 DM patients compared to healthy patients [16]. Research by Shah et al. (2012), using a retrospective analysis method involving 13,021 patients, stated that there was a significant increase in MPV in Type-2 DM patients compared to healthy patients [16].

Increased platelet activity causes an increase in MPV value. The increase in activity is influenced by many factors, including hyperglycemia, hyperlipidemia, insulin resistance and inflammation. Hyperglycemia conditions can induce non-enzymatic glycation of proteins on the surface of platelets and decrease fluidity so that platelets become active. Insulin resistance can inhibit collagen interactions and weaken platelet aggregation in sick or normal people. Inflammatory conditions in the blood vessels can cause the release of superoxide. Superoxide can increase calcium excretion. The increased calcium concentration stimulates the re-activation of platelets. In addition, superoxide can inhibit the activity of NO and prostacyclin (vasodilator) [17].

Patients with Type 2 DM experience dysregulation of platelet activity signals, resulting in abnormal platelet activity and thrombus formation. Increased MPV values may be associated with poor control of Type 2 DM, duration of DM, microalbuminuria, coronary heart disease and an increased number of DM complications. MPV values can return to normal when there is an improvement in the glycemic control of Type-2 DM [17]. The results of this study are not in line with those of Hekimsoy et al. (2004) with the cross-sectional method finding a significant decrease in MPV values between DM patients compared to normal. This is related to several related variables that affect platelets such as the survival of platelets, the level of their production and turnover in diabetes [12]. On the other hand, research by Akinsegun et al.

This study has limitations in the number of samples that are reduced due to the slightly increased mortality rate in groups 1 and 4. The increased mortality rate because the research technique is quite difficult.

PC and MPV values are one of the measuring tools to determine the effect of the duration of type 2 diabetes on ischemic stroke. The results of this study indicate that there is an influence between the duration of type 2 DM on ischemic stroke. This implies that PC and MPV assessments can be used as indicators in patients with type 2 DM for complications of ischemic stroke. Therefore, if the health management of patients with

Type 2 DM is carried out properly, routine health checks and measurements of PC and MPV (make sure they are within normal limits) can reduce the incidence of ischemic stroke and reduce mortality due to type 2 diabetes.

5 Conclusion

Experimental research using a post-test control group design method using experimental rats showed that there was a significant effect of the duration of Type-2 DM on PC and MPV values in an ischemic rat model.

Acknowledgments. We are really grateful because we managed to complete our research. This research cannot be completed without the effort and collaborate all author and UPPM (Unit Penelitian dan Pengabdian Masyarakat) Medical Faculty, Universitas Islam Indonesia.

Author's Contribution. ESH and RY conceived of the presented idea and planned experiments. N and ESH wrote the manuscript with support RY. All authors contributed to provide critical feedback and helped shape the research and analysis.

References

1. E.M. Clarke, E.A. Emerson, Design and synthesis of synchronization skeletons using branching time temporal logic, in: D. Kozen (Eds.), Workshop on Logics of Programs, Lecture Notes in Computer Science, vol. 131, Springer, Berlin, Heidelberg, 1981, pp. 52–71. DOI: https://doi.org/10.1007/BFb0025774
2. J.P. Queille, J. Sifakis, Specification and verification of concurrent systems in CESAR, in: M. Dezani-Ciancaglini and U. Montanari (Eds.), Proceedings of the 5th International Symposium on Programming, Lecture Notes in Computer Science, vol. 137, Springer, Berlin, Heidelberg, 1982, pp. 337–351. DOI: https://doi.org/10.1007/3-540-11494-7_22
3. C. Baier, J-P. Katoen, Principles of Model Checking, MIT Press, 2008.
4. M. Kwiatkowska, G. Norman, D. Parker, Stochastic model checking, in: M. Bernardo, J. Hillston (Eds.), Proceedings of the Formal Methods for the Design of Computer, Communication and Software Systems: Performance Evaluation (SFM), Springer, Berlin, Heidelberg, 2007, pp. 220–270. DOI: https://doi.org/10.1007/978-3-540-72522-0_6
5. V. Forejt, M. Kwiatkowska, G. Norman, D. Parker, Automated verification techniques for probabilistic systems, in: M. Bernardo, V. Issarny (Eds.), Proceedings of the Formal Methods for Eternal Networked Software Systems (SFM), Springer, Berlin, Heidelberg, 2011, pp. 53–113. DOI: https://doi.org/10.1007/978-3-642-21455-4_3
6. G.D. Penna, B. Intrigila, I. Melatti, E. Tronci, M.V. Zilli, Bounded probabilistic model checking with the muralpha verifier, in: A.J. Hu, A.K. Martin (Eds.), Proceedings of the Formal Methods in Computer-Aided Design, Springer, Berlin, Heidelberg, 2004, pp. 214–229. DOI: https://doi.org/10.1007/978-3-540-30494-4_16
7. E. Clarke, O. Grumberg, S. Jha, et al., Counterexample-guided abstraction refinement, in: E.A. Emerson, A.P. Sistla (Eds.), Computer Aided Verification, Springer, Berlin, Heidelberg, 2000, pp. 154–169. DOI: https://doi.org/10.1007/10722167_15

8. H. Barringer, R. Kuiper, A. Pnueli, Now you may compose temporal logic specifications, in: Proceedings of the Sixteenth Annual ACM Symposium on the Theory of Computing (STOC), ACM, 1984, pp. 51–63. DOI: https://doi.org/10.1145/800057.808665
9. A. Pnueli, In transition from global to modular temporal reasoning about programs, in: K.R. Apt (Ed.), Logics and Models of Concurrent Systems, Springer, Berlin, Heidelberg, 1984, pp. 123–144. DOI: https://doi.org/10.1007/978-3-642-82453-1_5
10. B. Meyer, Applying "Design by Contract", Computer 25(10) (1992) 40–51. DOI: https://doi.org/10.1109/2.161279
11. S. Bensalem, M. Bogza, A. Legay, T.H. Nguyen, J. Sifakis, R. Yan, Incremental component-based construction and verification using invariants, in: Proceedings of the Conference on Formal Methods in Computer Aided Design (FMCAD), IEEE Press, Piscataway, NJ, 2010, pp. 257–256.
12. H. Barringer, C.S. Pasareanu, D. Giannakopolou, Proof rules for automated compositional verification through learning, in Proc. of the 2nd International Workshop on Specification and Verification of Component Based Systems, 2003.
13. M.G. Bobaru, C.S. Pasareanu, D. Giannakopoulou, Automated assume-guarantee reasoning by abstraction refinement, in: A. Gupta, S. Malik (Eds.), Proceedings of the Computer Aided Verification, Springer, Berlin, Heidelberg, 2008, pp. 135–148. DOI: https://doi.org/10.1007/978-3-540-70545-1_14
14. Bath, P. et al. 'Association of Mean Platelet Volume With Risk of Stroke Among 3134 Individuals With History of Cerebrovascular Disease', pp. 622–626. doi: https://doi.org/10.1161/01.STR.0000116105.26237.EC. 2004
15. Handayani, E. S. *et al.* (2018) 'Effect of BCCAO Duration and Animal Models Sex on Brain Ischemic Volume After 24 Hours Reperfusion', *Bangladesh Journal of Medical Science*, 17(01), pp. 129–137. doi: https://doi.org/10.3329/bjms.v17i1.35293.
16. Shah, et al. The Relationship between Diabetes, Metabolic Syndrome, and Platelet Activity as Measured by Mean Platelet Volume: The National Health and Nutrition Examination Survey, 1999–2004. Diabetes Care. Pubmed.2012
17. Kim et al.'Clinical marker of platelet hyperreactivity in diabetes mellitus', Diabetes and Metabolism Journal, 37(6), pp. 423–428. doi: https://doi.org/10.4093/dmj.2013.37.6.423. 2013