A Preliminary Study on Platelet Reactivity in Normotensive Subjects with A Family History of Hypertension

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Abstract

Backgrounds: Platelets adhesion, activation, and aggregation play an important role in cardiovascular pathogenesis. These events emerge as consequences of the endothelial dysfunction and activation. Hypertension is known as a risk factor for cardiovascular disease.

Aim: To know whether endothelial dysfunction and activation have been occurred in prehypertension subjects.

Methods: We did a quasi experimental, ex vivo, human study, by examining platelet aggregation using the turbidimetric method in 10 undergraduate students with a family history of hypertension and 10 undergraduate students without a family history of hypertension.

Results: The two groups did not differ significantly in gender proportion, age, body mass index, resting blood pressure, and platelets counting. The two groups did not differ significantly in the percentage of maximal platelet aggregation to 2, 5, and 10 μM adenosine 5'-diphosphate (ADP). These results showed that normotensive subjects with family history of hypertension do not have exaggerated platelets reactivity as compared to their counterparts.

Conclusion: Under resting condition endothelial dysfunction may not manifest yet in the form of increased platelets aggregation in normotensive subjects with a family history of hypertension.

Relevance for patients: Normotensive subjects with a family history of hypertension can be considered as prehypertension subjects. To prevent the development of hypertension in these subjects and its collateral events, we need to explore the structural and functional changes in their cardiovascular system.

Key words: platelets reactivity, prehypertension, normotensive subjects, a family history of hypertension, endothelial dysfunction.

1. Introduction

Primary or "essential" hypertension accounts for 90-95% of hypertension worldwide. Its etiology is unknown; however, it results typically from an **increase in systemic vascular resistance** rather than exaggerated cardiac output [1-3].

Physiologically, the endothelium functions as an inhibitory mode to the vascular tone since it releases endothelium-derived relaxing factors, such as nitric oxide (NO) and prostacyclin (PGI_2), which influence the vascular smooth muscle cells. NO and PGI_2 also act as thromboregulators because they inhibit platelets activation and aggregation. Thus, in normal condition endothelium has the capacity as a vasodilator and **antithrombotic** [4-5].

However, endothelium may undergo several phenotypic modulations that lead to the impairment of mechanisms that maintain homeostasis in healthy circulation. The condition commonly named as "**endothelial dysfunction**" is associated with a proinflammatory phenotype, increased oxidative stress, and abnormal modulation of vasoactive pathways, which may lead to several manifestations, including impaired endothelium-dependent vasodilation and thrombotic-prone [6].

Essential hypertension is likely to aggregate in the family. Normotensive subjects having a blood relative, such as a mother, father, sister, or brother, who has high blood pressure before the age of 60 have two to three times the risk of developing hypertension in the later life [7-8].

2. Materials and Methods

2.1. Study design

If normotensives subjects with a family history of hypertension already have endothelial dysfunction, thus they will have exaggerated platelets aggregation. To test this hypothesis we did a quasi-experimental, ex vivo, human study. In this study, we compared platelet aggregation in 10 normotensive subjects with a family history of hypertension and other 10 normotensive subjects without a family history of hypertension.

2.2. Study subjects

We collected 20 study subjects from undergraduate students of Faculty of Medicine and Health Sciences, Universitas Muhammadiyah Yogyakarta, by a purposive sampling, with equal gender proportion. Normotensive subject was defined as having blood pressure ≤ 140/80 mm Hg measured in sitting position. A family history of hypertension was defined as having mother, father, or both with high blood pressure (systolic blood pressure {SBP} ≥ 140 mm Hg or and diastolic blood pressure {DBP} ≥ 90 mm Hg) based on a self-reported questionnaire. We collected and measured characteristics data of study subjects, such as age, body mass index (BMI), SBP, DBP, heart rate (HR), and platelets count. BMI was calculated from the formulae: (body weight in kilograms)^2/height in meters. SBP and DBP were measured using a non-

invasive, oscillometric method, automatic vital sign monitor device TM-2551 P (A & D Co. Ltd., Tokyo, Japan) from the brachial artery of the subjects' dominant hand. Heart rate was counted manually using a radial pulse of the subjects' dominant hand. All measurements were done two times, then be averaged, and blindly.

2.3. Platelets aggregation test

Whole blood was taken from subject's arm and collected in tubes containing sodium heparin. At least 2 hours before blood withdrawal, the subjects were refrained from eating, exercising, and drinking coffee. One week before blood withdrawal, the subjects were not taking any drugs. The percentage of maximal platelets aggregation in response to adenosine 5'-diphosphate (ADP) as the platelets agonist was measured using the turbidimetric method (Helena Lab.).

2.4. Statistical analysis

Subjects' characteristics as a whole sample were presented as minimum, maximum, and mean ± standard deviation (SD). Except for gender proportion, group differences in subjects' characteristics were presented as mean ± SD, and were analyzed using independent t-test. The group differences in the percentage of maximal platelets aggregation was analyzed using independent t-test. For further analysis, the results of maximal platelets aggregation were categorized into three levels, namely hypo aggregation, normal aggregation, and hyper aggregation. To analyze the group difference in the level of maximal platelets aggregation we

used chi-square. p value < 0.05 was considered significantly difference. Statistical analysis was done using SPPS 15.0 for Windows.

3. Results

3.1. Subjects' characteristics

The subjects of this study have the following characteristics: late adolescent to young adult age, underweight to normal body mass index, normal blood pressure, normal heart rate to slight tachycardia, and normal platelets counting (Table 1).

Table 1. Subjects' characteristics (n = 20)

Characteristics	Minimum	Maximum	Mean ± SD
Age (years)	18	23	20,05 ± 1,32
Body mass index (kg/m^2)	17,8	28,1	21,04 ± 2,75
Systolic blood pressure (mmHg)	94	126	112,6 ± 8,52
Diastolic blood pressure (mmHg)	54	83	71,3 ± 6,4
Heart rate (times/minute)	58	108	85,1 ± 11,63
Platelets count ($10^3/mm^3$)	244	451	318,1 ± 52,25

Note: SD = standard deviation

Normotensives subjects with a family history of hypertension did not differ significantly regarding gender proportion, age, body mass index, SBP, DBP, HR, and platelets counting (Table 2).

Table 2. Group comparison of subjects' characteristics

Characteristics	Normotensive without a FHoH (n = 10)	Normotensive with a FHoH (n = 10)	*p* value
Gender proportion (F:M)	1:1	1:1	-
Age (years)	20,3 ± 1,42	19,8 ± 1,23	0.41
Body mass index (kg/m^2)	21,72 ± 1,23	20,36 ± 2,39	0.28
Systolic blood pressure (mmHg)	112 ± 9,49	113,2 ± 7,91	0.76
Diastolic blood pressure (mmHg)	71 ± 5,05	71,6 ± 7,81	0.84
Heart rate (times/minute)	87,7 ± 7,21	82,5 ± 14,79	0.33
Platelets count ($10^3/mm^3$)	326,5 ± 47,92	309,7 ± 57,55	0.49

Note: FHoH = family history of hypertension. F = female. M = male. Data are summarized as a mean ± standard deviation, except for gender proportion.

3.2. Platelets aggregation test

The results of maximal platelets aggregation in response to different doses of ADP (2 μM, 5 μM, 10 μM) between normotensives subjects with a family history of hypertension and those without a family history of hypertension did not differ significantly (Table 3).

Table 3. Group comparison of maximal platelets aggregation

Agonist	Normotensive without a FHoH (n = 10)	Normotensive with a FHoH (n = 10)	*c* value
ADP 2 µM	31 ± 27,56	31,14 ± 14,74	0.99
ADP 5 µM	64,76 ± 24	65,43 ± 21,97	0.95
ADP 10 µM	81,4 ± 15,47	83 ± 10,7	0.79

Note: FHoH = family history of hypertension; ADP = adenosine 5'-diphosphate. Maximal platelets aggregation was measured as a percentage (%). Data are summarized as a mean ± standard deviation.

As the results of maximal platelets aggregation were categorized into hypo-, normal-, and hyper aggregation, the proportion of subjects with hypo aggregation, normal aggregation, and hyper aggregation did not differ significantly between normotensives subjects with a family history of hypertension and their counterparts. However, we can see a trend of increasing platelets aggregation in normotensive subjects without a family history of hypertension as compared to normotensive subjects with a family history of hypertension (Table 4).

Table 4. Group comparison of the level of maximal platelet aggregation

Agonist	Classification	Normotensive without a FHoH (n = 10)	Normotensive with a FHoH (n = 10)	Nilai *p*
ADP 2 µM	Hypo aggregation	3	2	0.23
	Normal aggregation	5	8	
	Hyper aggregation	2	0	
ADP 5 µM	Hypo aggregation	2	1	0.36
	Normal aggregation	2	5	
	Hyper aggregation	6	4	
ADP 10 µM	Hypo aggregation	1	1	0.87
	Normal aggregation	2	3	
	Hyper aggregation	7	6	

Note: FHoH = family history of hypertension; ADP = adenosine 5'-diphosphate

4. Discussion

Normotensive subjects with family history of hypertension may have preclinical cardiovascular disease state, in which the cardiovascular system undergoes structural and functional changes. This condition, without any further intervention, can lead to cardiovascular diseases state [9]. Previous studies showed that ED, one of the initiators of the development of hypertension, already occur in children and adolescents having risks of cardiovascular diseases, such as having a family history of hypertension [10]. Li et al. [11] measured endothelium-dependent vasodilatation by the percentage of brachial artery diameter in response to reactive hyperemia. They found that the brachial artery response to reactive hyperemia was significantly reduced in

late young adults (age 44.5 ± 11.2 years) normotensive subjects with a family history of hypertension as compared to the counterparts without a family history of hypertension. Teixeira et al. [12] also confirmed that young adult normotensive subjects with a family history of hypertension were associated with ED.

This study showed that late adolescence-early young adult normotensive subjects with a family history of hypertension have no heightened platelets aggregation in response to different doses of ADP as compared to their counterparts without a family history of hypertension (Table 3). The results of our study are not in line with Nara et al. [13] who reported that platelet aggregation induced by ADP was significantly higher in men with a family history of essential hypertension as compared to men without such a family history of essential hypertension taking low cholesterol and low salt diet. However, Akbar [14] reported that platelets from spontaneously hypertensive rats (SHR) showed greater platelets aggregation in response to thrombin and prostaglandin E_1 (PGE_1) but lesser platelets aggregation in response to ADP as compared to Wistar-Kyoto (WKY) rats. Akbar's study [14] supports our results regarding the platelets response to ADP. Akbar [14] showed that platelets aggregation to ADP is greater in healthy animal (WKY rats) as compared to hypertensive animal model (SHR rats). In our study an increasing trend in platelets aggregation in normotensive subjects without a family history of hypertension was found as compared to normotensive subjects with a family history of hypertension (Table 4).

Akbar [14] did not explain the causes of the different responses of platelets of the animal model of hypertension (SHR) to different agonists. ADP, thrombin, and PGE_1 activate platelets through the same pathway, namely G protein-coupled receptors (GPCR) [15]. Thus, the different responses to different agonists in Akbar's study may be caused by, in our opinion, the differences in the intracellular biochemical signaling after GPCR activation. It also explains why in our study there was a trend of heightened platelets aggregation in normotensive subjects without family history of hypertension as compared to normotensive subjects with family history of hypertension.

Our study, however, did not measure ED. Our study based on the assumption that ED occurs in normotensive subjects with family history of hypertension [10-12]. Moreover, Schlaich et al. [16] found that L-arginine transport in the endothelial cells was substantially reduced in normotensive subjects with a family history of hypertension compared to normotensive subjects without a family history of hypertension. L-arginine is needed in NO synthesizing in endothelial cells. The disturbance in L-arginine transport in normotensive subjects with a family history of hypertension can lead to the decrease of NO release from endothelial cells, then, in turn, it fails to inhibit platelets activation and aggregation. The ED that occurs early in this prehypertension model is associated with praecox atherosclerosis, as reported by Solini et al. [17]. They found that young adult (age 25.2 ± 2.4 years) normotensive subjects with a positive family history of hypertension is associated with an initial increase in markers of inflammation (such as adhesion molecule P-selectin) and plaque instability (such as protease matrix metalloproteinase {MMP}-9). The development and progress of atherosclerosis due to ED may give risk to

atherothrombosis in later life. The greater risk to develop thrombosis than to bleed in hypertensive patients is known as “the thrombotic paradox” [18].

5. Conclusion

Because the exaggerated platelets reactivity is not found, endothelial dysfunction may not occur yet in our human model of prehypertension, i.e. normotensive subjects with family history of hypertension.

However, in our opinion, the temporary conclusion inducted by this preliminary study needs further investigation. For example, platelets aggregation in normotensives subjects with a family history of hypertension can be measured after endothelial cells are activated or stimulated. Moreover, different platelets agonists can be used, such as ADP, thrombin, thromboxane, and epinephrine, which act through G protein-coupled receptors (GPCR), and collagen, which acts through tyrosine kinase [15, 19].

CONFLICT OF INTEREST

The authors have no conflict to disclosure.

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