ORIGINAL ARTICLE

Platelet Cytosolic Free Calcium Levels among Normotensive and Hypertensive Individuals

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ABSTRACT

Aim: To determine the platelet cytosolic free calcium levels among normotensive and hypertensive Individuals.
Method: A cross-sectional research design was used in the study and a total of 79 participants were included. Fifty-three (53) hypertensive individuals from the out-patient department and twenty-six (26) normotensive individuals were recruited for the study. Whole blood sample were collected using citrated tubes and platelet poor and platelet rich plasma were separated through centrifugation. Platelets were subjected to repeated freeze-thawing to lyse and release the cytosolic calcium. Ortho-cresolpthalein complexone colorimetric method was used to measure the levels of platelet cytosolic free calcium from the platelet lysate. Data was analyzed using frequency, percentage, mean, standard deviation, one-way ANOVA and Pearson's r correlation.
Results: The platelet cytosolic free calcium levels in the four classification of hypertension showed a significant difference having an F-value of 145.869 and p-value of 0.000. The correlation coefficient for systolic blood pressure (SBP) and diastolic blood pressure (DBP) was 0.822 and 0.716 respectively with a p-value of 0.000. Based on the data gathered, the study revealed a significant positive correlation between platelet cytosolic free calcium levels and the systolic and diastolic blood pressures.
Conclusion: Determination of cytosolic free calcium levels may be suitable for assessing the level of blood pressure of patients with hypertension
Keywords: Cytosolic free calcium, hypertension, platelet,

INTRODUCTION

According to the World Heart Federation[1], there are about 330 million people in the developed world and around 640 million people in the developing world who have hypertension. The World Health Organization (WHO) rates hypertension as one of the most important causes of premature deaths worldwide and the problem is growing. In 2025, it is estimated that there will be about 1.56 billion adults living with high blood pressure[1]. In the Philippines, prevalence of hypertension has significantly grown in recent years accounting to 21 percent of adult Filipinos and the need to address it should all the more be prioritized, especially with the lack of awareness, treatment, compliance, and blood pressure control rates among hypertensive patients[2].

Hypertension is an increase pressure in the blood vessels which is a risk factor for coronary heart disease and the single most important risk factor for stroke. It causes about 50% of ischemic strokes and increases the risk of hemorrhagic stroke. Hypertension stresses the blood vessels, causing it to clog or weaken which can then lead to atherosclerosis and narrowing of the blood vessels making them more likely to block from blood clots or bits of fatty material breaking off from the lining of the blood vessel wall[1].

Hypertensive patients appear to have increased in vitro platelet activation, as evidenced by increased adhesion and increased aggregation in ADP. Platelet activation results to the change in shape of the platelets from normal disc shape to a sphere with long, dendritic extensions that is responsible for adhesion[3].

A number of agonists such as adenosine diphosphate (ADP) and collagen present at the sites of vascular injury will activate the platelets. The agonists bind to specific receptors on the platelet surface for activation. Once occupancy of these receptors is done, it then leads to a series of downstream events that ultimately alters the intra cytoplasmic concentration of calcium ions. The changes in platelet intracellular calcium occurs through release from intracellular stores and calcium influx through the plasma membrane[4].

Free cytosolic calcium has been shown to play an important role in the regulation of the cellular process in a variety of cells including platelets[5]. Calcium levels are found to be altered in essential hypertension. Elevated basal cytosolic free calcium levels, as well as defective membrane binding and transport kinetics of calcium, have been identified in platelet, erythrocytes, lymphocytes, and adipocytes of hypertensive subjects, in whom blood pressure were closely and directly related to the cytosolic free calcium content[6].

Despite many therapeutic advances that have led to increasingly effective anti-hypertensive drug treatments, the precise pathophysiological mechanisms of hypertension and its complications are still poorly understood. This prompted the researcher to study the variations of platelet cytosolic free calcium levels in patients with hypertension.

MATERIALS AND METHODS

Design and Setting. This cross-sectional study was conducted among 79 participants of which, 53 were hypertensive and twenty-six (26) were normotensive in an outpatient department of a selected hospital in Metro Manila, Philippines.

Participants of the Study. The participants of the study were grouped according to their systolic and diastolic blood pressure. Individuals were considered as hypertensive

stage 1 if the systolic blood pressure (SBP) falls between 130-139 mmHg and a diastolic blood pressure (DBP) of 80-89 mmHg while the hypertensive stage 2 individuals have an SBP of ≥140 mmHg and a DBP of ≥90 mmHg. Those individuals with SBP of ≥180 mmHg and a DBP of ≥120 mmHg was considered as hypertensive crisis[6]. Further, in order to be considered in the study, participants must be 30-65 years old, have no history of diabetes, must not be taking non-steroidal anti-inflammatory drugs (NSAIDs), oral contraceptive pills, thyroxine, multivitamins and calcium supplements as these can interfere with the results.

Specimen Collection: Whole blood (5 ml) sample using citrated tubes were collected from each participant by venipuncture. The samples were immediately transported to Centro Escolar University Research laboratory for processing.

Sample Processing: Each whole blood samples were centrifuged twice to prepare the platelet rich plasma followed by the isolation of platelets. Isolation was done by collecting the platelet pellets at the bottom of the tubes after the second centrifugation. The platelet poor plasma was separated into another tube for further testing and was homogenized by thoroughly mixing it with 1ml of plasma. In order to lyse the platelets, freeze and thaw cycle was done. It is important to lyse the platelets in order for the cytosolic free calcium to be released. After successful platelet lysis, the Ortho-Cresolpthalein Complexone method for calcium determination was performed following the manufacturer's manual. The homogenized platelet samples were then tested for the total calcium while the platelet poor plasma samples were tested for plasma calcium. Measurement of the absorbance of total calcium and plasma calcium was done using a Beckman Coulter UV-Vis spectrophotometer at 600 nm and in order to get the cytosolic free calcium concentration, the plasma calcium was subtracted from the total calcium.

Ethical Consideration: Prior to the conduct of the study, an ethical clearance was secured from Institutional Ethics Review Committee of Centro Escolar University. The objectives of the study were explained to the participants and consent form secured prior to sample collection. To ensure anonymity, code names were used to represent the participants.

Data Analysis. In order to analyze the gathered data, frequency, percentage, mean, standard deviation, one-way ANOVA and Pearson's r correlation was computed using IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp.

RESULTS

Table 1 shows the profile of the participants in terms of age, gender, SBP, DBP and cytosolic free calcium levels. The mean age was 55.24 while majority of the participants were females. The mean systolic and diastolic blood pressure were 139.615 and 88.718 respectively and the mean cytosolic free calcium levels was 4.290 (Table 1).

The difference between the levels of platelet cytosolic free calcium and degrees of hypertension was tested using one way ANOVA.It is revealed that there is a significant difference between levels of platelet cytosolic free calcium and degrees of hypertension. The computed mean cytosolic free calcium for normotensive, hypertensive stage 1, stage 2 and crisis were 0.848, 3.882, 7.229 and 10.584 respectively (Table 2).

Table 3 shows the platelet cytosolic free calcium levels with the degrees of hypertension which shows a significant difference having an F-value of 145.869 and p-value of 0.000. Multiple comparison test showed the same results with Normotensive vs Hypertensive Stage 1, Normotensive vs Hypertensive Stage 2, Normotensive vs Hypertensive Crisis, Hypertensive Stage 1 vs Hypertensive Stage 2 and Hypertensive Stage 1 vs Hypertensive Crisis and Hypertensive Stage 2 vs Hypertensive Crisis having a p-value of 0.000 (Table 3).

Pearson's r correlation was used to determine the relationship between platelet cytosolic free calcium levels and the degrees of hypertension. The r coefficient for SBP and DBP was 0.822 and 0.716 respectively. A significant correlation was noted between platelet cytosolic free calcium levels and the degrees of hypertension with a p-value of 0.000. (Table 4).

Table 1. Profile of the Participants

Profile (n=79)	n	%	Mean	SD
Age			55.244	11.813
Male Female	35 33	44 56		
Systolic BP			139.615	21.828
Diastolic BP			88.718	10.612
Cytosolic Calcium (mg/dl)			4.290	0.395

Table 2. Mean Cytosolic Free Calcium Levels with the Degrees of Hypertension (n=79)

Degrees of Hypertension	n	Mean	SD
Normotensive	26	0.848	0.606
Hypertensive Stage 1	31	3.882	1.836
Hypertensive Stage 2	12	7.229	0.881
Hypertensive Crisis	10	10.584	1.508

Table 3. Difference between Blood Pressure with Mean Cytosolic Free Calcium Levels

Blood Pressure (n=79)	Mean	SD	F-value	p-value	Post-hoc	p-value
Normotensive (n=26)	0.848	0.606	145.869	0.000*	Normotensive vs Hypertensive Stage 1 Normotensive vs Hypertensive Stage 2	0.000*
Hypertensive Stage 1 (n=31)	3.882	1.836			Hypertensive Stage 1 vs Hypertensive Stage 2 Hypertensive Stage 1 vs Hypertensive crisis	0.000*
Hypertensive Stage 2 (n=12)	7.229	0.881			Hypertensive Stage 2 vs Hypertensive Crisis	0.000*
Hypertensive Crisis (n=10)	10.584	1.508			Normotensive vs Hypertensive Crisis	0.000*

Table 4. Relationship between Platelet Cytosolic Calcium and Degrees of Hypertension

	Correlation coefficient	p value	Interpretation
SBP	0.822*	0.000	Significant
DBP	0.716*	0.000	Significant

*p value is significant at 0.05 level

DISCUSSION

Hypertension is defined as a persistently elevated blood pressure; that is a pressure that exceeds a randomly set level of normalcy. Among the other factors, enhanced platelet activity contributes significantly to this phenomenon[8]. A number of agonists such as adenosine diphosphate (ADP) and collagen present at the sites of vascular injury will activate the platelets. The agonists bind to specific receptors on the platelet surface for activation. Once occupancy of these receptors is done, it then leads to a series of downstream events that ultimately increases the intra cytoplasmic concentration of calcium ions[4].

The calcium ion plays a major role as intracellular second messenger during muscular activity of cardiac and smooth muscle cells. Thus, peripheral vascular resistance is determined by free intracellular calcium concentration. Abnormal calcium metabolism has been identified as one of the significant contributor for essential hypertension by many authors. Furthermore, how the vascular smooth muscle cell respond to calcium is critical for vascular tone and blood pressure[9].

According to Pawade et al.[6], the physiology of calcium is becoming evidently altered in essential hypertension. The membrane fluxes and intracellular concentrations of calcium have known a role for the normal function of both cardiac and the vascular smooth muscle. From the data of animal models suggested in hypertension, the smooth muscle was hyper-responsive to changes in extracellular calcium concentrations and that the vascular membrane permeability to calcium was increased.

The gathered data showed that 44 individuals or 56% of the sample population were female while 35 individuals or the remaining 44% were male. Several studies have shown that men younger than 65 consistently have higher levels of hypertension compared to women of the same age group. This difference is particularly pronounced in early adulthood—for instance, one study found that among 18- to 29-year-old white adults, just 1.5 percent of women but over 5 percent of men reported hypertension (for black women and men, the proportions were 4% and 10%, respectively[10]. According to Reckelhoff[11], men are at greater risk for cardiovascular and renal disease than are age-matched, premenopausal women. Recent studies using the technique of 24-hour ambulatory blood pressure monitoring have shown that blood pressure is higher in men than in women at similar ages. After menopause, however, blood pressure increases in women to levels even higher than in men.

The participants who were divided into four groups according to their blood pressure revealed different cytosolic calcium concentration. The normotensive patients, who have a systolic of less than 120 and a diastolic of less than 80, present a cytosolic free calcium concentration mean of 0.848**.** The Hypertensive Stage 1 patients present a cytosolic free calcium concentration mean of 3.882 which is slightly greater than the normotensive patients and was slightly above the normal range. The Hypertensive Stage 2 patients has a concentration mean of 7.229 which is greater than the two former groups and was above the normal range. Finally, the Hypertensive crisis patients have highest cytosolic free calcium levels of 10.584 and were markedly above the normal range. The findings were supported by Gupta et al.[12]which concluded a significant difference in platelet aggregation between normotensivesand hypertensive indicating that patients with hypertension have a state of platelet hyperactivity as compared to normal subjects. Erne et al[1] have shown that an elevation of platelet cytosolic free calcium and a close correlation of the calcium levels in platelets and blood pressure in untreated hypertensive patients. Accordingly, our data revealed that the higher the blood pressure, the greater the cytosolic free calcium levels.

According to Rao[5], platelets of hypertensive individuals are capable of inducing a greater hydrolysis of PIP2 and subsequentgeneration of IP3, (inositol phosphate). Together with diacylglycerol (DAG), IP3 is a second messenger molecule used in signal transduction in biological cells. While DAG stays inside the membrane, IP3 is soluble and diffuses through the cell, where it binds to its receptor, which is a calcium channel located in the endoplasmic reticulum. When IP3 binds its receptor, calcium is released into the cytosol, thereby activating various calcium regulated intracellular signals.

In agreement with our findings, Rumbaut and Thiagarajan[4] have documented that the increase in platelet intracellular calcium occurs through release from intracellular stores and calcium influx through the plasma membrane. Receptors coupled to G-proteins such as those to ADP, thromboxane A2 (TXA2) and thrombin, activates phospholipase Cβ (PLCβ), whereas receptors acting via the non-receptor tyrosine kinase pathways such as collagen receptor GpVI preferentially activates phospholipase Cγ (PLCγ). Both the activation of PLCβ or PLCγ results in the production of two second messengers: diacylglycerol (DAG) and inositol trisphosphate (IP3). Diacylglycerol (DAG) mediates calcium influx while triphosphate (IP3) liberates calcium from intracellular stores. In addition, calcium influx may be induced directly by certain agonists, such as ATP binding to the ligand-gated ion channel receptor, P2X1. And with these, it can actually explain why this study had produced these results in which there were a rise in the cytosolic free calcium concentration as the blood pressure of an individual increases. Other possible mechanisms accounting for altered cytosolic calcium in hypertension include increased agonist-induced calcium influx, activation of voltage-operated calcium channels, altered inositol 1,4,5 - triphosphate induced calcium efflux from internal calcium stores[13]and a decreased activity of sodium calcium exchange present in the platelet plasma membrane[14-15]

The study showed a significant correlation between cytosolic calcium free levels and the degrees of hypertension. The finding was supported by the study of Lechi et al[16] which stated that increased free cytosolic

calcium concentration, is related to an increase in active tension of smooth muscle cells and is therefore responsible for an increase arteriolar resistance. Further, studies have concluded that platelet intracellular free calcium concentration has been shown to correlate positively with blood pressure in normal and hypertensive subjects[17-18]. In another similar study, Fu et al.[19]assessed the correlation between plasma and intracellular calcium in normotensive and hypertensive subjects, the participants include 55 patients with essential hypertension and 32 normotensive controls. They found that, hypertensive group consistently demonstrated significantly lower plasma calcium and higher cytosolic calcium levels when compared with those in normotensive group. They concluded that, patients with essential hypertension have widespread depression of calcium -ATPase activity with plasma calcium depletion and cytosolic calcium overload, which may reflect an underlying membrane abnormality in essential hypertension. Few data published regarding the association of free cytosolic calcium and degree of hypertension. We found a significant positive correlation of serum calcium with levels of hypertension.

CONCLUSION

Determination of cytosolic free calcium levels may be suitable for assessing the degrees of hypertension among patients as it was revealed that as the blood pressure rises, the cytosolic free calcium levels increases.

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