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EFFECT OF OBESITY AND STATIN;

NEWLY EMERGING CARDIOVASCULAR RISK FACTOR; MEAN PLATELET VOLUME IN MALE AND FEMALE SPRAGUE DAWLEY RATS.

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ABSTRACT… Objectives: To observe the effect of obesity and subsequent atorvastatin administration on MPV in high fat diet induced obese male and female Sprague Dawley rats. **Study Design:** Randomized control trial (RCT). **Setting:** Department of Physiology, Army Medical College, Rawalpindi. Animal procurement and blood sampling was done at National Institute of Health (NIH), Islamabad and biochemical assays were performed at Centre for Research in Experimental and Applied Medicine (CREAM), Army Medical College, Rawalpindi. **Period:** The study was completed in 12 months. **Material and Methods:** Ninety healthy Sprague Dawley (male and female) rats were purchased and divided randomly into three equal groups. Rats in normal control group (Group I) were given normal chow diet for three weeks. Rats in obese control group (Group II) were given high fat diet for three weeks. Rats in obese treated group (Group III) were administered atorvastatin for three weeks in a dose of 10 mg/kg/day orally by gavage method after obesity induction. Terminal sampling was done at the end of the study by intra-cardiac puncture. MPV is a part of blood complete picture that was analysed by KX 21 Sysmex Hematology Analyzer. **Results:** High fat diet induced obesity resulted in a significant ($p < 0.05$) increase in MPV. The MPV was significantly ($p < 0.05$) decreased after atorvastatin administration. The result was comparable for both genders. **Conclusions:** Obesity increases MPV and hence the risk of adverse cardiovascular outcome. Atorvastatin apart from its known lipid lowering effect, decreases MPV and can play a beneficial role in decreasing cardiovascular morbidity and mortality.

Key words: Obesity, Mean platelet volume, statins, rats, cardiovascular risk, platelet reactivity.

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INTRODUCTION

Overweight and obesity constitute a major burden of public health problems in today's world. Initially considered to be a problem of affluent countries is also increasing in middle and low income countries now.[1] Two main predicted contributing factors are urbanisation and economic transition that result in a low level of physical activity and altered dietary preferences.[2] Overweight and obesity contribute a major share towards the occurrence of non-communicable diseases like diabetes mellitus and cardiovascular diseases due to which 68% of deaths occur worldwide.[3]

Obesity adversely affects functioning of various body systems including cardiovascular system in which platelets perform a key role. Platelets are small, non-nucleated, colourless, refractile, disc shaped, formed elements of the blood that contain various organelle and perform key functions in hemostasis.[4] Max Schultze first recognized platelets as a normal constituent of blood during a study mainly concerned with white blood cells. Bizzozero studied them microscopically and performed experiments that revealed that they were the first blood component to adhere to damaged vessel wall.[5]

Platelets circulate in blood and survey the integrity of vascular system. At sites of vascular injury, they adhere to blood vessel wall and release substances that lead to accumulation of more and more platelets, thus forming a platelet plug which together with coagulation system re-establishes blood flow in the damaged vessel. Platelets as well as other hemostatic components cannot

distinguish between an injured or diseased blood vessel like atherosclerosed vessel. Uncontrolled platelet accumulation and activation in diseased vessels can lead to the arterial blockage and subsequent infarction of vital organs. Thrombus formation at the site of disruption of endothelium involves a series of events including platelet adhesion, followed by activation, release of granular contents and coagulant activity.[6]

It has been documented that large platelets are more reactive and aggregate easily, suggesting their role in atherothrombosis. The granules present in them are denser, secrete greater quantities of serotonin and beta thromboglobulin, and synthesize more thromboxane A_2 than smaller platelets.[7]

Mean platelet volume (MPV) is emerging as a new predictor of adverse cardiovascular and cerebrovascular events. Patients having pre-existing coronary artery disease and raised MPV display greater chances of myocardial infarction[8], and patients who had suffered an attack of myocardial infarction, would be prone to recurrent attacks and death.[9] Thus raised MPV is considered as the risk factor of myocardial infarction in patients of coronary artery disease and for recurrent attacks or death after myocardial infarction. In susceptible individuals, large platelets may aggravate the thrombotic event. Their role in cerebral thrombosis has been mentioned and patients having an episode of acute stroke were found to have greater MPV that persisted longer after the stroke.[10] Elevated MPV has been found in patients of diabetes mellitus especially in patients having vascular complications.[11]

The advent of automated cell counters has made the determination of MPV a simple, inexpensive and easily obtainable procedure as compared to the other investigations of platelet reactivity that require specialized equipment, blood processing, and isolation of platelets.[12] Routine hematological analysis by virtue of MPV can easily identify subjects having large platelets. These subjects are prone to develop acute cardiovascular events because large platelets are known for being more reactive. Therefore, this simple and cost effective tool needs to be explored and implied upon extensively especially in our country to predict and prevent possible acute events.[13]

In humans, MPV has been positively correlated with BMI in obesity. Obese individuals have greater BMI and higher MPV when compared to their non-obese healthy controls.[14] Weight loss in obese individuals has been found beneficial to decrease MPV and platelet reactivity, hence reducing the incidence of sudden cardiovascular and neurological events.[15]

Statins are widely in use as lipid lowering drugs nowadays. They inhibit 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase; improve lipid profiles and have a beneficial effect in reducing morbidity and mortality due to cardiovascular system diseases.[16]

The aim of the present study was to observe the effect of high fat diet induced obesity followed by atorvastatin administration on newly emerging cardiovascular risk factor i.e., mean platelet volume in Sprague Dawley rats.

MATERIAL AND METHODS

The study was conducted at Department of Physiology, Army Medical College, Rawalpindi and completed in 12 months. It was a randomized control trial. 90 healthy Sprague-Dawley rats (45 male; 45 female) were purchased from National Institute of Health, Islamabad. The inclusion criteria were disease free rats having an average weight of 220 ± 30 grams. All the rats were acclimatized by giving normal diet and water for five days before the start of the experiment. Twelve hours of light and 12 hours of dark photoperiod was given and room temperature was maintained at 23 ± 5 °C. recording of body weight was made twice in a week throughout the study. Rats were divided into three groups.

Normal control (Group I rats)

given normal diet and water ad libitum for three weeks.

Obese control (Group II rats)

given high fat diet and water ad libitum for three weeks. Obesity was considered as 20 percent or more weight gain as compared to the initial weight at the start of the study.[17]

Obese treated (Group III rats)

given atorvastatin (10 mg/kg/day)[18] by oral gavage method after obesity induction for three weeks along with continuation of high fat diet.

At the end of the study blood sampling was done by intra cardiac puncture. This was done at the end of three weeks in group I and II rats and at the end of 6 six weeks in group III rats. From each rat approximately 5 ml of blood was taken. To perform blood complete picture by Sysmex KX-21N Hematology Analyzer, 1 to 1.5 ml of blood was shifted to potassium EDTA tubes and stored at room temperature. The rest of the blood was shifted to serum gel and clot activator tubes for measurement of various other parameters present in serum.

Data analysis was done using SPSS version 22. Quantitative variables were expressed as mean ± standard deviation. One-way Analysis of Variance (ANOVA) was used to compare among groups followed by post hoc tukey's test for individual comparisons. Independent sample t-test was used for comparison of results among both the sexes. A p-value of less than 0.05 was considered statistically significant.

RESULTS

In normal control rats, the mean value of MPV was 8.14 ± 0.619 in males and 8.08 ± 0.650 in females. The value was significantly ($p<0.05$) greater 10.01 ± 0.834 in males and 9.87 ± 1.090 in female obese control rats who took high fat diet for three weeks. Atorvastatin administration to obese rats resulted in a significant decrease ($p<0.05$) in MPV 8.23 ± 0.753 in male and 8.20 ± 0.942 in female rats as shown in Table-II and III.

MPV of obese control rats was significantly ($p < 0.05$) different from that of normal control and obese treated rats, whereas no significant ($p=0.870$) difference between MPV of obese treated and normal control groups was observed. The mean platelet volume was comparable among the male and female rats in all the groups as mentioned in Table-I and Figure-1.

Groups	MPV (fl) (Male)	MPV (fl) (Female)	Mean difference	p-value (t-test)
Group I	8.14 ± 0.619	8.08 ± 0.650	0.06 ± 0.031	0.749
Group II	10.01 ± 0.834	9.87 ± 1.090	0.14 ± 0.255	0.700
Group III	8.23 ± 0.753	8.20 ± 0.942	0.04 ± 0.188	0.904

Table-I. Comparison of Mean platelet volume among male and female rats in three groups.
*** Significant difference ($p < 0.05$)**
All values expressed as mean ± standard deviation.

Groups	MPV (fl)	p-Value (ANOVA)
Group I	8.109 ± 0.625	0.000*
Group II	9.944 ± 0.957	
Group III	8.216 ± 0.839	

Table-II. Comparison of Mean platelet volume among three groups using one-way ANOVA.
*** Significant difference ($p < 0.05$)**
All values expressed as mean ± standard deviation.

Inter group comparison(s)	MPV Mean difference	p-value
Group I vs. II	1.834 ± 0.211	0.000*
Group I vs. III	1.063 ± 0.123	0.870
Group II vs. III	1.728 ± 0.164	0.000*

Table-III. Group comparison of mean platelet volume by post hoc Tukey's test

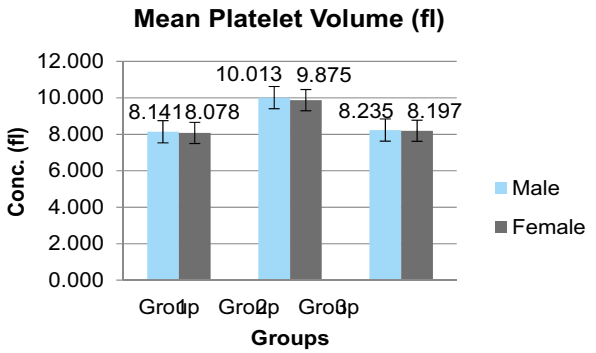

Figure-1. Comparison of Mean Platelet Volume in male and female rats among the three groups.

DISCUSSION

Mean platelet volume has been documented as the newly emerging predictor of adverse cardiovascular outcomes, however, it has not been studied extensively in relation to obesity in animal models. In humans, measurement of MPV is part of routine blood complete picture and is easily obtainable, therefore its correlation with obesity and various disease conditions has been widely worked upon. In our study MPV was found to be significantly increased in obese rats. The studies conducted in humans also revealed a positive correlation between obesity and increase in MPV.[14,19]

Coban et al., compared MPV of obese with non-obese control subjects. 100 obese and equal numbers of non-obese subjects were selected for the study. Determination of MPV in both the groups revealed significantly higher values in the obese group. MPV was hence positively correlated with BMI>[14] Coban et al., also observed a similar correlation between obesity and MPV when they conducted study in a different way. They observed the effect of weight loss on MPV. For this purpose, 30 obese and equal numbers of non-obese subjects were selected. Obese subjects were administered a specific weight reducing diet for three months. Body weight and MPV were measured before and after the diet treatment. The results showed that body weight as well as MPV was significantly decreased after the diet treatment.[15]

Arslan and Makay investigated MPV levels in relation to obesity and nonalcoholic fatty liver disease (NAFLD). Three groups of adolescents; non-obese healthy control, obese control and obese NAFLD patients were selected. MPV was found to be significantly raised in the obese adolescents as compared to non-obese healthy controls. The study revealed a positive correlation between obesity and MPV. Another important finding in this study was an inverse correlation between MPV and platelet count i.e. obese adolescents had higher MPV but a lower platelet count.[19]

Atorvastatin administration significantly decreased MPV in obese atorvastatin treated rats of our study. Effect of statins in reducing atherothrombotic events has been published widely in bio medical literature, and statins are known to decrease platelet reactivity; however, this effect on newly proposed platelet reactivity marker (i.e. MPV) has not been studied extensively so far. The study of Coban E et al., documented a similar correlation. They studied the effect of treatment of rosuvastatin together with hypolipedemic diet on MPV in dyslipidemic patients. Equal number of control and patients of uncontrolled primary dyslipidemia on hypolipidemic diet were selected. Rosuvastatin was administered in the dose of 10 mg/kg for 12 weeks. The MPV significantly decreased in the treatment group. The change in MPV however was not correlated with the change in serum lipid levels.[20]

CONCLUSION

High fat diet obesity in Sprague Dawley rats results in an increase in MPV which decreases on treatment with atorvastatin. Thus statins can have additional cardio-protective effects by causing a reduction in newly emerging cardiac risk factor marker i.e. MPV.

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